ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

REMOVAL OF VOLATILE ORGANIC SULFUR COMPOUNDS (VOSCs) EMISSIONS FROM DIFFERENT SOURCES VIA ANOXIC BIO-SCRUBBER

Ph.D. THESIS

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

FARKLI KAYNAKLARDAN OLUŞAN UÇUCU ORGANİK KÜKÜRT BİLEŞİKLERİ (VOSC'ler) EMİSYONLARININ ANOKSİK BİYO-SCRUBBER VASITASIYLA GİDERİLMESİ

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ŞUBAT 2019

Rasha Khalid Sabri Mhemid, a Ph.D. student of İTU Graduate School of Science Engineering and Technology student ID 501142711 successfully defended the thesis/dissertation entitled "REMOVAL OF VOLATILE ORGANIC SULFUR COMPOUNDS (VOSCs) EMISSIONS FROM DIFFERENT SOURCES VIA ANOXIC BIO-SCRUBBER.", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 20 December 2018

Date of Defense : 06 February 2019

To my spouse and children,

FORWARD

Firstly, I would like to express my deepest gratitude to my supervisor Prof. Dr. Kadir ALP for his continuous advice and guidance. Without his support and encouragement, this research would not have been possible.

Great appreciation goes to steering committee members, Assoc. Prof. Dr. Mustafa TURKER and Prof. Dr. Hayrettin Güçlü İNSEL, for their vital conversations.

The author is grateful to Res. Asst. Ilker AKMIRZA and Mohammed Salim SHIHAB for their high team work spirit and cooperation.

Special thanks to the Environmental Engineering laboratory technical staff and researchers.

I gratefully acknowledge the Iraqi Ministry of Higher Education and Scientific Research for the granted scholarship.

Finally, and most importantly, I would love to express my greatest gratitude to my parents, husband, children, and my brothers for their prayers, supplication, permanent support, infinite patience and meticulous care.

Thank you all, I am indebted to you all and will forever remain grateful.

February 2019

Rasha Khalid Sabri MHEMID
(Environmental Engineer)

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ABBREVIATIONS

BT : Butanethiol

COD : Chemical oxygen demand

DES : Diethyl sulfide

DMDS : Dimethyl disulphide

DMS : Dimethyl sulphide

DMSP : Dimethylsulphoniopropionate

EBRT : Empty bed residence time

EC : Elimination capacity

ET : Ethanethiol

HRT : Hydraulic Retention Time

IL : Inlet loads

MPS : Methyl phenyl sulphide

MT : Methanthiol

OTV : Odour threshold values

pH : Hydorgen ion concentration

PT : Propanethiol

RE : Removal efficiency

S.D. : Standard deviation

SD : Spraydensity

VOSCs : Volatile organic sulfur compounds

VSCs : Volatile sulfur compounds

VSS : Volatile suspended solids

SYMBOLS

CH_4	: Methane
CO_2	: Carbon dioxide
H_2S	: Hydrogen Sulfide
K_s	: Half-saturation coefficient
μ_{max}	: Maximal specific growth rate
NO_3^-	: Nitrate
q_{max}	: Maximal specific degradation rate
S°	: Elemental sulfur
SO_4^{2-}	: Sulfate
$Y_{ET/NO3^-}$	: Yield values (mol ET/mol NO_3^-)
Y_{XS}	: Biomass yield coefficient
ΔG_f^o	: Standard Gibbs free energy
ΔG_r^o	: Gibbs free energy for reaction (kcal/mol)

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SUMMARY

The air and water quality are affected by a technological development in a daily life and increased industrialization. The emission of pollutants and particulate materials into the atmosphere can be considered as a major impact of industries on the environment, leading to air and water pollution. The polluted streams comprise criteria pollutants and hazardous pollutants (as characterized by EPA). The criteria pollutants include sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), ozone (O_3), suspended particulate matter (SPM), lead (Pb); while the hazardous air pollutants include Volatile Organic Compounds (VOCs), ammonia (NH_3), hydrogen sulfide (H_2S), etc. VOCs are the major pollutants released by the industries, contaminating the atmospheric air and the fresh water resources. One of the VOCs are Volatile organic sulfur compounds (VOSCs). They have negative effects on human health and environmental even at very low concentrations. In addition, VOCs are main pollutants that release odour to the environment, which include dimethyl sulfide DMS (CH_3SCH_3), methanethiol MT (CH_3SH), ethanethiol ET (CH_3CH_2SH), dimethyl disulfide DMDS (CH_3SSCH_3). VOSCs are described by their potential corrosive influence, odour threshold value (OTV) and high toxicity. The emissions of odor are accepted as an important air contaminating parameter. Organic or inorganic odorous compounds are released into atmosphere from different industrial activities in gas or particle phase (as dissolved in liquid drops) forms. Because of their characteristics, these pollutants diffuse to the environment in a very short time and cause various disturbances. In addition, there is increasing concern about the environment as well as more stringent national and international regulations. Thus, there is a need to remove these emissions since they can cause real harms to humans and environment. The present study deals with the bio-degradation and bio-scrubber system for the removal of VOSCs from waste gas. The potential of microorganisms in consuming the VOSCs as carbon source makes biodegradation an attractive option for the removal of pollutants from the waste gas streams. Bio-scrubber is one of the bio-based techniques to prevent from the contaminated gas streams. Unlike physical and chemical technologies such as (chemical scrubbers, adsorption with activated carbon or clean waterscrubb, incineration, thermal oxidation, condensation, catalytic oxidation and ozonation) bio-scrubber offers considerable advantages over other physical and chemical methods for treating odorous waste gas that have water-soluble compounds as well as its low pressure drops during operation, avoiding the products accumulation, and prevent losing high amount of water spray column, absence of a secondary pollution and providing better control of reaction conditions (pH, nutrients). This study is focused on the batch anoxic bio-degradation process of single and the mixture of VOSCs such as ET and DMS and anoxic bio-scrubber system for the removal of ET or/and DMS. The examined biodegradation experiments in laboratory scale are carried out using acclimated mixed culture for the treatment of pollutants such as ET and DMS.

The batch experiments are executed to investigate the effect of target gas concentration (ET or/and DMS) and effect of biomass concentration on bio-degradation time of these compounds. Also, it was aimed to understand the behavior of microorganisms in bio-degradation the VOSCs and generate the data to find growth kinetic parameters using Monod model. The decreasing of VOSCs concentration with time refer to the microorganisms utilize the VOSCs as a carbon source. The maximum value of specific growth rate (μmax) is obtained as 0.14 and 0.2 1/h as sole compound for ET and DMS, respectively, while it is obtained as 0.036 and 0.03 as mixture of ET and DMS, respectively; in binary system, at 840 mg/m^3 and 850 mg/m^3 for initial concentration of ET and DMS as single compound respectively, and at 876 ± 30 mg/m^3 and 860 ± 10 mg/m^3 as dual compounds of ET and DMS respectively.

The bio-scrubber technology under anoxic condition is carried out for the removal of pollutants such as ET and DMS on a laboratory scale bio-scrubber (of 10 cm diameter and 50 cm long). The acclimation period of ET and DMS is achieved as 36 and 38 days, respectively, until reaching stability in the output concentrations. The operation time for the ET and DMS are 300 and 315 days, respectively. Various operational conditions related problems, such as pollutant concentrations, spraying density (SD) and empty bed residence time (EBRT) affected the bio-scrubber performance for removing single and dual compounds.

The performance of ET removal in an anoxic lab-scale bio-scrubber is investigated under various operating parameters and conditions for 300 days. The removal efficiency (RE) of ET is examined as a function of inlet concentration, EBRT and SD of irrigation. The results showed the best operation conditions and operation characteristics of the bio-scrubber for this study are obtained at an inlet concentration of 150 mg/m^3, an SD of 0.23 m^3/m^2 h and an EBRT of 90 s. An average RE of 91% and elimination capacity (EC) of 24.74 g/m^3 h is found for all inlet ET concentrations. Variations in SD higher than 0.23 m^3/m^2 h had no effect on ET RE at different ET concentrations. In addition, the anoxic lab-scale bio scrubber is studied to examine the DMS removal under various operation conditions for 315 days. DMS removal in bio-scrubber system is executed by controlling and changing the operation parameters, such as, inlet concentration, EBRT and SD of irrigation. Best conditions in the system are obtained for SD of 0.18 m^3/m^2h within EBRT of 40 s at 150 mg/m^3 inlet gas concentration with 92% of RE. The lab-scale bio-scrubber system can remove dual pollutant (ET and DMS) mixture. The optimal ratio was 3:1 for mixture at an EBRT of 90 s and a SD of 0.23 m^3/m^2 h. According to aforementioned results, bio-scrubber has significant ability of remove the compounds (ET, DMS) separately or as mixture under anoxic conditions. The average experimental yield molar ratio value was closer to the theoretical value ($Y_{compound}/NO_3^-$) of 0.74 that is derived from stoichiometric equations when the main product was elemental sulfur S^o. This indicates that S^o and other sulfur forms were produced rather than sulfate SO_4^{2-} as the end product.

The study also deals with the molecular analysis. The bacterial communities' profile was examined by the 16S™ NGS (next generation sequence) of sample that taken from bio-tank in the bio-scrubber system for removal ET and DMS. Based on 16S™ NGS results revealed the predominate microbial phylum *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* were distinguished by increased relative abundance around 1.33 times of its relative abundance in the

biomass samples taken from the bio-scrubber system. As the operation continued, they were capable of degradation of carbohydrates, mercaptans groups and inorganic sulfide. The microbial profile indicated also that other microorganisms (non-inoculated) namely *Antibacterial* and *Firmicutes* were generated during the operation of the bio-scrubber. The class *Chlorobea* within the *chlorobi* family, is capable of oxidating the reduced sulfur compounds to S^o. The strains of mixed biomass resisting in the bio-scrubber system have been successfully applied for bio-degradation of ET or/and DMS.

FARKLI KAYNAKLARDAN OLUŞAN UÇUCU ORGANİK KÜKÜRT BİLEŞİKLERİ (VOSC'ler) EMİSYONLARININ ANOKSİK BİYO-SCRUBBER VASITASIYLA GİDERİLMESİ

ÖZET

Kullanıma elverişli hava ve su kalitesi, gündelik hayattaki teknolojik gelişmelerden ve artan endüstrileşmeden etkilenmektedir. Gaz, sıvı ve partikül maddelerin atmosfere verdiği emisyon, hava ve su kirliliğine yol açan ve endüstrinin sebep olduğu büyük etki olarak düşünülebilir. Kirlenen akımlar, kriter kirleticileri ve tehlikeli kirleticilerden oluşmaktadırlar (EPA'nın nitelendirdiği şekilde). Kriter kirleticiler arasında kükürt dioksit (SO_2), azot dioksit (NO_2), karbon monoksit (CO), ozon (O_3), askıda partikül madde (SPM), kurşun (Pb) yer alırken, tehlikeli hava kirleticiler arasında ise Uçucu Organik Bileşikler (VOC), amonyak (NH_3), hidrojen sülfür (H_2S) vb. yer almaktadır. VOC'ler, atmosferi ve temiz su kaynaklarını kirleten ve endüstriler tarafından salınan başlıca kirleticilerdir. VOC'lerden bir tanesi uçucu organik kükürt bileşikleridir (VOSC'ler). Bunların çok düşük konsantrasyonlarda dahi insan sağlığı ve çevre üzerinde olumsuz etkileri bulunmaktadır. Ayrıca VOSC'ler, aralarında metanetiyol MT (CH_3SH), dimetil sülfür DMS (CH_3SCH_3), dimetil disülfür DMDS (CH_3SSCH_3), etanetiyol ET (CH_3CH_2SH) yer alan temel çevresel koku kirleticilerdir. VOSC'ler, yüksek toksisiteleri, potansiyel aşındırıcı etkileri ve çok düşük koku eşik değerleri (OTV) ile nitelendirilirler. Koku emisyonları, önemli bir hava kirletici parametresi olarak kabul edilmektedir. Organik ve inorganik kokulu bileşikler, gaz veya partikül fazlarda (sıvı damlaları içerisinde çözünmüş olarak) farklı endüstriyel faaliyetler tarafından atmosfere salınırlar. Bu kirleticiler, özelliklerinden dolayı çok kısa bir sürede çevreye yayılır ve çeşitli sıkıntılara sebep olurlar. Ayrıca, çevre ile ilgili artmakta olan kaygılardan dolayı daha sıkı ulusal ve uluslararası düzenlemeler mevcuttur. Bundan dolayı, insanlara ve çevreye karşı ciddi zararlar verdiklerinden dolayı bu emisyonların giderilmesi gerekliliği doğmuştur. Bu çalışmanın konusu, biyolojik bozunma ve VOSC'lerin atık gazdan arındırılmasına yönelik biyo-gaz yıkama sistemidir. Karbon kaynağı gibi, VOSC'leri tüketen mikroorganizmaların potansiyeli, biyolojik bozunmayı atık gaz akımlarının kirleticilerden arındırılmasına yönelik cazip bir seçenek haline getirmektedir. Biyo-gaz yıkama, kontamine gaz akımlarını önlemeye yönelik biyolojik temelli tekniklerden biridir. Yoğuşma, aktif karbon ile temizlenme veya temiz su yıkayıcılar, yakma, kimyasal gaz yıkayıcılar, termal oksidasyon, katalitik oksidasyon ve ozonlama gibi fiziksel ve kimyasal tekniklerin aksine, biyo-gaz yıkayıcı, operasyon sırasında düşük basınç düşüşleri,

ürün birikiminden kaçınma ve büyük miktarda su püskürtme kolonu önlenmesi, ikincil bir kirlenmenin meydana gelmemesi ve reaksiyon koşullarının (pH, besin ögeleri) daha iyi kontrolünün sağlanması açısından suda çözünebilen bileşiklere sahip kokulu atık gazları arındırmaya yönelik diğer fiziksel ve kimyasal yöntemlere göre kayda değer avantajlar sağlamaktadır. Bu çalışmanın odaklandığı konu, ET ve DMS gibi tekli ve karışım VOSC'lerin toplu biyolojik bozunma prosesine ve ET ve/veya DMS giderimine yönelik anoksik biyo-gaz yıkayıcı sistemidir. Laboratuvar ölçeğinde incelenen biyolojik bozunma deneyleri, ET ve DMS gibi kirleticilerin arındırılması için ortama alıştırılmış karışık kültür kullanılarak gerçekleştirilmiştir.

Toplu deneyler, hedef gaz konsantrasyonunun (ET ve/veya DMS) ve biyokütle konsantrasyonunun bu bileşiklerin biyolojik bozunma zamanına olan etkisinin araştırılması amacıyla gerçekleştirilmiştir. Ayrıca, VOSC'lerin biyolojik bozunmalarında yer alan mikroorganizmaların davranışlarının anlaşılması ve Monod modeli kullanılarak kinetik büyüme parametrelerinin bulunması için veri oluşturulması amaçlanmıştır. VOSC'lerin konsantrasyonlarının zamanla azalması, mikroorganizmaların VOSC'leri bir karbon kaynağı olarak kullanmaları anlamına gelmektedir. Maksimum özgül büyüme oranı (μmax), ET ve DMS için tekil bileşikler olarak sırasıyla 0,14 ve 0,21/h, bunun yanı sıra ET ve DMS karışımı için sırasıyla 0,036 ve 0,03; ikili sayı sisteminde ET ve DMS ilk konsantrasyonunda tekil bileşik olarak sırasıyla 840 mg/m^3 ve 850 mg/m^3, çift bileşikler olarak ise sırasıyla 876 ± 30 mg/m^3 ve 860 ± 10 mg/m^3 olarak bulunmuştur. Biyo- gaz yıkama teknolojisi, ET ve DMS gibi kirleticilerin giderilmesi için anoksik koşullarda, laboratuvar ölçekli (10 cm çapında ve 50 cm uzunluğunda) bir biyo-gaz yıkayıcıda uygulanmıştır. ET ve DMS'nin ortama alıştırma süresi, çıkış konsantrasyonlarında istikrara erişilene kadar, sırasıyla 36 ve 38 gün olarak elde edilmiştir. ET ve DMS için operasyon süresi 300 ve 315 gündür. Kirletici konsantrasyonları, püskürtme yoğunluğu (SD) ve boş yatak kalma süresi (EBRT) gibi çeşitli operasyonel koşullarla bağlantılı sorunlar, tek ve çift bileşikler için biyo-gaz yıkayıcı performansını etkilemiştir. Anoksik bir laboratuvar ölçekli bir biyo-gaz yıkayıcıda uygulanan ET giderme performansı, 300 gün boyunca farklı operasyon parametreleri ve koşulları altında araştırılmıştır. ET giderim verimi (RE), sulamaya ait giriş konsantrasyonu, EBRT ve SD'nin bir fonksiyonu olarak incelenmiştir. Sonuçlar en iyi operasyon koşullarını göstermiş ve bu çalışma için biyo-gaz yıkayıcının operasyon karakteristikleri, giriş konsantrasyonunda 150 mg/m^3, SD'de 0,23 m^3/m^2 h ve EBRT'de 90 s olarak bulunmuştur. Tüm giriş ET konsantrasyonları için RE ortalaması %91 ve eleme kapasitesi (EC) 24,74 g/m^3 h olarak bulunmuştur. SD'deki 0,23 m^3/m^2 h üzerindeki değişimlerin farklı ET konsantrasyonlarında ET RE üzerinde etkisi olmamıştır. Ayrıca, anoksik laboratuvar ölçekli biyo-gaz yıkayıcı üzerinde 315 gün boyunca çeşitli operasyon koşullarında DMS giderimini incelemek amacıyla çalışılmıştır. Sulamaya ait giriş konsantrasyonu, EBRT ve SD gibi operasyon parametrelerinin kontrol edilmesi ve değiştirilmesi

için biyo-gaz yıkayıcı sisteminde DMS giderimi gerçekleştirilmiştir. Sistemden elde edilen en iyi sonuçlar, %92 RE ile 150 mg/m^3 giriş gaz konsantrasyonunda 40s EBRT içerisinde SD için 0,18 m^3/m^2h olarak bulunmuştur. Laboratuvar ölçekli biyo-gaz yıkayıcı sistemi, çift kirletici karışımını (ET ve DMS) giderebilmektedir. 90 s EBRT ve 0.23 m^3/m^2 h SD'deki karışım için optimum oran 3:1 olarak bulunmuştur. Yukarıda belirtilen sonuçlara göre, anoksik koşullarda biyo-gaz yıkayıcının bileşikleri (ET, DMS) ayrı ayrı ya da karışım olarak kayda değer bir giderim kabiliyeti bulunmaktadır. Ortalama deneysel verim molar oranı değeri, ana ürün element kükürt S^o olduğunda, stoikiometrik denklemlerden türetilen 0,74 teorik değerine ($Y_{bileşik/\ NO3-}$) daha yakın çıkmıştır. Bu durum, son ürün olarak sülfat SO_4^{2-}'den ziyade S^o ve diğer kükürt formlarının üretildiğini göstermektedir. Bu çalışma ayrıca, moleküler analizi ele almaktadır. Bakteriyel toplulukların profili, ET ve DMS giderimi için biyo-gaz yıkayıcıdaki biyo-tanktan alınan numunede 16S™ NGS (yeni nesil dizi) ile incelenmiştir. 16S™ NGS sonuçlarına dayanarak, baskın mikrobiyal filum *Proteobakteriler*, *Bakteroidetler* ve *Aktinobakteriler*, biyo-gaz yıkayıcı sisteminden alınan biyokütle örneklerinde göreceli bolluğunun 1,33 katı civarında artan göreceli bolluktan ayırt edildiğini göstermiştir. Operasyonun devam etmesiyle, karbonhidratların, merkaptanlar gruplarının ve inorganik sülfitin azaltılmasını sağlamışlardır. Mikrobiyal profil ayrıca biyo-gaz yıkayıcı operasyonu sırasında *Antibakteriyel* ve *Firmicutes* adı verilen diğer mikroorganizmaların (aşılanmamış) ortaya çıktığını göstermiştir. *Chlorobi* ailesine ait *Chlorobea* sınıfı, azaltılmış kükürt bileşiklerini S^o'a okside etme kabiliyetine sahiptir. Biyo-gaz yıkayıcı sisteminde direnen karışık biyokütle zorlamaları, ET ve/veya DMS'nin biyolojik bozunması için başarıyla uygulanmıştır.

1. INTRODUCTION

Volatile organic sulfur compounds (VOSCs) are considered in the main environmental odor pollutants, which include methanethiol (CH_3SH), dimethyl sulfide DMS (CH_3SCH_3), dimethyl disulfide DMDS (CH_3SSCH_3), Propanethiol PT ($CH_3CH_2CH_2SH$), Butanethiol BT ($CH_3(CH_2)_3SH$) and ethanethiol ET (CH_3CH_2SH). VOSCs are characterized by their high toxicity, potential corrosive effect and very low olfactory threshold values; e.g. 0.6-40 ppbv and 0.01-350 ppbv for (CH_3SCH_3, DMS) and (CH_3CH_2SH, ET), respectively. (Sun et al, 2016) (Demeestere et al, 2005) & (Tangerman et al, 2009) (Albert Janssen et al., 2013). Moreover, these compounds may cause poisoning, headaches, skin and respiratory irritations, human health threats and vomiting (Giri et al, 2014).

Volatile sulfur compounds have a bad smell, toxicity, and corrosive properties. This is why their presence in the atmosphere is undesirable. H_2S and MT are present as a gas at ambient conditions, whereas, under the same conditions many other VOSCs present in a liquid state. Low odour three shold OTV (ppb range), high toxicity, and potential corrosive effects are the most important characteristics of these compounds. Because of the range of OTV is in the parts per billion, VOSCs will have a role in odour problems even in small amounts emission. In the parts per billion range, MT, DMDS and DMS compounds are entered into the food as an important flavor component such as strawberries, milk, radishes, beer, wine, tea, cocoa, oysters, cheese, coffee and many cooked vegetables. H_2S, MT, and DMS can also be emitted from decaying proteins but in Trace amounts. It is possible to take advantage of this emission as a warning signal of spoiled foods due to the presence of unpleasant odor quality.

In addition, research shown that natural human breath contains ET at the level of 1-6 $\times 10^{-9}$ g /L, while, the sea's smell beyond to the presence of DMS partially. The chemical and physical properties of some sulfur compounds can be seen in Table 1.1 (Albert Janssen et al, 2013).

Table 1.1: Chemical and Physical properties of some sulfur (organic/inorganic) compounds.

Sulfur compound	Odorant character	Boiling point (◦C)	Melting point (◦C)	Odor threshold (ppbv)	Henry's constant at 25°C	Solubility at 25°C (%)
H_2S	Rotten eggs	−60.7	−85.5	8.5–1000	0.41	0.34
CH_3SH	Decayed cabbage	6.2	−123.0	0.90–8.5	0.10	2.3
CH_3CH_2SH	Skunk	35.0	−147.9	0.01−350	0.15	0.676
$CH_3(CH_2)_2SH$	Rotten cabbage, skunk	67.8	−113.1	3.1	0.17	—
$(CH_3)_2S$	Decayed vegetables	37.3	−98	0.6−40	0.073	2.0
$(CH_3)_2S_2$	Irritating, putrefaction, foul	109.7	−85	0.1−3.6	0.045	—
$(CH_3CH_2)_2S$	Garlic	92.1	-103.9	0.033	—	—

Among the VOSCs, ET and DMS are a volatile organic sulfur compound (VOSC) with an intensive rotten cabbage odor, a colorless liquid, the vapor and liquid states of which are highly flammable, and if inhaled, highly toxic. DMS and ET are released into the atmosphere from both through natural and anthropogenic sources, including production by phytoplankton in the oceans, salt marsh, Swamps, Soil and plants, sewage treatment plants, waste treatment or disposal facilities, paint facilities, petroleum refineries, landfill gas, liquefied petroleum gas, anaerobic brewery WWTP, synthetic natural gas, rendering plants, pulp mills, plastic and resin manufacturers and chemical industries as shown in Table 1.2. ET and DMS are commonly used in raw materials in the plastics, antioxidants and insecticides industries and are also used as a warning material to detect natural gas leaks into the environment (An et al, 2010). Compared with other volatile organic sulfur pollutants or mercaptans (ethyl mercaptan, CH_3SH, propyl mercaptan, and butyl mercaptan). They can even lead to death due to respiratory paralysis and has potential corrosive effects even at

low concentrations (Li et al, 2015). The human nose can detect the VOSCs at low concentrations (1 ppb of air). (Bajpai, 2014).

Table 1.2: Some emission sources and concentrations of ET and DMS

Type of gas stream / Typical concentration source	Unit	ET	DMS
Landfill gas	ppb(v)		1500
Landfill gas	ppb(v)		2600
Landfill gas	ppb(v)		1700
Landfill gas	ppb(v)		7
Landfill gas	ppb(v)		77
Synthetic natural gas	ppb(v)	400	220
Wastewater plant	mg/m^3		23.6-308.1
Liquefied petroleum gas	ppm(v)	220	
Liquefied petroleum gas	ppm(v)	5000	
Liquefied petroleum gas	ppm(v)	500	
commercial LPG feed containing 28 wt% propane and 71% n-butane	ppm	217	615
Propane	ppm(v)	10	
n-butane	ppm(v)	10000	
Gas samples from WWTP	ppb(v)		610
Low pressure fuel gas	ppm(v)	50	
Condensate from chiller separator	ppm(v)	139	
Waste air of wastewater treatment plants	mg/m^3		0.66-5.41
Rendering cookers	ppm(v)		
Kraft paper production	ppm(v)		17-20
Oceans	Tg/year		38-40
Salt marsh	Tg/year		0.58
Swamps	Tg/year		0.84
Soil and plants	Tg/year		0.2-4
Atmospheric concentration	ppb		0.11
aerobic brewery wastewater treatment plant	ppm(v)		30
aeration tank of a sewage treatment plant	µg/L		10-200
production of compost	ppb(v)	24-840	24-840

aerobic composting of biowaste	ppm(v)		3.2
hydrothermal reactor	mg/m^3	0.41	0.19
natural human breath	g/L	1-6 $\times 10^{-9}$	

In addition, these compounds have toxic environmental effects, causes global environmental problems such as acid rain formation and ocean acidification, and is very toxic to aquatic organisms (Li et al, 2015; Kachina et al, 2006). The Toxicity of sulfur compounds to humans and animals described via the maximum concentration values in them workplace (MAC) for volatile (organic) sulfur compounds are less than or equal to 20 ppmv as presented in Table (1.3), where the ET is more toxic than H_2S, DMS and DMDS as reported in the inhalation studies with rats (Janssen et al., 2013; van Leerdam, 2007).

Table 1.3: Toxicological properties of some volatile (organic) sulfur compounds and Maximum allowable concentration (Janssen et al., 2013; van Leerdam, 2007)

Sulfur compound	LC_{50} inhalation	LD_{50} oral	MAC (ppmv)
H_2S	444	-	1.6
MT	675-1664	-	0.5
ET	4970	1034	0.5
PT	7300	1730	-
BT	4460	2575	-
DMS	40,250	-	20
DMDS	805	-	<20

LC_{50}: Concentration, which is lethal to 50% of the animals tested, LD_{50}: Dose, which is lethal to 50% of the animals tested, MAC: Maximum concentration value in workplace conditions

As well, DMS influences the climate regulation by affecting atmospheric chemistry, the heat balance of the atmosphere and acid rain formation (Wei et al, 2013; Luvsanjamba et al, 2008; Gypens et al, 2014; Lomans et al, 1997). DMS is highly water-soluble, has a high potential for volatility, has a low potential of bioaccumulation due to a log Kow (octanol–water partition coefficient) < 3 (i.e. that a substance is easily to sorb and is not expected to bioaccumulate), is readily biodegradable and is harmful to aquatic life. Therefore, the removal of these

pollutants from different emissions of waste gas streams are highly desirable in the field of environmental engineering (Rappert et al, 2010). Various physical and chemical technologies have been invented to eliminate odours and gases to remove VOSCs from the air. Three methods, namely physical technologies (condensation, adsorption with activated carbon or clean water scrubbers, etc.), chemical technologies (chemical scrubbers, thermal oxidation, catalytic oxidation, ozonation, etc.) and biological technologies (bio-filters, bio-scrubbers, bio-trickling filter, etc.) have often been used for this purpose (Kennes et al, 1998; Burgess et al, 2001). Among these techniques, the physical and chemical methods have been identified as the most effective; however, the secondary pollutants and high costs and energy demand are unavoidable. In contrast, the biological waste air treatment techniques such as bio-filter, bio-scrubber and bio-trickling filter have been found to be very promising technologies for the removal of odorous and/or toxic volatile organic compound waste gases because of low operational and investment costs, low energy requirements and environmentally friendly technology as well as the absence of a secondary waste stream (Giri et al, 2013; Akmirza et al, 2016). Bio-filter, bio-srubber and bio-trickling filter are considering the major bioreactor design and they are usually used until now. The difference between them represented by the presence or absence of a carrier material and of a mobile liquid phase (Table 1.4). (Christian and Fre, 1998).

Table 1.4: Different Characteristics of the Three Main Biological Gas-Cleaning Technologies

Reactor design	Mobile phase	Carrier	Active biomass
Bio-scrubber	Liquid and gas	None	Dispersed
Trickling bio-filter	Liquid and gas	Synthetic	Fixed
Bio-filter	Gas	Organic/synthetic	Fixed

Bio-filters contain microorganisms in a biofilm form installed on a packed bed consisting of materials such as soil, manure, peat, and synthetic materials or combinations of these (Figure 1.1, b), in bio-trickling filters, a layer of inactive filler material is continuously sprayed through a liquid phase spreading from bottom to top of the column. Bio-scrubber concept consists of absorption the gas

phase to the liquid phase (physical separation), followed by the biological treatment as a liquid-phase in bio-reactor (Figure 1.1, a). (Dumont, 2015), (MAHMOOD et al, 2007).

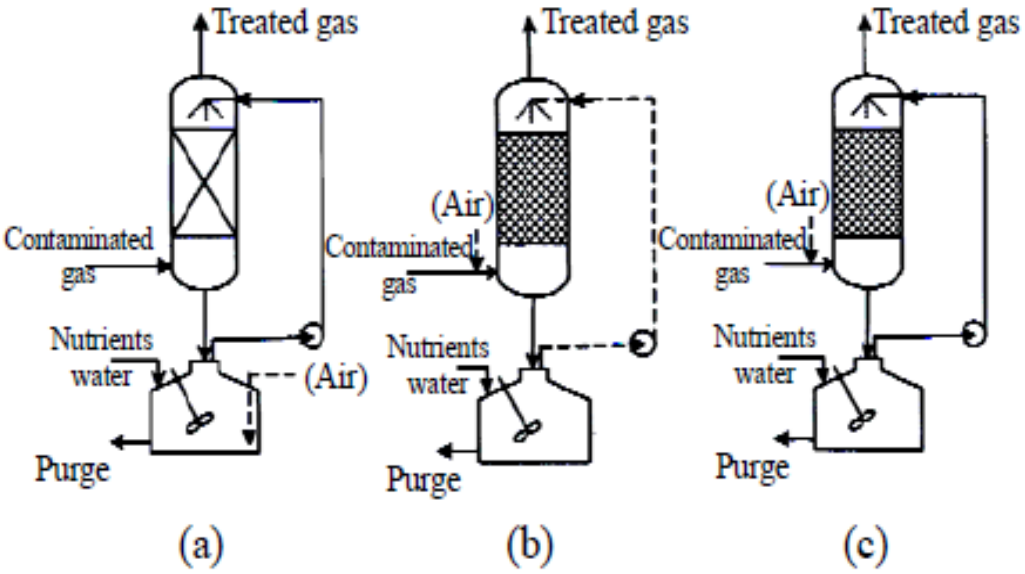

Figure 1.1: Bioreactors. (a) Bioscrubber; (b) Biofilter; (c) Biotrickling filter

Despite the advantages of these biotechnology alternatives on VOSCs removal, most of the studies focused on aerobic waste gas treatment, and that resulted in higher capital costs for operations (particularly energy for pumps or aerators) and aeration equipment and higher maintenance requirements, and they possibly have monitoring requirements to check the dissolved oxygen levels in the liquid (Qaisar et al, 2007). Up to now, only limited number of studies assessing the performance under anoxic conditions, and therefore, the development of biotreatment alternatives able to efficiently treat VOSCs emissions under anoxic conditions will significantly improve the economic and environmental sustainability of VOSC treatment in the petrochemical industry (Muñoz et al,

2013; Akmirza et al, 2017). Anoxic VOSC mineralization via denitrification offers a potential solution for the removal of VOSC from emissions in addition to nitrogen removal. There is no need for any addition of air and there is no limite of gas liquid mass transfer in anoxic systems because of the NO_3^- form in the liquid medium already content dissolved oxygen. As a result, anoxic bio-processes could be an appropriate choice to overcome the disadvantages of aerobic bio-processes that combine denitrification with VOSC removal (Dumont, 2015). In recent years, using NO_3^- as electron acceptors of sulfur compounds oxidation has been studied by several researchers. For example, Yavuz et al. (2007) studied the removal of sulfide from industrial wastewater using oxygen and NO_3^- as electron acceptors in lab-scale batch experiments. They found that 80% sulfide removal occurred when oxygen was used as an electron acceptor with activated sludge, and 100% sulfide removal was achieved when NO_3^- was used. Turker et al. (2012) investigated the removal of H_2S in biogas to S^o or SO_4^{2-} using NO_3^- and nitrite NO_2^- present in feed wastewater in a pilot-scale bio-scrubber system under denitrifying conditions. The results showed that biogas desulfurization was combined with nitrogen removal, normally required in most industrial wastewater treatment plants, and more than 90% of the H_2S in the biogas was removed with simultaneous nitrogen removal at wastewater/biogas ratios between 2 and 3.

Among the biological treatment alternatives, bio-scrubbers have been known to be an efficient technology for odour treatment in so far as resulting low pressure drops in operation, possibilities of avoiding accumulation of products and better control of reaction conditions (pH, nutrients) with water-soluble compounds in order to avoid high spray columns and large water flows, thus, it requires less attention than other bioreactors. Inaddition, emissions should not be wetted before treatment. This can save the cost of installing the moisturizing process. The bio-scrubber has a smaller footprint than the other bioreactor. Also, Bio-scrubber can treat emissions containing particulate matter and it can handling high pollutant concentrations and large gas flows (Li et al, 2015). However, there is a limited number of studies, and no report published related with biodegradation process of ET and DMS as a sole target and as mixture in an anoxic bio-scrubber. Therefore, it would be interesting to investigate the conditions for anoxic removal of each of ET and DMS separately and as mixture.

1.1 Purpose of the Thesis

Increasing number of residential areas next to industrial processes and improvement by lifestyle expectations of people have led to an increase in public complaints especially against odorous waste gases (toxic compounds) that contains organic sulfur compounds. industrial processes, wastewater treatment, and landfill areas are primary sources for VOCs and may cause undesirable issues in adjacent areas and contribute to atmospheric pollution. In order to reduce their undesirable effect, control and management of these VOCs emissions come into prominence

The anoxic bio-scrubber system was established to treat ET (CH_3CH_2SH) and DMS (CH_3SCH_3). Since their toxic effect on human health and global environmental problems even low concentration and their low OTV potential corrosive effects. With this aim of concept lab-scale bio-scrubber operation was started for single component waste gas flow and treatment efficiencies for each component was estimated. In the next stage of study, mixed synthetic waste gas streams constituted and synergic or detrimental effects of mixtures was investigated. The batch experiments were conducted as a first step to study the kinetics of the mixed culture of the lab-scale bio-scrubber and determine the bio-degradation capability of ET or/and DMS by acclimated mixed culture under anoxic conditions. Moreover, the kinetics parameters of ET or/and DMS were estimated for the first time using the Monod model under anoxic conditions. Experimental investigations were then as a second step conducted to remove the target gases, ET or/and DMS, in a lab-scale bio-scrubber under anoxic conditions and examined the influences of EBRT, SD and biological oxidation on the performance of the lab-scale bio-scrubber system. In addition to this treatment the production of S° as an end-product from the biodegradtion of each ET and DMS under anoxic conditions have economical values, where S° can be recovered, thus less sludge generation and minimizes the cost of sludge disposal.

1.2 Literature Review

The purpose of this section is to review the literature on removal and fate of sulfur compounds in enivronmental and to explain major studies done on these

subjects. Moreovre, increasing well being level of people and stringent legistations of countries against indutrial environmental pollution makes obligatory to design new environmental friendly and cost effective treatment technologies.

1.2.1 Microbial degradation of sulfur compounds

1.2.1.1 Aerobic and anoxic degradation

Kyeoung et al. (1991) tested the ability of the degradation of H_2S, MT, DMS and DMDS by *Thiobacillus thioparus* DW44 isolated from peat biofilter. The Michaelis-Menten model provided an accurate fitting for the experimental data to find the maximum removal rates (V_m; g-S/ kg-dry peat. d) for single compounds via the peat biofilter; V_m (H_2S) = 5.52, V_m (MT) = 1.16, V_m (DMS) = 0.50 and V_m (DMDS) = 1.02. Visscher and Taylor (1993) studied aerobic and anaerobic degradation of a range of alkyl sulfides (DMS, diethyl sulfide (DES), ET, dipropyl sulfide, dibutyl sulfide, and dibutyl disulfide) by a denitrifying marine bacterium. They found the isolate bacterium was identified as a Thiobacillus sp. and designated strain ASN-1. It could have metabolized alkyl sulfides by induced or derepressed cells with oxygen, NO_3^-, or NO_2^- as electron acceptor.

In Table 1.5 a summary of reactions is given of the microbial conversions of MT and DMS under aerobic and anoxic conditions as mentioned by (Albert Janssen et al, 2013).

Table 1.5 : Microbial conversions of VOSCs

Oxidation	$DMS/MT + O_2 \rightarrow MT + CH_2O \rightarrow CO_2 + SO_4^{2-}$
	$DMS/MT + O_2 \rightarrow CO_2 + S_2O_3^{2-}$
Phototrophic oxidation	$DMS/MT \rightarrow DMSO$
Oxidation to DMSO	$DMS + O_2 \rightarrow DMSO$
Denitrification	$DMS/MT + NO_3^- \rightarrow N_2 + CO_2$
Methanogenesis	$DMS/MT \rightarrow CH_4 + CO_2 + HS^-$
Sulfate reduction	$DMS/MT + SO_4^{2-} \rightarrow CO_2 + HS^-$

From literature, ET can be degraded biologically via *pseudomans* sp. as a sole source of carbon and energy under aerobic condition, the aerobic oxidation of ET

into the end products of SO_4^{2-} was depicted in Figure 1.2, despite the multiple advantages of aerobically biological treatment of sulphur compounds possible undesirable by-product production due to their rapid conversion to H_2S in the absence of oxygen is considered as one of the drawbacks of the aerobic systems (Wang et al, 2015).

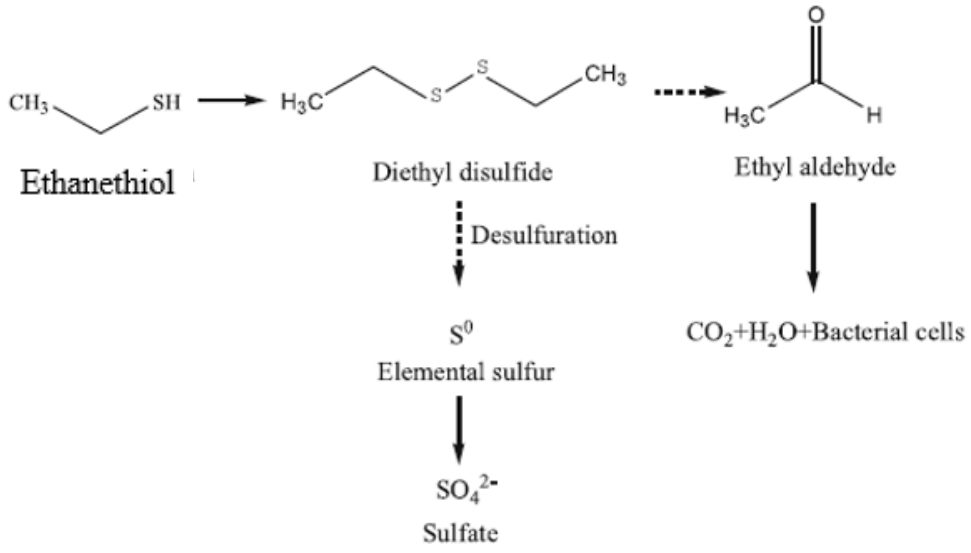

Figure 1.2: Aerobic pathways of ethanethiol degradation by *Pseudomonas* sp. strain WL2. Solid-arrow: identified intermediate (products); dashed-arrow: possible intermediate.

The effects of different sulfur to nitrogen (S/N) ratios on the specific autotrophic denitrification activity of the sludge was studied by (Campos et al, 2008) in batch experiments using thiosulfate and NO_3^- as substrates. They conculded that NO_2^- was the main end product when S/N ratios of 3.7 and 6.67 g/g output from the the higher specific reduction rate of NO_3^- compared to that of NO_2^-. While, NO_3^- was the main end product when S/N ratios of 1.16 and 2.44 g/g were examined. In addition, they determined the influnce of endogenous (NO_3^- ,NO_2^- ,$S_2O_3^{2-}$ and SO_4^{2-}) and exogenous compounds (acetate and NaCl) on the specific denitrifying activity. NO_2^- and SO_4^{2-} had inhibitory effects over the process whereas $S_2O_3^{-2}$, acetate and NaCl did not have strong effects at the concentrations tested.

1.2.1.2 Anaerobic degradation

Lomans et al. (2002) indicated the importance of studying DMS and MT through Microbial cycling of VOSCs because these compounds play an intensive role in the processes of global warming, the global sulfur cycle and acid precipitation. Also they mentioned in freshwater sediments VOSC concentrations are relatively low due to the mass balance between the formation and degradation of these compounds. while in marine ecosystems, where dimethylsulfoniopropionate (DMSP) can be found in marine phytoplankton. DMSP is broken down by marine microbes to form two major volatile sulfur products, MT and DMS. Kiene et al. (1986) described the path of DMS Under anaerobic conditions via a pure microorganisms of an Estuarine Methanogen, where is first converted to methane (CH_4) and MT, then subsequently disproportionated to produce the end products of CH_4 , CO_2, and H_2S as shown below.

$$(CH_3)_2S + 2H^+ + 2e^- \rightarrow CH_4 + CH_3SH \qquad \Delta G^\circ_f = -72.8 \text{ kJ/mol}$$

$$CH_3SH + H_2O \rightarrow 0.5CH_4 + 0.5CO_2 + H_2S + 2H^+ + 2e^- \qquad \Delta G^\circ_f = -1.0 \text{ kJ/mol}$$

$$\text{Sum: } (CH_3)_2S + H_2O \rightarrow 1.5CH_4 + 0.5CO_2 + H_2S \qquad \Delta G^\circ_f = -73.8 \text{ kJ/mol}$$

To investigate the degradation of H_2S by using two new strains of microorganisms (*Acinetobacter sp.* MU1_03 and *Alcaligenes faecalis* MU2_03) under certain conditions, Potivichayanon et al. (2005) found a mixture of the two strains was capable of 98% H_2S degradation. RE% increased with decreasing inlet gas flow rates, increasing the height of packing and EBRT. The H_2S converts to SO_4^{2-} under aerobic condition, where SO_4^{2-} production increased when the RE% increased.

1.2.2 The technologies of treating gas streams containing sulfur compounds.

The different technologies that used for the gaseous emissions treatment classified into physical, chemical and biological. a number of considerations must be take into accounts. For the selection of an appropriate treatment technique, such as the composition and property of the waste stream, its quantity and concentration,

characteristics of the air or waste gas stream and the generation of by-products from the selected process.

1.2.2.1 Physical-chemical technologies

Rafson et al. (1998) reported the physical and chemical air pollution control technologies for treatment sulfur compounds including incineration, regenerative thermal oxidation, and wet scrubbers. They found that thermal elimination and incineration methods have high operating costs, produce a greenhouse gas carbon dioxide (CO_2) and require SO_2 scrubbing.

Kastner and Das (2002) investigated chemical wet scrubbers for volatile organic compound removal; they found that this system requires expensive oxidizing chemicals such as ClO_2 or NaOCl, requires large amounts of water, and can produce chlorinated hydrocarbons if not properly controlled.

Mercaptans groups (ET, MT, DMS, propyl mercaptan, and butyl mercaptan) from gas emissions of refinery combustion sources can be removed by absorption into amines and caustics (Jones et al, 2014).

In absorption process, there are different types that used for absorbents such as water scrubbing, organic physical scrubbing, and chemical scrubbing. Normally, for hydrophilic waste gases water is the most scrubbing absorbent that using while, silicone oil or polyethylene glycol is more adequate for hydrophobic waste gases (López et al., 2012; van Leerdam, 2007). Furthermore, the scrubbing of VOSCs due to the high Henry constant of these compounds needs high water flow rate to gas flow rate (L/G) (Smet and Van Langenhove, 1998). It is worth noting that light thiols MT, ET and propyl mercaptan can be scrubbed by an alkaline liquid with pH at least 11.0 (van Leerdam, 2007). Nevertheless, Hypochlorite is the most active and efficient oxidant for the chemical scrubbing of VOSCs is (OCl^-, pH >6) (Smet and Van Langenhove, 1998).

Generally, the adsorption mechanism is efficient for abatement of VOCs with low vapor pressures and high molecular weights (Devinny et al., 1999; Kennes,

2013). Frequently, activated carbon has high sorption capacity in VOSCs and H_2S removal based on physical adsorption mechanism, whereas DMS, DMDS, and CS_2 was removed very effectively (van Leerdam, 2007).

Condensation systems are based on the conversion of gases waste into a liquid by pressurization and/or by temperature reduction. These techniques have efficiently proved for VOCs controlling. Mainly, VOCs with a boiling point above 40°C, concentration more than 5000 ppmv, and relatively low mass flow rates (Gómez, 2016; Kennes, 2013; Rene et al., 2012).

Incineration does not allow the recovery of the contaminants, it requires a high operating and capital cost technique due to the high-temperature input that necessary for complete combustion and oxidation (Devinny et al., 1999; Gómez, 2016; Kennes, 2013; Rene et al., 2012).

The main drawbacks of the Physical-chemical technologies are the high capital and operating costs, high energy requirements and non-environmentally friendly nature (Sedighi et al, 2013).

1.2.2.2 Biological technologies under aerobic and anaerobic

Traditionally, bio-filters, bio-scrubbers, and bio-trickling filters are the main categories for air phase treatment technologies that based on absorbed volatile off-gases on aqueous, frequently, water followed by biodegradation (Smet et al., 1998).

Bio-filter

Biological treatment of DMS was described by (Giri et al, 2013) in a bench-scale bio-filter, packed with compost with wood chips, and enriched with DMS degrading microorganism *Bacillus* sphaericus. The bio-filter could remove 62–74% of the inlet DMS from ambient air, at an optimum loading of 0.484 g/m^3 h with optimum EBRT of 384 s and an average moisture range of 65–70%. The bio-degradative products of DMS were sulfide, thiosulfate and SO_4^{2-}. Evaluation of microbiological status of the bio-filter indicated the presence of other bacterial cultures viz. *Paenibacillus polymyxa*, and *Bacillus megaterium*, besides B.

sphaericus. Removal of H_2S and NH_3 using two bio-filters proposed by Kim et al. (2002), the first one packed with wood chips while the second one paked with granular activated carbon (GAC) and spray the packing material was by a mixture of activated sludge (as a source of nitrifying bacteria) with *Thiobacillus thioparus* (for sulfur oxidation), also, the drain solution of the bio-filter was recirculated to increase the acclimation of microorganisms. 99.9% REs were obtained in the inital operation of both filters but with time progress the RE% decreased beacuse of the clogging effect of S° and ammonium sulfate on the surface of packing materials to 75% and 30% for H_2S and NH_3, respectively. Chung et al. (1996) sutdied the effect of immobilized *Thiobacillu thioparus* CH11 obtaining from swine wastewater with Ca-alginate producing pellet packing material for the bio-filter system to removal of H_2S. More than 98% of RE was found at a 28 s optimal retention time and the S^o or SO_4^{2-} was produced depending on the amount of inlet H_2S concentration. Typical applications of bio-filters are used to treat malodorous gases i.e. NH_3, VOCs, H_2S, DMS, DMDS, MT, etc. emitted from the processing of food wastes, wastewater treatment facilities, and composting and rendering plants (Hort et al, 2009). Bench and pilot scale studies using bio-filtration techniques have shown that organic and inorganic sulfur compounds, such as H_2S, SO_2, and VOSCs pollutants in industrial and municipal gaseous emissions can be successfully treated (Jaber et al., 2017; Luvsanjamba et al., 2008; Zhang et al., 2015).

Bio-scrubber

The bio-scrubber was used by Nishimura and Yoda (1997) to remove H_2S from the biogas produced by an anaerobic wastewater treatment process, where H_2S production from the biogas was absorbed in scrubbing column then transferred into the biological aeration tank and degraded to SO_4^{2-} by sulfur oxidizing bacteria. The results showed that more than 99% RE was achieved. Mesa et al. (2002) used a bio-scrubber of removing H_2S emitted from biogas via a combination of chemical and biological processes. Where, H_2S removal can be achieved first by absorption in a ferric sulfate solution (chemical process) lead to produce ferrous SO_4^{2-} and S°. While in biological process using Acidithiobacillus ferroxidase to regenerate the Ferric. Removal of H_2S from natural gas described

by Benschop et al. (2002) using a full-scale bio-scrubber system. H_2S is removed from a gaseous stream by absorption into a sodium carbonate/bicarbonate solution then the sulfide as aqueous phase containing scrubbing liquid is transferred to the biological tank. Less than 3.5% of the sulfide is converted to SO_4^{2-} and mostly converted biologically to S°. Also, the trace H_2S present in the spent air from the bioreactor can be treated using compost filter as researchers mentioned. Where, lower than 4×10^{-6} (v/v) outlet H_2S concentration is reached when handling with natural gas containing 2000×10^{-6} (v/v) H_2S. Barbusinski et al, (2017) mentioned in the bio-scrubber system the effluent from bio-tank is recirculated to the top of the absorber column, providing efficient gas cleaning of highly soluble pollutant. Moreover, the bio-scrubber was applied for handling high pollutant concentrations and high gas flows rate.

Bio-trickling filter

Wange et al. (2015) used microorganisms fixed on iron oxide-based porous ceramsite in a bio-trickling filter for removal of waste gas containing ET under aerobic conditions. Sedighi et al. (2016) investigated the degradability of ET via phenol utilizing cells of *Ralstonia eutropha* in both suspended and immobilized culture systems in a gas trickle-bed reactor packed with kissiris particles. ET can be degraded biologically as a sole source of carbon and energy or as mixture with DMDS and methyl phenyl sulfide (MPS) by inoculated the microorganisms of the B350 group in a bio-trickling filter (Wan et al, 2011). Ramírez et al. (2011) used tow bio-trickling filters in series for H_2S, MM, DMS and DMDS treatment. The first one was inoculated with Acidithiobacillus thiooxidans (BAT) and the second one with Thiobacillus thioparus (BTT). They highlighted the effect of H2S on biodegradation of the VOSCs i.e. MM, DMS, and DMDS. H_2S concentration increment from 23 to 293 ppmv had an inhibitor effect on VOSCs degradation. Where the REs levelled off from 97 to 84%, 86 to76%, and from 83 to 67% for DMS, DMDS, and MM, respectively. The sequence order of the ECs of these VOSCs was as following: H_2S > DMDS > DMS > MM. Hence, ECs of H_2S-S, DMDS-S, and MM-S were 57.5, 36, and 9.8 g/m^3 h, respectively.

The removal performance of aerobic bio-trickling filter immobilized with *Lysinibacillus* sphaericus RG-1 was evaluated by Wan et al. (2011) for treating ET, DMDS, and thioanisole in single, binary and ternary gas mixture pattern. They reported that the sole ET, DMS, and thioanisole was completely removed (i.e RE 100%) when inlet concentration less than 1.05, 0.81 and 0.33 mg/L, respectively, and EBRT 110 sec. Also, in binary (ET:DMDS 1:1) and ternary (ET:DMDS:thioanisole 3:2:1), complete removal (i.e. RE100%) was achieved. The kinetic parameters were calculated for ET, DMDS, and thioanisole, where the maximum removal rate (Vmax) was 56.18, 57.14 and 22.78 g/m3/h, respectively. Accordingly, RG-1 strain has a significant potential for degradaing sole, even mixture of ET, DMDS and thioanisole. The aerobic co-treatment properties of single, dual and ternary mixture gas of ET, DMDS and thioanisole in a bio-trickling filter inoculated with *Lysinibacillussphaericus* RG-1 was reported by Shungang et al. (2011). The results showed for sole ET, DMDS and thioanisole, 100% RE was obtained at operation condition, inlet concentration below 1.05, 0.81 and 0.33 mg/L, respectively, and EBRT 110s. Also at the same operation condition the RE of binary ET and DMDS (1:1) and ternary ET, DMDS and thioanisole (3:2:1) was 100%. The maximum removal rate (Vmax) using Michaelis–Menten equation was found as 56.18, 57.14 and 22.78 g/m^3 h for sole ET, DMDS and thioanisole, respectively, whereas, the Vmax was 41.84 and 14.56 g/m^3 h for binary and ternary systems, respectively. Therefore, according to the results, the strain RG-1 was more efficient for removing ET, DMDS and thioanisole with high concentrations of ET, DMDS and thioanisole, even the mixture of them. Compare the twin-bio-trickling filter columns performance of ET by inoculated and immobilized the strain RG-1 and B350 on ceramic particles. The maximum elimination capacities were 39.93 (RE= 60.30%) and 30.34 g/m3 h (RE= 46.20%) for RG-1 and B350, respectively, at an inlet concentration of 2.03 mg/L. Depending on the results, the strain RG-1 was a better choice than strain B350 for the biodegradation of ET. SO_4^{2-} was the main end product of sulfur of ET under anaerobic condition (Taicheng et al, 2010).

Several authors have proposed the possibility of aerobically degrading these pollutant VOSCs in different biological reactor configurations, including bio-filters, bio-scrubbers, and bio-trickling filters. Although it is environmentally

friendly, these aerobic biological processes have high capital costs for aeration equipment, high operating costs (particularly energy for pumps or aerators), and high maintenance requirements has been submitted by (Fernández et al, 2013; Kennes et al, 1998; Qaisar et al, 2007).

1.2.3 Anaerobic and aerobic bio-degradation of removal sulfur compounds

The aerobic and anaerobic degradation of DMS and MT in sediments slurries of minerotrophic peatland were studied by Lomans et al. (1999). The results refer to that the maximum degradation capacity of DMS under aerobic conditions was 4.0 nmol/mL of sediment slurry/h. While under anaerobic conditions i.e. N_2 or H_2 shaken bottles, the maximum degrading capacities were 0.37 and 0.32 nmol/mL of sediment slurry/h, respectively. Kinetic analysis result in Ks values 6 to 8 mM and 3 to 8 mM for aerobic and anaerobic DMS degradation, respectively.

An alternative process for the abatement and desulfurization of H_2S and VOSCs containing waste streams investigated by Manconi and Lens (2009) which employs a silicon-based membrane to eliminate H_2S and VOSCs simultaneously. Sulfide and the VOSCs studied, i.e. MT, ET, and DMS were removed from the synthetic wastewater using a silicone rubber membrane. MT showed the highest mass transfer coefficient (kov) (8.72×10^{-6} m/s) and sulfide the lowest kov value (1.23×10^{-6} m/s). VOSCs adsorption had a passive effect on the overall (kov). When using Fe (III) EDTA as extractant kov for sulfide extraction was (5.81×10^{-7} m/s) i.e. one fold less than with anaerobic water (2.54×10^{-6} m/s). On the other hand, the removal efficiency of the sulfide increased from 60% up to 84%. Moreover, the formation of S^o that attached on the membrane surface in anaerobic water limited mass transfer. Overall, membrane extraction process for sulfide and VOSCs from a synthetic wastewater solution showed a reliable alternative to treat VOSCs emissions efficiently.

1.2.4 Anoxic bio-degradation of removal sulfur compounds

Study that examined by Soreanu et al. (2008) dealing with the anoxic biological removal of H_2S from biogas under real-time operating conditions, they studied bio-trickling filter packed with plastic fibres and nutritive solution for the bio-

trickling filter was NO_3^- rich wastewater from a pilot scale sequencing batch reactor effluent. The biogas produced from a pilot anaerobic digester, the flow rate and concentrations ranged between 10 to 70 L/h and 1000 to 4000 ppmv, respectively. Also, the study showed with increasing the flow rate of H_2S up to 40 L/h and > 3000 ppmv lead to decrease RE% to less than 80% with maintaining the NO_3^- concentration around 200 mg N-NO_3^-/L. >99% a maximum RE was recorded at (40 L/h; 1000 ppmv H_2S). This technology could be used for full-scale because of the stability in working was observed during long term operation period. To study the influence of sulfide to NO_3^- (S/N) ratio on sulfide removal where NO_3^- was used as electron acceptor in the completely stirred tank reactor, several S/N ratios were examined for this purpose ranging from 0.3 to 2.4 mol/mol. 80% RE was achieved at best S/N ratio 0.85 mol/mol with the autotrophic denitrification, where the main product of sulfide oxidation was SO_4^{2-}. While, S° was the main end product at S/N ratio was recorded higher than 1.3 mol/mol (Petr et al, 2015). A review study investegated by Dumont (2015) aimed to provide an overview of the bio-technologies under aerobic and anoxic conditions, the bio-reactors (bio-trickling filters, bio-scrubbers), and a combination of (chemical- scrubbers and bio-reactors) used for the bio-degradation of H_2S from biogas. At lab-scale, biological processes are effective for biogas purifying and provide the same achievement. S^o and biomass accumulation leading to block of the packed bed, which represents the main disadvantage of bio-technologies. Elimination capacities (EC) was found high value at lab-scale,while at industrial-scale the EC must not be higher than 90 g/m^3 h because the limit blocking effects. At full-scale, the aerobic processes need to mointor the oxygen level for mass transfer accurately remains a key issue for their development, therefore, the aerobic processes alone are may not the most suitable biotechnologies for the treatment of biogas highly loaded with H_2S. For anoxic biotechnologies using NO_3^- as an electron acceptor, convert of the lab-process to a full-scale plant remains a challenge, where using NO_3^- wastewater of treatment plants was represented a cheap source of NO_3^- and an interesting opportunity for the development of innovative bioprocesses enabling the removal of H_2S and NO_3^- at the same time.

Lomans et al. (1997), were detect MT concentrations between 4 - 40 mM in 32 sample of sediment slurries that collected from different freshwater estuaries. The maximum production rates of MT was 1.44 μmol/L of sediment slurry/day which attained after methanogenic and sulfate-reducing organisms were inhibited. Due to the high sulfide concentrations in the sediment slurries, MT production rates increased dramatically from 5 to 80 pmol of MT/mL of sediment slurry/h. Evidently, it was found that H_2S and DMS generated in methylation mechanism resulting in MT and DMS in freshwater sediments, respectively. The experiments supported that high concentration of H_2S (methyl acceptor) is the main factor which stimulates the methyl groups to follow MT or DMS route rather than other possible compounds. Mahmood et al. (2007) used a lab scale anoxic-sulfide oxidizing bioreactor for treatment of synthetic wastewaters containing sulfide and nitrite. The maximal removal rates were 13.82 and 16.311 kg/m^3/day for S^{2-} and NO_2^-, respectively, at HRT 2.4 hr. The results showed that under high sulfide concentrations i.e. 1920 mg/L, RE was equal to even more than 89%. At NO_2^-/ S^{2-} 0.93, there was partial oxidation of S^{2-} and higher concentrations of NO_2^- released in the bioreactor up to 2265 mg/L. When HRT decreased from 36 to 18 hr, NO_2^- was more critical than S^{2-} in bio-oxidation process. The results of the batch degradation and stoichiometric analyses showed that 89–90% of S^{2-} was reduced by NO_2^-. When NO_2^- concentrations were above 2265 mg/L, considerable amount of NH_3 i.e. 200–550 mg/L was accumulated in the bioreactor results process inhibition.

Cai et al. (2008), investigated the effect of S^{2-}/NO_3^- molar ratio in the simultaneous removal of S^{2-} and NO_3^-. Where three S^{2-}/NO_3^- molar ratios 2.5, 1.0, and 0.625 were selected. The maximum volumetric removal rates i.e. 4.86 kg/m^3/d and 0.99 kg/m^3/d for S^{2-}-S and NO_3^--N, respectively, were achieved at S/N molar ratio 2.5. Moreover, S^0 and nitrogen were increased when the S/N molar ratio 2.5 rather than 1.0 or 0.625.

In another study, Campos et al. (2008) studied the influence of S/N ratio in batch experiments on the autotrophic denitrification activity using thiosulfate as substrates and NO_3^- as oxidant. When S/N ratios were between 1.16 and 2.44 g/g, NO_2^- was the main end product. The inhibitory effects of NO_2^- and SO_4^{2-} were clearly noticed over the process while $S_2O_3^{2-}$, acetate, and NaCl have no

significant effects at the concentrations tested. Similarly, under autotrophic denitrification experiments, sulfur was not favorable for these microorganisms as electron donor, in contrary sulfide was oxidized successfully.

Soreanu et al. (2008) studied the removal of H_2S from anaerobic digester in real-time operating conditions using anoxic bio-trickling filter fed with biogas flowrate ranged between 10 to 70 L/h and H_2S concentrations 1000 to 4000 ppmv. Nitrate was continuously fed in trickling liquid from batch reactor wastewater effluents as nutritive solution and electron acceptor. The study reported that better removal performance can be realized when NO_3^- concentration is about 200 mg NO_3-N/L. In another hand, increasing biogas flowrate from 40 L/h to 70 L/h and H_2S concentration from 1000 to 2000 ppmv results decreasing in RE from 99% to less than 80%. The observed results of this study showed that this unique biotechnology represents a platform for biogas removal in full-scale process.

The performance of the anoxic sulfide-xidizing reactor under pH range of 4–11 was investigated by Mahmood et al. (2008). Sulfide was partially oxidized to SO_4^{2-} at influent pH of 7–7.5 during S^{2-} and NO_2^- loading. At acidic pH 3, S^{2-} and NO_2^- removal rates dropped significantly, where microorganisms under acidic condition were more sensitive compared with alkaline pH. Hence, anoxic sulfide-oxidizing reactor can be operated in a pH range 5–11.

Montebello et al. (2012), tested the aerobic and anoxic conditions in two bio-trickling filters at neutral pH for H_2S and MT removal. Initially, MT concentration was gradually increased from 0 to 75–90 ppmv at constant H_2S loading rates of 53–63 g H_2S-S/m^3/h. Accordingly, ECs of around 1.8 g MT-S/m^3/h were resulted. After that, the MT loading rates were increased by decreasing EBRTs from 180 to 30 sec. Maximum ECs of H_2S was found for both filters were between 100 and 140 g H_2S-S/m^3/h. High H_2S loading rates had a negative impact on MT's ECs in both filters. Sulfur mass balances showed that the impact of S^o accumulation was reduced since MT and S^o can react at neutral pH. Finally, the co-treatment of H_2S and MT as a feasible operation in biogas abatement under aerobic and anoxic conditions can be considered.

In a lab-scale continuous flow stirred tank reactor Doğan et al. (2012) studied sulfide bio-oxidation using NO_2^- as an electron acceptor. The removal efficiency of sulfide remained above 80% throughout the study period, although sulfide loading rates increased from 0.47 to 2.16 kg S^{-2}/m^3/ day. The thermodynamic calculations of S^{2-}/NO_2^- molar ratios suggesting main end-product S^0 and SO_4^{2-} were 0.44 and 1.74, respectively. Hence, when S^{2-}/NO_2^- molar ratio was above 1.48, the end product was mainly SO_4^{2-}. Otherwise, a mixture of S^0 and SO_4^{2-} can be distributed as end-products under the 1.48 ratio.

In real scale bio-scrubber, Turker et al. (2012) carried out biogas desulfurization using aerobically treated wastewater containing a mixture of NO_3^- and $NO_2^-$from full-scale activated sludge treatment plant. When the volumetric sulfide loading rates ranged between 2 and 4 kg $S^{2-}/m^3/d$ more than 95% of H_2S removed from biogas. The optimum wastewater/biogas ratio was 2-3 m^3/m^3 and minimum EBRT around 10 min for maximum removal rates of H_2S. The study work revealed that sulfide and nitrogen removal can be integrated efficiently with wastewater treatment plants.

Fernández et al. (2013a), conducted a study using anoxic bio-trickling filter to remove H_2S. They studied the influence of the H_2S inlet concentration, nitrate feeding regime (manual and controlled) and liquid flow rate on the elimination capacity of the bio-trickling filter. The results showed that RE decreased from 99% to 85% when the H_2S inlet loads increased from 120 to 180 g $S/m^3/h$. The main by-product was S^0 i.e. 68.4 ± 15.7% with an SO_4-S/S^o ratio of 0.46 since bio-trickling filter was running at N/S molar ratio of 0.77 ± 0.32.

Cano-Santana (2015) also tested the effect of S/N molar ratios between 0.3-2.4 in a completely stirred tank reactor for sulfide removal using NO_3^-. The denitrification efficiency of the *Thiobacillus* and *Thiomicrospira* reached up to 80% while sulfide was completely removed at S/N molar ratio 0.85, and the final metabolites product was SO_4^{2-}. In contrast, nitrogen was reduced to ammonia at higher S/N molar ratio i.e. 1.3, hence accumulation in S^0 occurred. The specific autotrophic denitrification rates increased from 5 to 26 mg $N\text{-}NO_3$/gVSS/h when S/N ratios increased from 0.8 to 1.2, respectively.

A review study prepared by Dumont (2015) aimed to summerize performance, description, and application area of aerobic and anoxic operating systems used for biogas cleaning from H_2S. The ability of examination the aerobic and anoxic bio-reactors (bio-scrubbers, bio-trickling filters, and a combination of chemical scrubbers and bio-reactors) to degrade H_2S was presented. Using lab- scale biopresecees for biogas cleaning are feasible and provide the same performance regardless of their operating mode. The major drawback of these bioprocesses is clogging due to S^o deposition and biomass accumulation. In order to limit clogging effects, EC must not be greater than 90 $g/m^3/h$ at industrial-scale. For the development of aerobic processes at full-scale, the controlling of oxygen mass transfer playing a significant role during treatment process and remains a key issue. Therefore, the aerobic treatment solo may be not the most appropriate bio-technology treatment for the highly H_2S loaded of the biogas. Anoxic bio-technologies exploiting the nitrates produced from wastewater treatment plants as a free source represents an interesting opportunity for the development of innovative bio-technologies that enabling the removal of H_2S and NO_3^- in the same time.

Cano et al. (2016), stated that a lab-scale anoxic bio-trickling filters model can be efficiently clean up biogas from H_2S. The effects of wide ranges of operational parameters were investigated, among these, N/S molar ratio (0.33- 0.4). The summarized results declared that optimum trickling liquid velocity was 10 m/h, the maximum EC 282 g S/ m^3/h corresponding to RE 97.5% at EBRT 164 sec and H_2S concentrations10,000 ppmv. In addition, increasing the height: diameter ratio has significant improvement in the anoxic bio-trickling filters performance. For instance, at height: diameter ratio (9.8), the RE can be reached more than 99.55% at low EBRT 75 sec and for H_2S inlet concentration of 2000 ppmv. By increasing N/S molar ratio from 0.33 to 0.4, the maximum production of S^0 as metabolic products shifted from 91.5 to 98 %.

Another study conducted by Li et al. (2016) for simultaneous biogas desulfurization and wastewater denitrification. Tow biological system i.e. bio-trickling filter and a bio-bubble column were used in this study to examine the effect of S/N on biogas desulfurization performance. The obtained results confirm that S/N molar ratio is inversely proportional with removal efficiency of

H_2S. Where H_2S removal efficiency significantly raised from 66 up to 100 % when S/N molar ratio decreased from 3.6 to 0.7. In contrast, denitrification efficiency decreased from 100 to 70 % in both bio-reactors. The intermitted feeding mode of nitrate increased sulfate production while H_2S removal efficiency did not affect evidentially. Due to the differences in gas-liquid modes, bio-trickling filter performance surpassed bio-bubble column.

An evaluation study for H_2S removal from biogas using anoxic bio-filter was introduced by Jaber et al. (2017). Whatever, N/S molar ratio was i.e. 0.4, 0.89 and 1.6, the resulted end-product of desulfurization process was a mixture of SO_4^{2-} (55%) and S^0 (45%). However, the experimental results approved that H_2S concentrations up to 1100 ppmv are efficiently treated in anoxic bio-filters at an EBRT of 300 sec, corresponding to RE 100% and maximum EC = 30.3 $g/m^3/h$.

Finally, López et al. (2018) implemented the application of feedforward control strategy through nitrate addition in an aerobic and anoxic bio-trickling filter. At the highest H_2S loading rate (141 g $S\text{-}H_2S/m^3/h$), 68.4% and 62.6% of H_2S concentrations were removed in aerobic and anoxic bio-trickling filters, respectively. In the anoxic bio-trickling filters, the sulfate selectivity was kept constant at 32.8%±0.8% at loading rate above 49.2 g $H_2S\text{-}S/m^3/h$ and N/S_{in} molar ratio between 0.96 and 0.38, while it was 100% in aerobic one. However, in anoxic bio-trickling filters, the feedforward strategy in nitrate addition was shown to be a robust strategy for reducing load fluctuations impact.

1.3 Unique Aspect

Until now many of physic- chemical VOSCs treatment technologies such as thermal oxidation, condensation, incineration, chemical scrubber's catalytic oxidation processes are investigated for the treatment of VOSCs. But these processes represent nowadays a highly costly alternative for the treatment of VOSCs emissions both in terms of construction and high technical complexity and the high energy and chemical reagent consumption. In this context, biological treatment methods such anoxic bio-scrubbers offer a more cost-effective and environmentally friendly alternatives to these conventional physical/chemical treatment processes, also in anoxic systems the addition of air

is unnecessary and there is no limit of gas liquid mass transfer in anoxic systems because of the NO_3^- form in the liquid medium already content dissolved oxygen. Therefore, anoxic bio-degradation could be a suitable alternative to overcome the disadvantages of aerobic bio-degradation (Dumont, 2015). By the way there are lack of data in literature for the design criteria about biological treatment technologies and investigations about biological treatment process design parameters are privileged research points for odor treatment.

The following points were another novel aspect of the thesis: -

- Study the bio-degradation capability of ET or/and DMS via acclimated mixed culture under anoxic conditions through batch mode.
- nitrate ion NO_3^- was used as electron acceptor to oxidize VOSCs under anoxic condition. The degradation of these compounds leads to the formation of element sulfur or sulfate depending on the molar ratio between pollutant and NO_3^- (compound/NO_3^-).
- Using NO_3^- as an electron acceptor in bio-reactor under anoxic conditions, where it is available in most wastewater treatment plants that lead to reduce the costs of aeration and integrate waste gas treatment with wastewater treatment.
- Finding the molar ratio when the end product was S^o, which it can be recovered as raw material in sulfur industry or hydro sulfuric acid, thus less sludge generation and minimizes the cost of sludge disposal.
- The literature reviews about anoxic bio-scrubber in removal each of ethanethiol and dimethyl-sulfide are quite a few.

1.4 Impact

In this project anoxic lab-scale bio-scrubber for VOSCs (ethanethiol ET and dimethyl-sulfide DMS) removal have many contributions to economy, health and scientific knowledge as following: -

- Provide technical solutions to the VOSCs pollution problems encountered in various of industries
- Provide operation strategies that guarantee process stability

- The optimization of compound/ NO_3^- ratio can help to further understand the role of electron acceptor (NO_3^-) in terms of elemental sulfur production.
- Increase S^o ratio recovery (raw material usage) at selected industries
- Reduce operation and construction costs within proper design
- Reducing by-product gases that emitted in aerobic and/or anaerobic conditions such as sulfate SO_4^{2-}, Sulfur dioxide SO_2, Hydrogen sulfide H_2S and Methane CH_4 in which have passive effect on health and environment.

1.5 Hypothesis

Gas stream contaminated with ET or/and DMS was treated in a lab-scale bio-scrubber under anoxic conditions, where activated sludge containing an amount of NO_3^- (as an electron acceptor) was used in a bio-scrubber unit as irrigation fluid. The ET or/and DMS was absorbed by microorganisms and then degraded and transformed into inorganic sulfur using a limited NO_3^- source to obtain the S° for recovery in another purpose.

2. MATERIALS AND METHODS

2.1 Gas phase bio-degradability and Kinetic assay in batch system

Batch experiments were conducted to evaluate the bio-degradation of target gas by acclimated microbial communities when compound was present as a single and as a mixture substrate, and the kinetics of the mixed microbial population of the bio-scrubber under anoxic conditions were studied. Moreover, the compound degradation was evaluated at two concentrations of sludge, 300 and 3000 mg of VSS/L, to study the effect of sludge concentration on the target gas degradation process.

2.1.1 Experimental setup and acclimation procedure

Six 1-L air-sealed bottles (batch mode), three of them contained 100 mL of MSM, were inoculated with activated sludge from the MBR of Kemerburgaz Leachate Treatment Plant (Istanbul, Turkey) under anoxic denitrifying conditions, reaching a final concentration of ~300 mg of VSS/L. Another three bottles were inoculated with anoxic activated sludge, reaching a final concentration of ~3000 mg of VSS/L. The rubber stoppers were used to close the bottles and sealed with aluminium caps. The gas headspace of the bottles was washed with N_2 (purity > 99.999%) for at least 15 min to remove any molecular oxygen. Single and dual combinations of ET and DMS compounds have been injected into the bottles at the following initial concentrations: (1) ET: 840±30mg/m^3 (2) DMS: 850± 10 mg/m^3 (3) ET:DMS mixture (876 ± 30: 860 ± 10) mg/m^3. Single and dual ET/DMS combinations were prepared in duplicate in order to study the degradation of these pollutants individually and as dual mixtures. The bottles were stirred at 320 rpm constantly via magnetic stirring at room temperature as shown in Figure (2.1).

Figure 2.1: Experimental Setup of Batch System

Afterwards, samples were taken hourly from the headspace using a gas-tight syringe (Hamilton, USA), and the pollutant concentrations were analysed by Gas Chromatography/Mass Spectrometry (GC/MS) (Figure 2.2).

Figure 2.2: Gas Chromatography/Mass Spectrometry (GC/MS)

Every week, 30% of the liquid media was replaced with new MSM to provide guaranteed nutrients for microbial growth, the amount of NO_3^- was added periodically according to the theoretical yield $Y_{Componud/NO3^-}$ value of 0.54 and was monitored during the test using ion chromatography (IC) to maintain the batch mode under anoxic conditions. Five complete bio-degradation cycles were carried out for the acclimation of the microorganisms to the target gas as a single carbon source. One cycle was considered complete when the compound concentration in the headspace was totally degraded. At the end of each bio-degradation cycle, the compound content was replenished by adding the pertaining quantity of each compound to the bottles to restore the initial concentration.

2.1.2 Kinetic test

The kinetic parameters of the aqueous compound under anoxic conditions, such as the half saturation point (K_s), maximal growth rate (μ), and yield coefficient (Y_{xs}) was determined using kinetic test in batch mode, 30% of the media was exchanged with new MSM and $NaNO_3$ was added after completed five bio-degradation cycles of the acclimation period to ensure adequate nutrients and NO_3^- during the test. Following by adding the target gas (ETor/and DMS) to the headspace, and the gas samples were taken with time until the compound was completely degraded or until the bio-degradation stopped. Before and after the kinetic test, liquid samples were taken to determine NO_3^- consumption through the test, total suspended solids (TSS), and volatile suspended solids (VSS).

2.2 Experimental Setup and Technological Process

The lab-scale bio-scrubber setup is shown in Figures 2.3 and 2.4. The bio-scrubber column was made from cylindrical jacketed PVC (0.10 m inner diameter, 0.5 m height) packed with Kaldness rings (polyethylene, size 14 × 9.8 mm^2, specific surface area >800 m^2/m^3, porosity > 85%, and packing density 0.98 g/cm^3) to have a working volume of 2 L (0.25 m height). It was supported by a perforated sieve at the bottom and top of the column. The scrubbing column was interconnected with a 3 L working volume stirring tank (biological reactor) and magnetically agitated at 400 rpm (biological reactor, manufactured with PVC, 0.15 m inner diameter and 0.2 m height). The pollutant gas was absorbed into the liquid phase in the scrubbing

column followed by transfer to the biological reactor to reduce the pollutant (bio-transformation step) biologically to either S^o or SO_4^{2-} under anoxic conditions depending on the availability of NO_3^-. The activated sludge with a VSS concentration of 3000 mg/L in the biological reactor was obtained from the membrane biological reactor (MBR) at Kemerburgaz Leachate Treatment Plant (Istanbul, Turkey) and was adapted to the substrate as explain in (chapter 3). It was then connected to a 3 L working volume sedimentation tank (manufactured with PVC, the first part cylindrical with 0.15 m inner diameter x 0.15 m height and second part conical with 0.15 m inner diameter x 0.15 m height). The clean solution from the sedimentation tank was recycled into the scrubbing column. Every connection was carried out with a peristaltic pump to control the flowrate of the sludge and supernatant. Certain concentrations of target gas were prepared by injecting liquid of pollutant via a syringe pump (MODEL LSP02-1B, Dual Channels Syringe Pump) into a container connected with nitrogen gas (N_2) (Linde Turkey, purity >99.999%). In other words, a small amount of nitrogen was bubbled into the container to evaporate and produce the pure target gas, followed by the gas entering the mixing gas tank and being mixed with nitrogen gas to adjust the concentration to the desired level.

Exhaust gas has a negative effect on the environment; therefore, the exhaust gas compound that passed through the bio-scrubber was finally absorbed with 32% NaOH to convert unhandled gas into water-soluble thiolate salts, for instant of ET as shown in equation (2.1) (Frederick et al, 2013).

$$CH_3CH_2SH + NaOH \xrightarrow{H_2O} CH_3CH_2S^-Na^+ + H_2O \qquad (2.1)$$

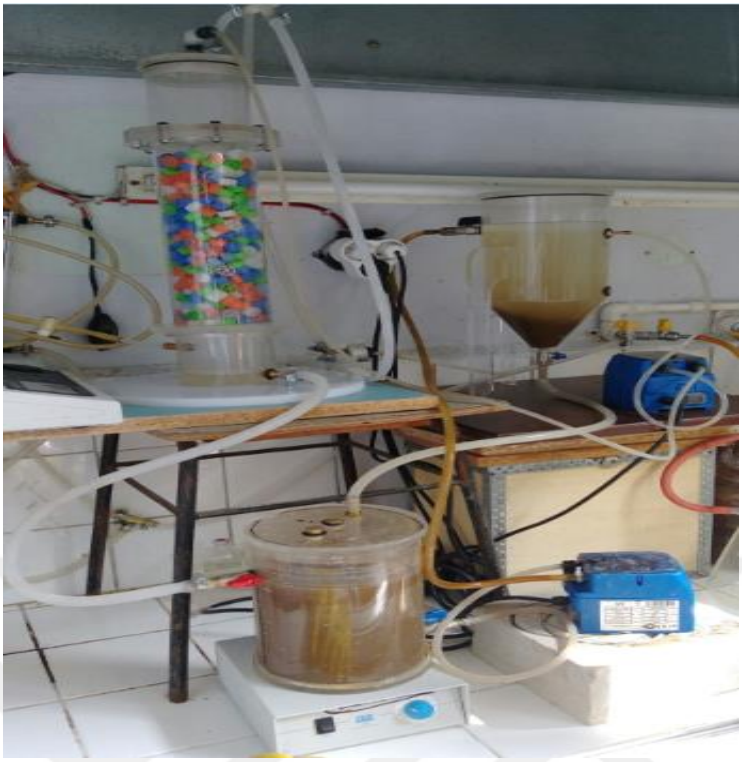

Figure 2.3: Real photos of the laboratory-scale of bio-scrubber system.

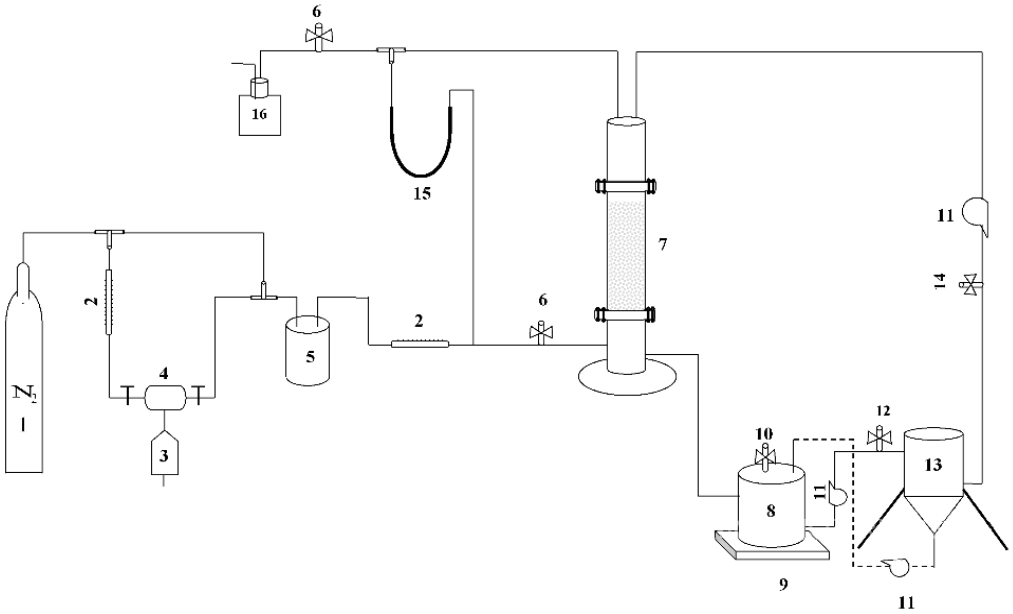

Figure 2.4: Schematic diagram of the lab-scale bio-scrubber: (1) N_2 gas bottle; (2) gas-flow meter (3) syringe pump; (4) evaporation container; (5) mixing gas tank; (6) gas sampling port for inlet and outlet gas; (7) scrubbing column; (8) biological tank; (9) magnetic stirrer; (10) CO_2 sampling port; (11) peristaltic pump; (12) liquid sampling port for VSS and TSS test; (13) sedimentation tank; (14) liquid sampling port for COD, SO_4^{2-}, and NO_3^- tests; (15) manometer; (16) NaOH absorption bottle.

The synthetic inlet gas stream was controlled by the gas-flow meter (AALBORG, NY 10962, USA) and entered from the bottom of the scrubbing column through the media and then outflew from the top. However, the supernatant from the sedimentation tank was circulated in a counter-current mode, sprayed from top to bottom and lifted to the biological reactor and then to the settling tank by the water pump (PR1 peristaltic pump, SEKO Italia S.P.A). Moreover, the manometer was used to measure the pressure drop (head-loss) between the input and output of the scrubbing column as an indicator of efficient transition between gas and water.

The experiments were conducted to study three factors in the lab-scale bio-scrubber: target gas inlet concentration (ET or DMS), SD and EBRT, as shown in Table 2.1, and they were designed to determine the optimal conditions of the lab-scale bio-scrubber required to achieve the highest RE.

Table 2.1: Experimental conditions of lab-scale bio-scrubber.

Experiments	Target gas inlet concentration (mg/m^3)	EBRT (s)	SD (m^3/m^2 h)	Irrigation flowrate (mL/min)	Hydraulic retention time HRT (h) of biological tank	Total operation days of all inlet concentrations	Gas/Liquid ratio	Target
Ethanethiol								
E1	50-1450	60	0.12	14	3.5	0-36	150	Start-up acclimation
E2	150 350 850 1450	60	0.12	14	3.5	36-66	150	Best SD (m^3/m^2 h)
			0.18	21	2.4	67-95	100	
			0.23	26	2.0	96-121	80	
			0.3	35	1.4	122-153	60	
			0.45	52	1.0	154-180	40	
E3	150 350 850 1450	30	Best SD from E2	Best irrigation flowrate	Best HRT (h)	181-207	Best Gas/Liquid ratio	Best EBRT (s)
		60				208-239		
		90				240-272		
		120				273-300		
Dimethyl sulfide								
E	50-	60	0.12	14	3.5	0-38	150	Start-up

4	1450							acclimation
E5	150 350 850 1450	60	0.1	12	4.0	38-63	180	Best SD (m^3/m^2 h)
			0.12	14	3.5	63-91	150	
			0.18	21	2.4	92-122	100	
			0.23	26	2.0	123-151	80	
			0.3	35	1.4	152-179	60	
			0.45	52	1.0	180-202	40	
E6	150 350 850 1450	30	Best SD from E5	Best irrigation flowrate	Best HRT (h)	203-231	Best Gas/Liquid ratio	Best EBRT (s)
		40				232-260		
		60				261-279		
		90				280-297		
		120				298-315		
ET: DMS mixture								
E7	Fixed ET concentration at 150 with different ET:DMS ratios	Best EBRT depending on E3 and E6	Fixed SD depending on E2 and E5	Best irrigation flowrate	Best HRT (h)	0-30	Best Gas/Liquid ratio	Best ET:DMS ratio

2.3 Chemical and mineral salt medium

The chemical ET and DMS (99.0% purity) was purchased from Sigma-Aldrich. All nutrient mineral salt media (MSM) were composed of (g/L) $Na_2HPO_4 \cdot 12H_2O$, 6.15; KH_2PO_4, 1.52; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.038; and 10 mL/L of a trace element solution containing (g/L) EDTA, 0.5; 6 $FeSO_4 \cdot 7H_2O$, 0.2; $ZnSO_4.7H_2O$, 0.01; $MnCl_2.4H_2O$, 0.003; H_3BO_3, $CoCl_2.6H_2O$, 0.02; $CuCl_2.2H_2O$, 0.001; $NiCl_2.6H_2O$, 0.002; and $NaMoO_4.2H_2O$, 0.003 as a nutrient for the microorganisms (Muñoz et al, 2013). Ethylenediaminetetraacetic acid (EDTA) was used in a low level of concentration as a chelating agent and mixed with trace element solution to remove free metal ions. Every week, 30% of the liquid media was exchanged with new MSM to offer adequate nutrients for microbial growth, and pH was kept neutral between 7.2 and 7.6 for all experiments by the daily renewal of MSM with pH 7.0.

$NaNO_3$, ranging between 5 g/L and 10 g/L, was prepared to supplement the electron acceptor as NO_3^- for gas oxidation and as a nitrogen source for microbial growth in a biological tank (Muñoz et al, 2013). Additionally, NO_3^- was added in accordance with the (ETorDMS)/NO_3^- molar ratio calculated from the stoichiometric equations (A.4, A.5, A.6 and A.7 in the Appendix (A.1)). Before the beginning of each experiment, the $Y_{compound/NO3^-}$ was fixed to a 0.54 average value between two stoichiometric ratios of 0.34 or 0.74 when the end product was SO_4^{2-} or S^o, respectively. The compound/NO_3^- ratio of 0.54 was selected to ensure sufficient available NO_3^- at the beginning of the operation. As the operating time progressed, NO_3^- consumption increased. As a result, the ratio of pollutant/NO_3^- was approached to 0.74, when the final product was S^o under NO_3^- limitation. Furthermore, the NO_3^- consumption was monitored via ion chromatograph during the experiment to maintain anoxic conditions.

2.4 Analytical methods

The measurements of the inlet and outlet gas concentrations in the gaseous phase from the scrubbing column and CO_2 concentrations from the biological tank were performed using a gas-tight syringe (Hamilton, USA, volume 100μL) with a gas chromatography-mass spectrometry (GC/MS) system (Agilent 5975C-Triple axes

with a mass spectrometry detector). The oven was started at an initial temperature at 40°C for 3 minutes increasing by 30°C/min up to 50°C, and then at 30°C/min until reaching a final temperature of 130°C. As the carrier gas, Helium gas at a flow rate of 1.37 mL/min was used. In addition, effluent and liquid samples from the biological tank inlet were drawn daily to determine the concentration of compunds and COD. The aqueous compound concentration of the recycling liquid was measured using the headspace method at the inlet and outlet of the biological tank in order to assess the denitrification capacity of the system. Hypovials containing liquid samples were placed in a water bath at 30°C for 10 minutes. Each vial was then pressurized with nitrogen through the septum to 68.9 Kpa. The hypovials were replaced in the bath and were shaken vigorously at 0, 15 and 30 min. The 100-μL headspace was removed from the hypovial using a gas tight syringe, and was injected into the gas chromatograph-mass spectrometer (Mundy, 1991). COD samples were measured according to the Standard Method (1998) (5220-B) open reflexed (Andrew et al, 1998). The concentration of NO_3^- and SO_4^{2-} of the irrigation liquid samples were measured using ICS-3000 on a chromatograph (DIONEX, USA). The pH of the sludge in the biological reactor was determined by taking liquid samples daily using a pH-meter (Orion 720 A^+, USA). Samples from the activated sludge at the end of each experiment were drawn and measured for the VSS and TSS according to Standard Methods 2540-E and 2540-D respectively. (Andrew et al, 1998; Vesilind et al, 2010). Calibration curves were drawn by GC-MS between (0-2000) mg/m^3 for ET, (0-1700) for DMS and (0-7500) mg/m^3 for CO_2 .Within this methodology high correlation coefficient (R^2=0.99) was found for each compound as shown in Figure (2.5). Furthermore, Carbon dioxide CO_2 concentration was measured for monitoring the biological assimilation of microorganism throughout producing CO_2 gas.

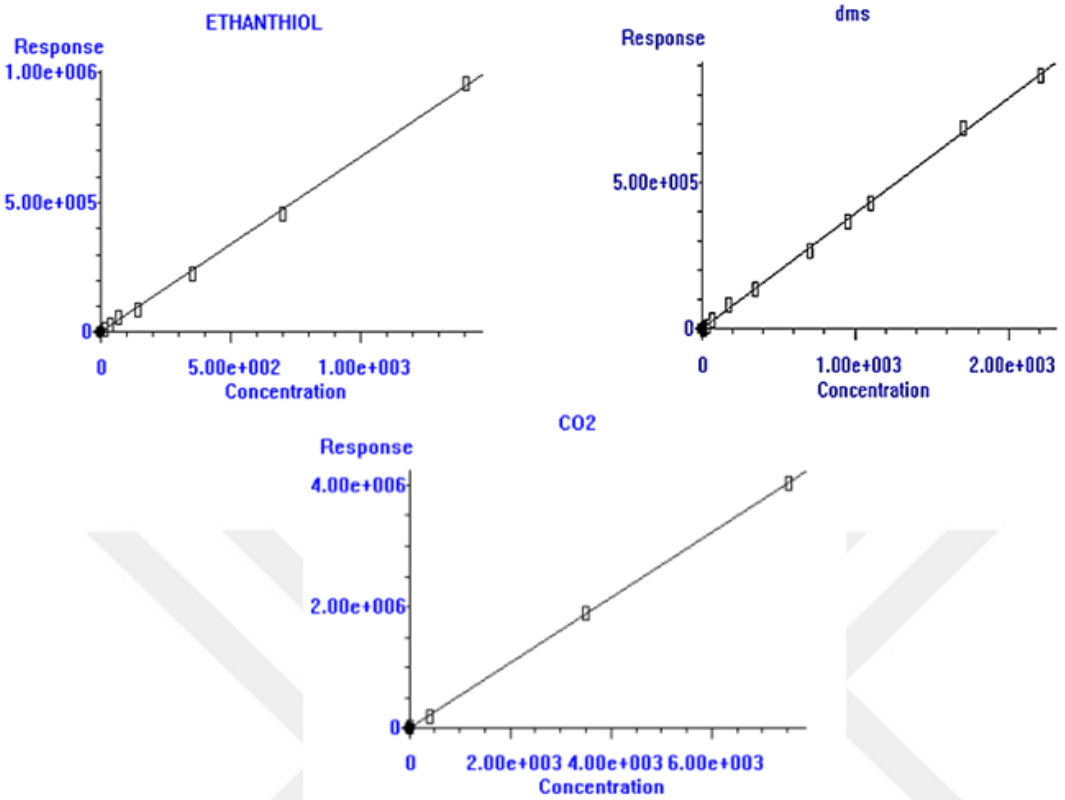

Figure 2.5: Calibration Curves for ET, DMS and CO_2

The performance of the studied bio-scrubber was generally evaluated as follows:

RE (%) was the fraction of the pollutant removed from the bio-treatment expressed as a percentage. It is defined as equation (2.2):

$$RE(\%) = \frac{C_{VOSC}^{in} - C_{VOSC}^{out}}{C_{VOSC}^{in}} \times 100, \tag{2.2}$$

Where C_{VOSC}^{in} (g/m^3) is the inlet concentration, and C_{VOSC}^{out} (g/m^3) is the outlet concentration of the gas compounds. However, the inlet loads IL (g/m^3 h), another significant parameter of bio-scrubber performance, are calculated by equation (2.3):

$$IL = \frac{C_{\text{in}} \times Q_g}{V}, \tag{2.3}$$

where Q_{g} (m^3/h) is the gas flowrate, and V (m^3) is the volume of the scrubbing column.

In addition, *EBRT*(*s*) and SD (m^3/m^2 h), two necessary parameters, can be calculated as:

$$EBRT(s) = \frac{V}{Q_g} \times 3600, \tag{2.4}$$

$$SD = \frac{4Ql}{\pi D^2}, \tag{2.5}$$

where *EBRT* is (*s*), Q_g and V in (2.4) are identically defined in (2.3); Q_l (m^3/h) and D (m) in (2.5) are the liquid spray flow and the inner diameter of the filter, respectively.

In addition, the volumes of NO_3^- removal rate (NRR) and VOSC removal rate (VOSC RR) (Kg/m^3 day) are defined as follows:

$$NRR = \frac{(C_N^{in} - C_N^{out})}{V} Ql, \tag{2.6}$$

$$VOSCRR = \frac{(C_{VOSC}^{in} - C_{VOSC}^{out})}{V} Qg, \tag{2.7}$$

Elimination capacity EC (g/m^3 hr) is

$$EC = \frac{(C_{VOSC}^{in} - C_{VOSC}^{out})}{V} Qg, \tag{2.8}$$

where Q_g, Q_l, C_{VOSC}^{in}, C_{VOSC}^{out}, and V in (2.6), (2.7) and (2.8) are as define in (2.2), (2.3), (2.4), and (2.5), whereas C_N^{in} and C_N^{out} are the concentrations of NO_3^- from liquid irrigation flow at the beginning and end of the experiment.

2.5 Molecular biological analysis

Biomass samples at the beginning of operation (raw sludge) and at the end of gas biodegradation in bio-scrubber system (ET/DMS inoculum of the sludge bio-tank) were collected and stored immediately at -4 C^{o} to evaluate the percentage of microbial community. The samples were processed and analyzed with the ZymoBIOMICS® Service-Targeted Metagenomic Sequencing (Zymo Research, Irvine, CA) as explained in Appendix (A.2).

3. RESULTS AND DISCUSSION

3.1 Bio-degradation and Kinetic assay in batch system for single and dual compounds (mixture gas).

3.1.1 Acclimation and biodegradability of ET and/or DMS in a batch system

Before the kinetic experiments, five cycles of biodegradation were completed in each bottle to adapt the microorganisms to the target compound. During the acclimation period, 30% of the MSM was periodically exchanged with a fresh medium to avoid the possibility of producing the microbial inhibition, probably due to the toxic metabolites excreted during the biodegradation process. Figure 3.1, shows five cycles of degradation trends of the ET, DMS and mixture (ET:DMS), respectively, in gas phase during the batch experiments with two different sludge concentrations of 300 mg of VSS/L (525 mg of TSS/L) and 3000 mg of VSS/L (5190 mg of TSS/L). The ET was completely removed in a shorter time (0.5 days) at VSS 3000 mg/L compared to VSS 300 mg/L (5.28 days) i.e., ET degradation time by mixed culture (3000 VSS mg/L) was approximately ten times lower than by sludge concentration (300 VSS mg/L) as shown in Figure 3.1 a and b. While DMS, the mixed culture with a concentration of VSS 3000 mg/L was taken (0.35 days) to completely removed DMS and this is considering a short time in comparison to mixed culture of 300 VSS mg/L, where the time of acclimation was (5 days) as depicted in Figure 3.1. The comparison between DMS time of degradation with ET showed that the DMS take lower time because DMS solubility in water is higher than ET. As for, the times of degradation the mixture of ET to DMS (1:1) (Figure 3.1) were slowly in degradation compare with using ET or DMS when present as single carbon and energy sources, which were 6.65 d at 300 VSS mg/L and 0.56 d at 3000 VSS mg/L, that refer to the supplementation of an additional carbon and energy source resulted always in a lower biodegradation rates and an increasing in biodegradation time compared to the biodegradation of ET or DMS as individual substrates. Moreover, Figure 3.1 a and b have been showed the degradation of compounds at VSS 3000 mg/L took shorter time to complete the degradation compare with VSS 300 mg/L because of the increasing in the competition among the microorganisms to survive, thereby leading to higher efficiency in removal of the compound in a shorter time. Therefore, the evaluation of the kinetic constants for

the ET and DMS biodegradation (kinetic parameters) were studied at 3000 mg VSS/L.

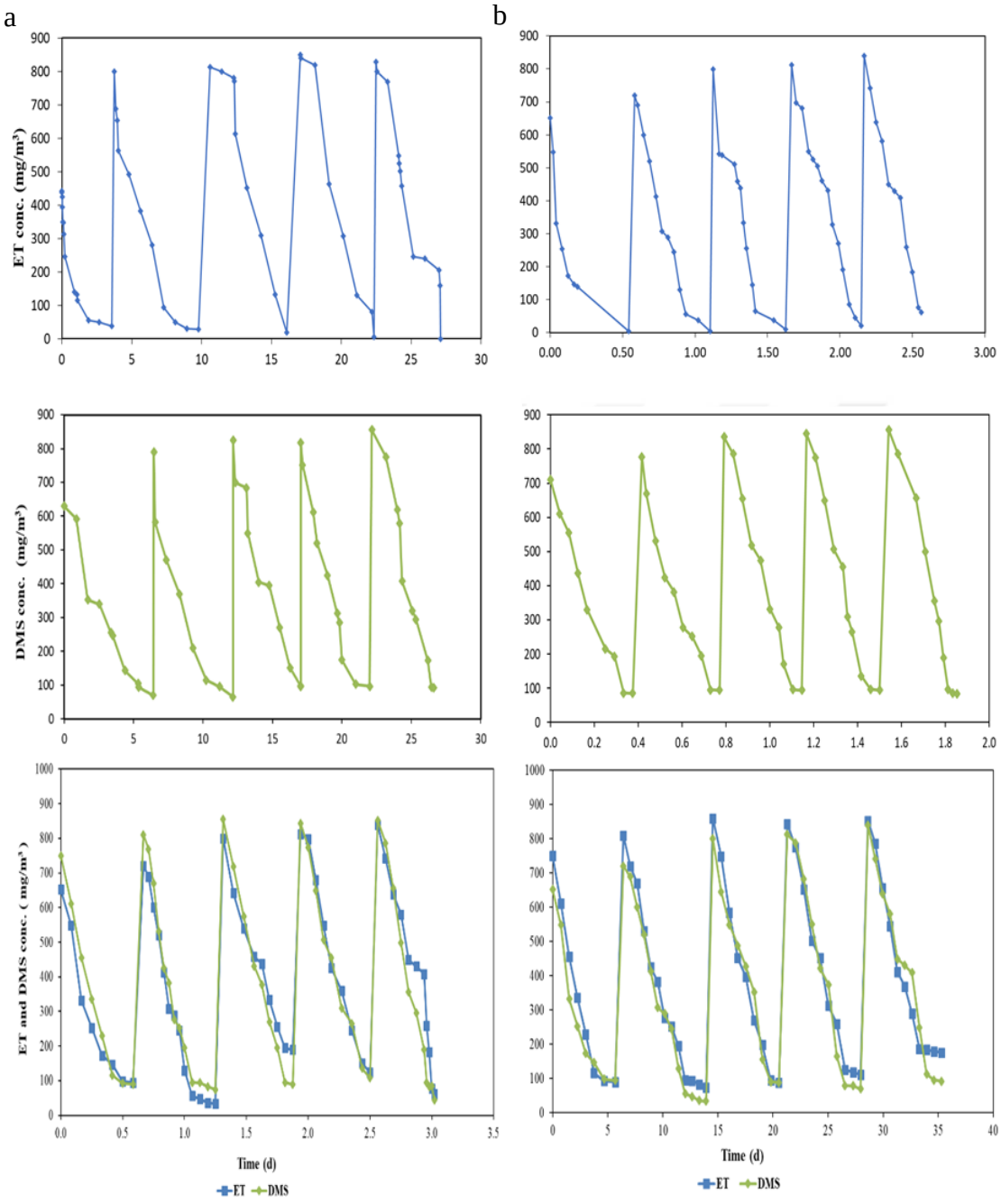

Figure 3.1: Time course of target gas concentration during the adaptation period (a) at 300 mg of VSS/L (525 mg of TSS/L) and (b) at 3000 mg of VSS /L (5190 mg of TSS/L).

Although SO_4^{2-} appeared in small quantities (data not shown) from the ET and/or DMS oxidation reaction in the present study, which directs the focal point towards enzymatic reactions involved in the energy production, and because an increase in biomass growth rate of 47 mg of TSS/L was observed, these compounds are

considered as the source of energy and biomass growth. As reported previously, oxidation of inorganic/organic sulfur compounds to SO_4^{2-} leads to energy production during the reaction (Tang et al., 2009). It was found that the ET at various initial concentrations ranging from 115 to 320 mg/m^3 was completely metabolized by *R. eutropha* under aerobic conditions within 120 to 168 h, and no clear increase in biomass concentration of 2000 mg of VSS/L was obtained, as described by (Sedighi et al., 2013). In addition, an et al. (2010) mentioned that a new *Lysinibacillus sphaericus* strain RG-1 could aerobically biodegrade 2000 to 4000 mg/m^3 of ET totally within 96 h. However, Yavuz et al. (2007) found that the sulfide removal rate through laboratory-scale batch experiments increased by approximately 88% with increasing activated sludge concentration with NO_3^- as an electron acceptor, and sulfide was completely removed within 6 min. The acclimation period of VOSCs such as ET was also reported by (Wang et al., 2015), in which the *Pseudomonas sp*. WL2 bacteria was used to mineralize ET in aerobic batch reactors, and ET spontaneously converted to DEDS during the acclimation period. The concentrations from 1107 to 4963.5 mg/m^3 of initial DEDS were then inoculated and biodegraded within 14-32 h and a growth rate of 9.7 to 31.1 mg of TSS/L, respectively. The results obtained from batch mode experiments compared with previous literature will lead to a better understanding of the behaviour of VOSCs degradation under anoxic conditions and indicate that their biodegradation required a short period and achieved high biomass production when NO_3^- was used as the electron acceptor and ETor/and DMS as the electron donor. The results can then be applied to an anoxic lab-scale bio-scrubber.

3.1.2 Evaluation of the kinetics constants for the VOSCs biodegradation

Microbial cells are considered as the catalyst to degrade a particular substrate. According to this theory, theoretical models including the Monod model have been developed to understand the saturation kinetics during biodegradation processes in batch mode. The Monod model is considered to be the best model for describing the biodegradation of a single substrate through batch growth mode and provides an opportunity to compare different biodegradation kinetic behaviour (Carvajal et al, 2018). The specific growth rate of bacterial cultures is often described using the Monod kinetics test (Giri et al, 2015). Moreover, Jennings et al. (1976) developed

theoretical models to describe the removal of solo pollutants that adopted the Monod-type rate equation. Upon two extreme conditions of Monod equations, zero-order and first-order, Ottengraf and Oever (1983) derived design equations to predict partial removal. The Monod equation has two limiting cases. The first case is at low substrate concentrations (relative to the half-saturation constant) $S << K$s, at which the growth rate becomes a function of the substrate concentration (first-order equation). The second limiting case occurs at high substrate concentrations where $S >> K$s, at which the degradation rate is independent of the substrate concentration (zero-order equation) (Jennings et al, 1976; Ottengraf et al, 1983; Okpokwasili et al, 2005). The mixed microbial population (suspended growth) was first studied in batch mode under anoxic conditions to determine the ability to degrade ETor/and DMS by kinetic parameters before using it in an anoxic bio-scrubber system. Models such as the Monod model are useful because they can help in estimation the growth constants such as *K*s that are difficult to determine experimentally and quickly describe how changes in any of the experimental parameters affect growth without performing a long and tedious set of experiments.

Batch experiments were performed to determine the maximal specific growth rate (μ_{max}, 1/h), half-saturation coefficient (K_s, mg/L), biomass yield coefficient (Y_{XS}, g_x/g_s), and maximal specific degradation rate (q_{max}, g_S/g_X h) for biodegradation of 5.6 ± 0.2 mg/L ET and 12.14± 0.143 mg/L DMS in the aqueous phase (aqueous phase was calculated according to Henry's constant), by mixed culture under anoxic conditions as described via the Monod expression (Carvajal et al, 2018):

$$\mu = \frac{\mu_{max} \times s}{K_s + s}, \tag{3.1}$$

where μ is the specific growth rate (1/h), and S is the substrate concentration in liquid phase (mg/L).

Moreover, the biomass growth rate of any organism is expressed by

$$\frac{dx}{dt} = \mu X, \tag{3.2}$$

where X is the total biomass concentration of microorganisms.

It is also necessary to relate biomass growth (X) versus substrate concentration (S) and time (t). Therefore, by using mass balances and biological kinetics, it is possible to obtain one equation for the variation of the substrate concentration versus time. Depletion of growth associated with substrates in a batch degradation can be described by

$$\frac{-ds}{dt} = \frac{\mu x}{Y_{xs}}, \tag{3.3}$$

where *Yxs* is the biomass yield coefficient. For instant *Yxs* of ET is equal to 0.52 g biomass produced/g substrate consumed, and the substrate ET consumed by microorganisms is 5.45 mg for 2.84 mg of biomass production. Notably, the value of Y_{XS} calculated in the batch experiment approached the theoretical value of Y_{XS} in stoichiometry equations (Appendix (A.1)).

Furthermore, the (q_{max}) was calculated according to the following equation:

$$q_{max} = \frac{1}{Y_{xs}} \times \mu_{max}, \tag{3.4}$$

A plot of 1/μ against 1/S should produce a straight line with the intercept on the y-axis at $1/\mu_{max}$ and a gradient equal to K_s/μ_{max}. Therefore, all key kinetic parameters can be readily determined, and when the values of K_s, μ_{max}, Y_{XS}, and q_{max} are known, a complete quantitative description can be given of the growth events occurring during a batch culture. The linear regression plots to determine the coefficient were created using Microsoft Excel 2010. For example, the values of K_s and μ_{max} of ET were shown in Figure 3.2, the experimental data were fitted to the model, and the value for the correlation coefficient (R^2) was calculated to be 0.97, which indicates a good correlation. The values of μ_{max}, K_s, Y_{XS}, and q_{max} were 0.14 1/h, 1.172 mg/L, 0.52 g_x/g_s, and 0.26 g_s/g_x h, respectively. The ET biodegradation process under anoxic conditions confirmed that ET is readily biodegradable when present as the sole carbon and energy source. Moreover, ET biodegradation is more consistent with the Monod model.

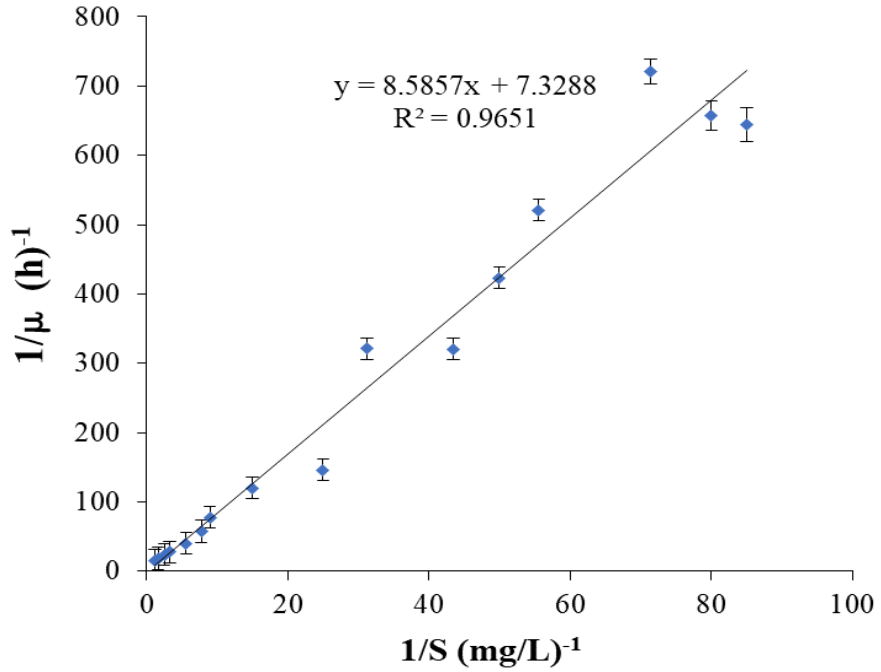

Figure 3.2 : Representation of degradation kinetics of ET in batch experiment under anoxic conditions.

As previously mentioned, the Monod equation has been fitted to the biodegradation kinetic parameter data for an ET compound in batch mode using Graph Pad Prism 5 software, wherein q_{max} and K_S were 0.00023 g_s/g_x h and 1.379 mg/L, respectively, at initial ET concentrations of 1-4 mg/L by *Ralstonia eutropha* under aerobic conditions (Sedighi et al, 2013). Comparatively, *R. eutropha* aerobically degraded ET at a q_{max} 1.130 x 10^3 times lower than that for ET by a mixed culture under anoxic conditions in our study, and the values of K_S were closer to 1.379 mg/L for ET degradation by *R. eutropha* but were 1.172 mg/L in the present study. Furthermore, μ_{max} of 0.0308 1/h for ET degradation by *L. sphaericus* strain RG-1 in batch mode as reported by Wan et al (2010) was achieved using the pseudo-first-order model, which was 0.22 times lower than that by the mixed culture under anoxic conditions (0.14 1/h). The results showed that anoxic ET degradation by the mixed culture was more efficient than by other aerobic pure cultures from previous studies. The Monod model predicted that the applied mixed microorganisms can degrade higher concentrations of ET according to the kinetic parameter ($S<<K_s$). Furthermore, the biomass to substrate Y_{XS} was used in the bio-scrubber modelling to avoid further adjustments and assumptions for X and Y_{XS}. Using the Monod model

for single and dual compounds, kinetics parameters were estimated as shown in Table 3.1.

Table 3.1: Kinetics parameters estimated for single and dual compounds (ET or/and DMS) degradation tests

Compounds	μ_{max} (1/h)	K_S (mg/L)	Y_{XS} (g_x/g_s)	q_{max} (g_s/g_x h)	R^2
ET	0.14	1.172	0.52	0.26	0.965
DMS	0.2	2.194	0.53	0.38	0.98
Mixture (ET:DMS) ET DMS	0.036 0.03	1.1845 0.68	0.54 0.55	0.067 0.054	0.97 0.92

The results showed that for individual substrates ET and DMS were more rapidly biodegradable compounds when present as single carbon and energy sources than present simultaneously, their biodegradation being characterized by μ_{max} values of ET or/and DMS 0.14 1/h ,0.2 1/h, 0.036 1/h and 0.031/h, respectively, and degradation periods were recorded during the acclimation period, 12 h and 8 h (Figure 3.1), respectively, that confirmed those contaminants were the most easily biodegradable under anoxic conditions than as dual compounds ET:DMS because the supplementation of an additional carbon and energy source always resulted in lower pollutant biodegradation rates, which confirmed the occurrence of a competitive inhibition and an increased biodegradation time compared to the biodegradation of VOSCs as individual substrates.

3.2 Removal of VOSCs in bio-scrubber system for single and dual compounds (mixture gas).

3.2.1 Removal of solo VOSC (ET)

3.2.1.1 Bio-scrubber start-up and running performance

The bio-scrubber was started and ran for 36 days (E1 in Table 2.1) to adapt microorganisms on the ET and until steady-state conditions were attained (i.e., RE

and output gas concentrations attained a regular pattern). The process was operated under conditions in which the experimental temperature was 20-25 °C, the inlet concentration of the ET was approximately 50–1500 mg/m^3, the SD was 0.12 m^3/m^2 h with a flowrate of 0.12 m^3/h and the EBRT was 60 s. Outlet gas samples were determined when the bio-system was stabilized for 24 h at a certain inlet ET concentration. Figure 3.3 shows the performance of the bio-scrubber during the acclimation period for ET gas removal over 36 days. The ET REs were 53.90%, 64.40% and 85.80% on the 1st, 10th and 18th days, respectively, with ET concentrations of less than 486.23 mg/m^3. The REs stabilized at an average of 86% between the 19th and 28th days of steady running of the bio-scrubber with increased ET inlet concentrations from 469 to 875 mg/m^3. Afterwards, the inoculation of microorganisms to ET was achieved after 28 d. To understand interruptions in the system operation (such as shutdown in weekend, higher loading rates, and excessive biomass growth) that often lead to a temporary lack of feed of waste gas, nutrient medium, NO_3^- and low gas diffusion through the microbial community in the bio-tank, the system was fed with high concentrations of ET from 900 to 1500 mg/m^3 in a non-permanent irrigation liquid during the final eight days. By day 29, the RE began to decrease slightly, and it reached 79.20% on the 36th day. It thus appears that re-acclimation of the pollutant plays an important role in preventing lower pollutant elimination in the system.

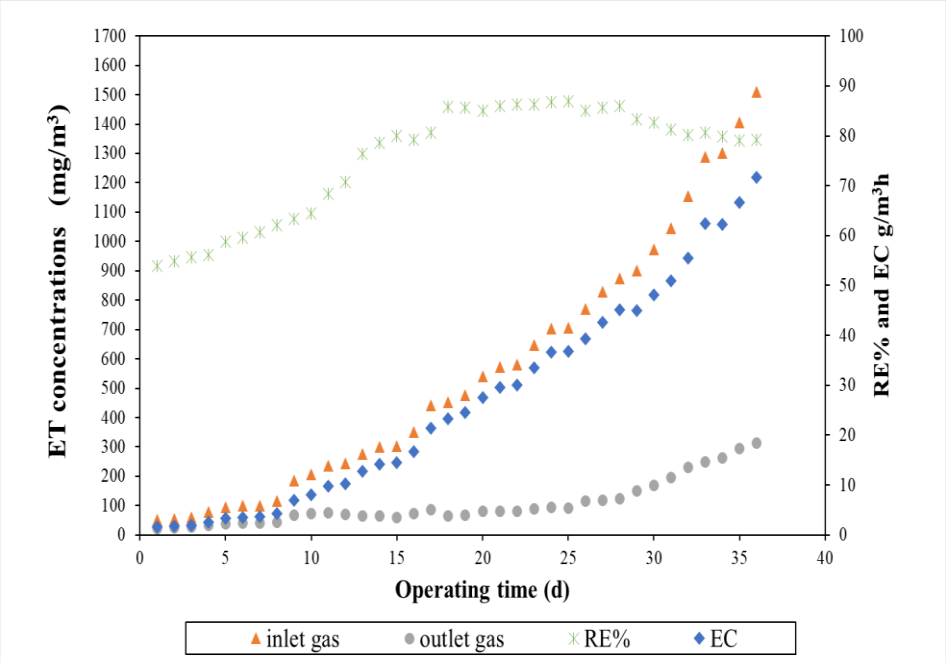

Figure 3.3: Performance of bio-scrubber at ET inlet, outlet concentration, and RE during start-up period (36 days).

The results also showed that the ECs of ET were increased from 1.6 to 71.7 g/m^3 h with increasing inlet concentrations from 50 mg/m^3 (IL= 3 g/m^3h) to 1500 mg/m^3 (IL = 90.51 g/m^3h), which caused the COD values to increase from 30 to 820 mg/L and indicated that ET was bio-degraded to its end products in the bio-reactor.

3.2.1.2 Effect of inlet concentration and SD on RE% and EC.

In all biological desulfurization processes, the initial concentration and SD are significant parameters for RE (Fortuny et al, 2011). In this study, different concentrations of 150 mg/m^3, 350 mg/m^3, 850 mg/m^3 and 1450 mg/m^3 were supplied in a lab-scale bio-scrubber under the following experimental operating conditions: spray densities of 0.12, 0.18, 0.23, 0.3 and 0.45 m^3/m^2 h; flowrate of 0.124 m^3/h and EBRT of 60 s (E2 in Table 2.1).

Figure 3.4 a-d shows the effects of inlet ET concentrations and spray densities on RE and EC. There was a decrease in the REs of ET with an increase in the inlet concentration in all cases, and vice versa for EC. When the SD ranged from 0.12 to 0.45 m^3/m^2 h at a low inlet concentration of 150 mg/m^3 (Figure 3.4 a), the RE rose from 85% (EC = 7.68 g/m^3 h) to 91.29% (EC = 8.25 g/m^3 h); at a high inlet concentration of 1450 mg/m^3 (Figure 3.4 d), RE ranged from 71% (EC = 62 g/m^3 h) to 76.61% (EC = 65.22 g/m^3 h); and at inlet concentrations of 350 mg/m^3 and 850 mg/m^3 (Figure 3.4 b and c), the average REs were approximately 84.54% (EC = 17.82 g/m^3 h) and 77.32% (EC = 39.59 g/m^3 h), respectively. Furthermore, the RE and EC decreased at a higher SD of 0.45 m^3/m^2 h when the SD was reduced to 0.12 m^3/m^2 h; this occurred because the decrease in the liquid flowrate helped to reduce the mass transfer rate between the gas and liquid (Potivichayanon et al, 2006) and hence decrease the ET RE and EC, and vice versa. The reason for the reduction of RE with increasing inlet concentrations is summarized as follows. While the increase by IL resulted in an increase in ET absorption in the scrubber column (due to enhancement by mass transfer), the possible substance inhibition in the liquid bioreactor tank at high IL decelerated the biological activity and resulted in a decrease of RE (Rappert et al, 2005).

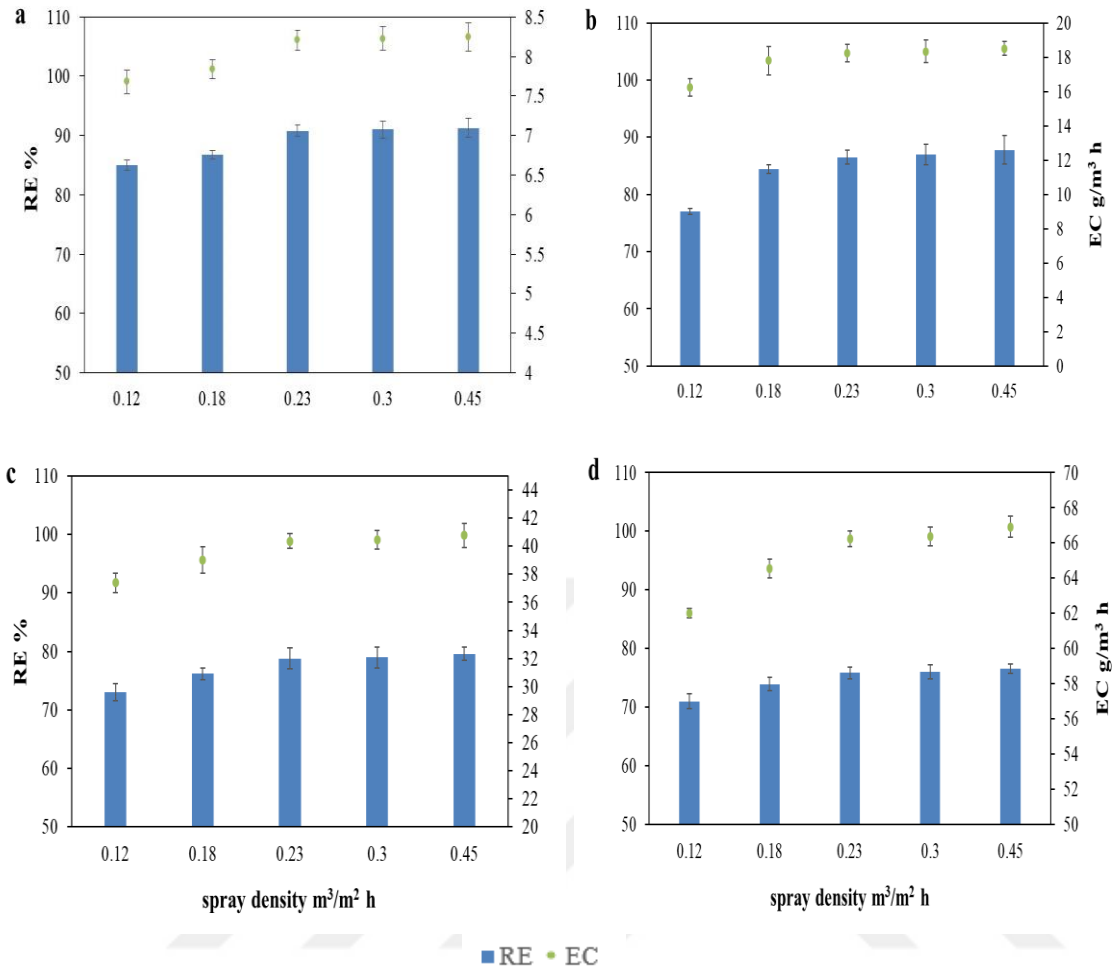

Figure 3.4: Effect of spray density on ET RE% and EC of bio-scrubber at different ET inlet concentrations and fixed EBRT of 60 s: (a) 150 mg/m^3, (b) 350 mg/m^3, (c) 850 mg/m^3, and (d) 1450 mg/m^3. Error bars indicate standard deviation (S.D.) of duplicate samples.

However, it is possible that biomass accumulations may have been enhanced in the biological reactor owing to an increase in the ET inlet concentrations with various ILs ranging from 9.32 to 87.10 g/m^3 h during the process. An increase of approximately 30% in the biomass concentration (in terms of VSS mg/L) in the biological reactor was observed every six consecutive days. Figure 3.4 a–d shows that the ET REs and EC of four inlet concentration groups were unstable when the SD was less than 0.23 m^3/m^2 h. Correspondingly, stability of the REs and EC was accomplished at a SD greater than 0.23 m^3/m^2 h. Thereafter, there was hardly any increase in the REs, even when the SD was increased, for the following reasons: the ET reached a saturation point, there were moderately soluble properties in the water (Henry's coefficient: 0.15–0.21) (Wang et al, 2015) and it is considered that the excessive biomass thickness in the bio-tank could have affected inside diffusion of

the ET gas molecules. Therefore, the microorganisms were unable to adequately decompose ET and were probably returned in the irrigation liquid. Otherwise, the increasing SD caused decreasing HRT from 2 h at 0.23 m^3/m^2 h to 1.4 h at 0.3 m^3/m^2 h and 1 h at 0.45 m^3/m^2 h. Thus, if the ET bio-degradation time is reduced in a biological tank, quantities of incomplete ET decomposition are likely to be returned in the irrigation water. Our results conclude that the best SD was 0.23 m^3/m^2 h (HRT = 2 h), and this achieved an average 83% removal of all ET concentrations (EC = 33.26 g/m^3 h).

A previous study determined the effect of SD on ET removal from an aerobic bio-trickling filter, and reported that the REs of three ET concentration groups (110, 200 and 300 mg/m^3) greatly improved when the SD was increased to 0.24 m^3/m^2 h, and RE values of approximately 60% were attained. In addition, when the SD was greater than 0.24 m^3/m^2 h, a stable RE trend was obtained in the three groups, because the increase in the moisture content of the packing material surface led to an excessive increase in the thickness of the biomass, thus the inside diffusion within the microorganisms for the ET molecule obstructed ET degradation (Wang et al, 2015). In contrast, the anoxic bio-scrubber studied here is more able to effectively eliminate ET (RE > 83%, EC > 33 g/m^3 h) with a lower irrigation flowrate of 26 mL/min (SD = 0.23 m^3/m^2 h).

3.2.1.3 Influence of inlet concentration and EBRT on RE% and EC

EBRT is also a significant parameter in any bio-treatment desulfurization process, and it can be evaluated as the time needed to reach the concentration limit (Pokorna D. et al, 2015). The influence of EBRT on the RE and EC of ET is presented in Figure 3.5 a-d; these results were obtained under experimental conditions in which the temperature was approximately 20–25 °C, the best SD was 0.23 m^3/m^2 h, Various inlet concentrations were used in the bio-treatment system with different EBRT as shown in (E3 in Table 2.1).

The results of ET RE indicated that a longer residence time is a benefit to ET removal when EBRT is too short to obtain biological oxidization of ET into the by-product before release. A comparison of four different inlet concentrations with respect to the influence of EBRT on RE and EC showed that high RE values were

similar (at approximately 99%) at ET concentrations of 150 and 350 mg/m^3 and at ET concentrations of 850 and 1450 mg/m^3 (at approximately 98%) with EBRT of 120 s. Maximum EC values were 14.40, 33.10, 76.50 and 125.28 g/m^3 h for 150, 350, 850 and 1450 mg/m^3 at a lower EBRT of 30 s, respectively. In addition, better RE values were found with comparative values of the four inlet ET concentrations: 150 > 350> 850 > 1450 mg/m^3, and vice versa for EC. It is of note that the high REs for the four inlet concentrations (Figure 3.5 a-d) were approximately 91% and 99%, which corresponds to average ECs of 24.74 and 20.60 g/m^3 h at an EBRT of 90 s and 120 s, respectively.

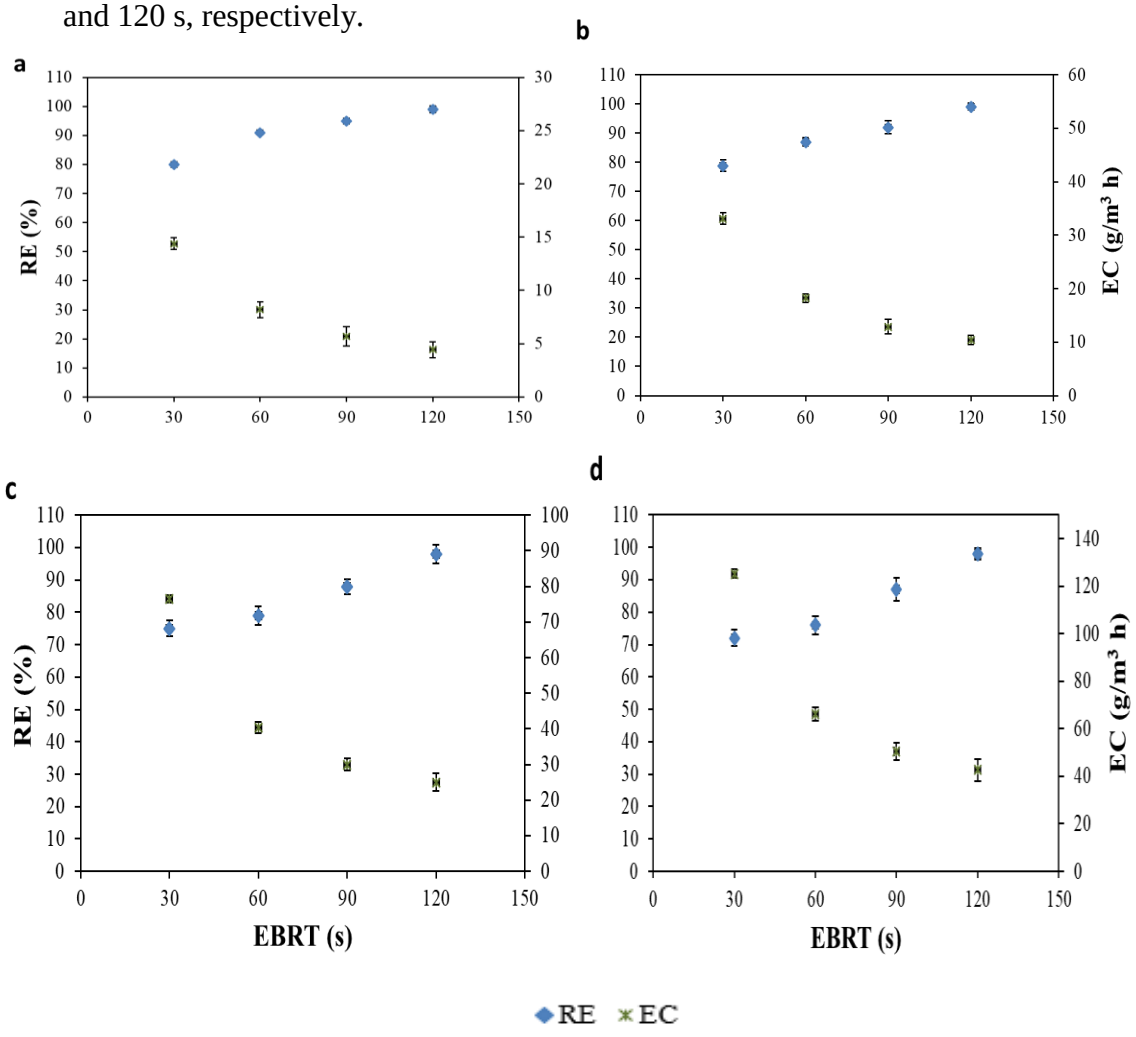

Figure 3.5 : Influence of EBRT on ET RE% and EC of bio-scrubber at different ET inlet concentrations and fixed spray density of 0.23 m^3/m^2 h: (a) 150 mg/m^3, (b) 350 mg/m^3, (c) 850 mg/m^3 and (d) 1450 mg/m^3. Error bars indicate S.D. of duplicate samples.

Therefore, according to REs and ECs, the best EBRT is 90 s of ET treatment in the anoxic bio-scrubber system at a fixed SD 0.23 m^3/m^2 h. Furthermore, the best gas-to-liquid ratio is 53, according to an EBRT of 90 s and SD of 0.23 m^3/m^2 h. In comparison with other studies, all the results in this work showed a much higher ET RE and EC with a lower EBRT under anoxic conductions and a mixed culture, and without the use of a specific type of bacteria. For example, the study of An et al. (An et al, 2010) seeded mixed microorganisms with strains RG-1 and B350 in twin bio-trickling filter columns to purify ET under aerobic conditions, and the maximal ECs for RG-1 and B350 were 38.36 g/m^3 h with 89.20% RE and 25.82 g/m^3 h with 57.10% RE, respectively, at EBRT of 83 s. In another study, the maximum EC was only 3.70 g/m^3 h with a RE of 50% for ET at EBRT of 40 s in an aerobic bio-trickling filter inoculated with alkaliphilic sulfo-oxidizing bacteria under alkaline conditions (Garcia et al, 2010). A comparison of bio-scrubber performances with various bio-reactors for removing sulfur compounds under anoxic conditions is provided in Table 3.2 Most practical experiments have used lab-scale and pilot-scale traditional bio-reactors for sulfur compound removal.

Table 3.2: Bio-reactors for sulfur-containing compound removal under anoxic conditions.

Bio-reactor type	Target pollutants	Scale	C_{in}/IL	RE/EC	EBRT (s)	Irrigation flowrate (mL/min)	References
Bio-trickling filter	H_2S	Lab-scale	78 g/m^3 h	99%	144	180	(Fernandez et al, 2013)
Bio-trickling filter + Bubble column	H_2S	Lab-scale	2164–2225 mg/m^3	100% /54.5 gH_2S/m^3 h	300	5	(Li et al, 2016)
Bio-trickling filter	MT	Lab-scale	160–192 mg/ m^3	82% /1.8 gS^{2-}MM/ m^3 h	180	100	(Montebello et al, 2012)
Bio-trickling filter	H_2S	Pilot-scale	400–800 gH_2S/ m^3 h	100% /270–300 gH_2S/ m^3 h	1080	500	(Soreanu et al, 2008)
Bio-filter	H_2S	Pilot-scale	1527 mg/ m^3 1249 mg/ m^3	100% /30.3 gH_2S/ m^3 h 100% /25.2 gH_2S/ m^3 h	300 240	1.33	(Jaber et al, 2017)
Bio-scrubber	ET	Lab-scale	150 mg/ m^3 6 g/ m^3 h 1450 mg/ m^3 58 g/ m^3 h	95% /5.7 g/ m^3 h 87% /50.46 g/ m^3 h	90	26	This study

Table 3.2 shows results of calculated RE%, EC, EBRT and irrigation flowrates obtained from this study and other studies. As reported, the RE%, EC, EBRT and irrigation flowrate values are all different between studies because of the different types of bio-reactors, pollutants, scales, inlet concentrations, loading rates, liquid flowrates and another experimental condition employed. Moreover, in this study, the best RE% and EC ranged from 95–87% and 5.70–50.46 g/ m^3 h at an EBRT of 90 s, irrigation flowrate of 26 mL/min (SD = 0.23 m^3/m^2 h) and an inlet ET concentration ranging from 150 to 1450 mg/m^3.

3.2.1.4 Metabolic product analysis and mass balance of carbon, sulfur and nitrogen

To ensure that ET was not only removed into the irrigation liquid by physical absorption, but also biologically oxidized, the SO_4^{2-} concentrations, biomass growth of the liquid samples and CO_2 of the gas samples from the bio-reactor at an EBRT of 90 s and SD of 0.23 m^3/m^2 h were monitored daily during 32 days of observation under steady-state conditions. An initial gas ET injection amount of 150 to 1450 mg/m^3 was eventually converted in terms of mass (g/d) into an aqueous phase (expressed as ET-aqueous: the ET transferred from gas to liquid after the scrubbing ET gas phase by the irrigation liquid), and the aqueous ET was calculated according to Henry's coefficient constant of 0.15 (Wang et al, 2015). In Figure 3.6, aqueous ET is utilized as the sole carbon source by a mixed culture and is biologically oxidized to either CO_2, SO_4^{2-} or another intermediate, which are then utilized by the cells to produce biomass. Stoichiometrically, 1 g/d of ET converts to 0.516 g/d of S^o, 0.31 g/d of CO_2 and 0.565 g/d of bacterial growth (according to Equation A.4), and according to A.6 in the Appendix (A.1), 1 g/d of ET converts to 1.548 g/d of SO_4^{2-}, 0.57 g/d of CO_2 and 0.44 g/d of bacterial growth.

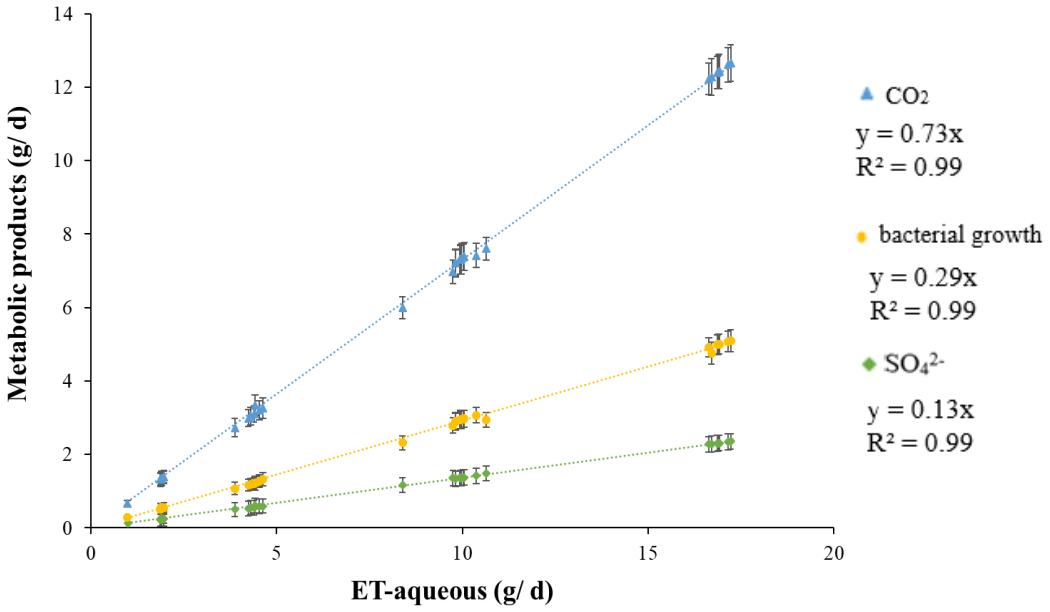

Figure 3.6: Relationship between by-products (bacterial growth, CO_2 production and SO_4^{2-}) with mass of initial ET-dissolved. Error bars indicate the S.D. of duplicate samples.

The results show that the CO_2 production from converting 1.91 to 17.22 g/d dissolved ET increased from 1.345 to 12.59 g/d, and the bacterial growth ranged from 0.56 to 5.09 g/d. Furthermore, SO_4^{2-} values biologically produced from ET degradation were 0.26 to 2.36 g/d. These results prove that the main end products fluctuated between S^o and SO_4^{2-}, because the empirical results of production biomass, CO_2 and SO_4^{2-} lay between two stoichiometric equations (A.4) and (A.6) as shown in Appendix (A.1). Previous research confirmed mineralization during ET aerobic degradation by *pseudomonas sp.* WL2 to CO_2, in addition to bacterial yield and SO_4^{2-} production in a batch reactor, where it was shown that for 4.2–25.2 mg of initial ET degradation, 8.9–29.0 mg of CO_2 and 1.2–3.5 mg TSS were produced after the substrate was consumed completely in 24 h. In addition, the SO_4^{2-} production from oxidation of initial ET values of 6.7, 15.1 and 23.5 mg was 100.3, 32.5 and 15.2 mg/L, respectively (Wang, X. et al, 2015). Comparatively, the results obtained in this study were more efficient in terms of ET removal at a low energy consumption, in addition to the reduced production of SO_4^{2-}, which indicates that the system operates under a limited NO_3^- source.

The carbon mass balance was conducted with respect to the carbon molecules in ET used by microorganisms for energy metabolism and cell synthesis. The carbon balance for the lab-scale bio-scrubber system can be defined by

$$C_I = C_o + C_{biom} + C_G, \tag{3.5}$$

where C_I represents the influent ET amount as a carbon source (suffix as ET-C) in g/d within the bio-tank, C_{biom} represents the carbon assimilated for bacterial growth, C_G is the amount of carbon converted to produce gas and C_o represents the outlet ET-C calculated from the COD measurement divided by a factor of 1.43 (which represents the COD of ET).

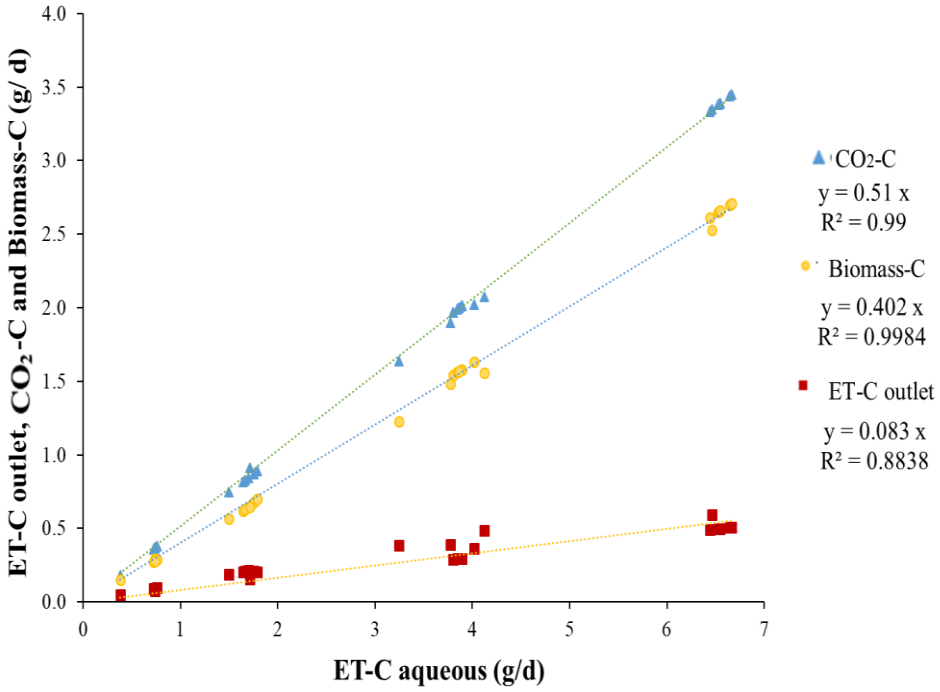

Figure 3.7: Carbon mass balance.

According to the calculated mass balance, 0.38 to 6.67 g/d of incoming ET-C was consumed by the cells for biomass growth and CO_2 production, which equalled approximately 40% and 52% of the total ET, respectively. The remaining part of ET-C was approximately 8%, as reported in Figure 3.7. In addition, there were no intermediate compounds containing carbon molecules.

Furthermore, the bio-degradation of the ET was mainly oxidized to either S^o or SO_4^{2-} according to Equations (A.4) and (A.6), and it was thus assumed that the solo

end products were S^o and SO_4^{2-}, in accordance with stoichiometric equations. In this respect, a sulfur mass balance was conducted using subtraction (Montebello et al, 2012) to estimate the quantity of sulfur produced in the system. As studied previously, the pathway of ET degradation under aerobic conditions by strain WL2: diethyl disulfide (DEDS) was formed and then converted to SO_4^{2-} at complete mineralization of ET, while S^o occurs inside the bacterial cells by the transformation of DEDS to S^o prior to being further oxidized to SO_4^{2-}. Therefore, the only final products were S^o and SO_4^{2-} (Wang, X. et al, 2015).

In this study, the relationship between ET removed and NO_3^- consumed is presented as ET_{rem} /$NO_{3\,cons}^-$, and the mass balance of sulfur, SO_4^{2-} production is expressed as SO_4^{2-}-S. The S^o formation in the lab-scale bio-scrubber system from oxidation of aqueous ET (expressed as ET-S) was calculated and is depicted in Figure 3.8 a and b. Initially, different amounts of ET were input with time (from 0.288 to 2.86 g/d) and NO_3^- feeding was maintained at a molar ratio of 0.54 at each ET inlet renewal rate. The empirical molar ratio data fall between two limits of stoichiometric equations 0.74 and 0.34, where the $ET_{rem}/NO_{3\,cons}^-$ molar ratio was approximately 0.58 between the 1st and 25th days. However, after the 26th day, the ratio decreased to approximately 0.48 mol/mol during the operation period, which may be due to accumulation of remaining NO_3^- with time and variations in the amount of ET removed to NO_3^- consumed. The accumulated SO_4^{2-}-S increased markedly from 0.09 g/d (at day 1) to 12.21 g/d (by day 32); an increasing amount of sulfur was obtained as time progressed, and the molar ratio $ET_{rem}/NO_{3\,cons}^-$ decreased from 0.60 to 0.47, respectively. The results obtained from calculated S^o range between 1.37 and 127.13 g/d. It is clear that most ET oxidation was converted to S^o rather than SO_4^{2-}. This was also confirmed by the estimated percentage of S^o/ET-S from the sulfur mass balance, which was approximately 90% of ET-S biologically converted to S^o. The percentage of SO_4^{2-}/ET-S was approximately only 9% of the ET-S transferred to SO_4^{2-} Figure 3.8 b.

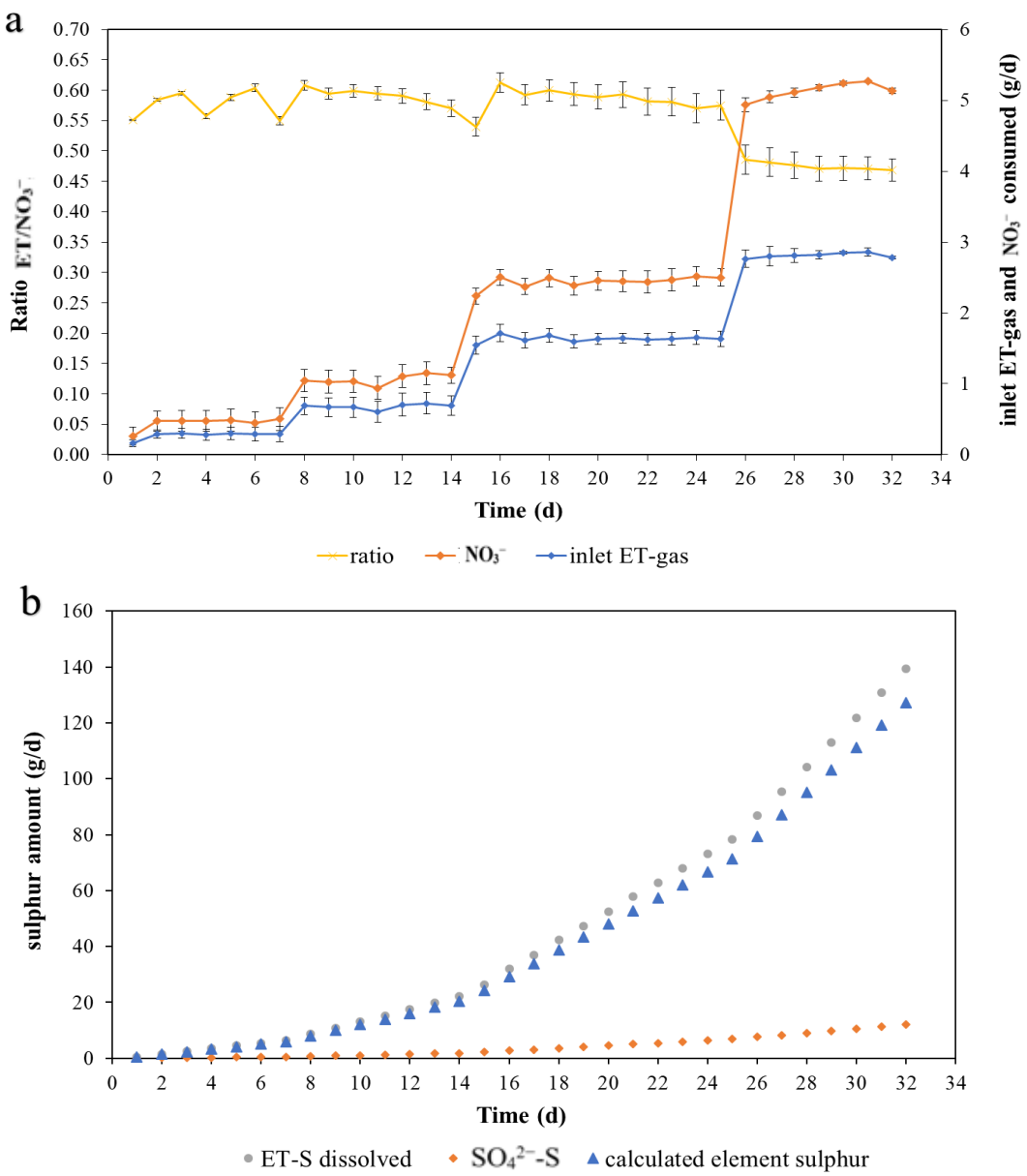

Figure 3.8: Time course of (a) ratio of ET/NO_3^-, ET inlet loading, NO_3^-consumption rate and (b) sulfur mass balance (i.e., SO_4^{2-}-S and calculated sulfur formation in the bio-scrubber system from ET-S dissolved loading rate). Error bars indicate the S.D. of duplicate samples.

However, there was a certain pale-yellow substance (that had some S^o material characteristics) within the biomass of the reactor. This indicated that the desulfurization process had occurred in the bio-reactor for the ET remaining in an aqueous phase, and it confirmed that the remaining ET had been converted into S^o and other forms of sulfur, rather than SO_4^{2-}, via the desulfurization process (Sedighi et al, 2016). A previous study found that degradation of the H_2S-S loading rate fluctuated during anoxic bio-trickling filtering to S^o or SO_4^{2-} because of variations in

the molar ratio of $N_{supplied}/S_{removed}$ and H_2S ILs (Lebrero et al, 2015). Through the sulfur mass balance, the study also found that the ratio of $SO_4^{2-}S/ S^o$ was increased from 5% to 70% when H_2S-S was converted to SO_4^{2-}. In addition, S^o was noticed in the polyurethane foam. Several studies have reported the important role of the N_{sup}/S_{remo} molar ratio in controlling desulfurization products, and this has been adjusted while the IL has been fixed (Li et al, 2016) and (Soreanu et al, 2008). However, controlling the amount of NO_3^- supplied for the complete oxidation of sulfide to SO_4^{2-} in real operating conditions is inflexible owing to rapid variations in ILs, which results in the partial oxidation of sulfide to S^o (Fernandez et al, 2014). Janssen et al. (1997) mentioned that the production of S^o is related to the amount of dissolved oxygen, where S^o is the major end product of sulfide oxidation under oxygen-limiting conditions. However, in current study, the correlation between the $ET_{rem}/NO_3^-{}_{cons}$ molar ratio and forms of S^o production were evident in the end products, and this result is consistent with those in the studies mentioned above.

A nitrogen mass balance (expressed as NO_3^--N) was conducted according to the actual inputs of feeding nitrogen and the concentrations of measured nitrogen in the system. The nitrogen balance for the whole system was calculated by

$$N_I = N_o + N_{biom} + N_G, \tag{3.6}$$

where N_I represents the amount of nitrogen input to the system, N_o represents the amount of nitrogen output from the biological tank, N_{biom} represents the amount of nitrogen consumed for biomass growth and N_G is nitrogen gas produced during the denitrification process, which is assumed by taking the average from the stoichiometric equations (A.4) and (A.6); however, as it was the same as the carrier gas contaminant, it was difficult to measure. To calculate the mass balance, a mass flow diagram was prepared (in Figure 3.9) for one case under certain system operation conditions (ET inlet 150 mg/m^3, SD of 0.23 m^3/m^2 h and EBRT of 90 s) for 7 days. The initial nitrogen load into the bio-reactor was a rate of 121.90 mg N/day, and approximately 13% of this was found to be outgoing (with a value of 15.73 mg N/d).

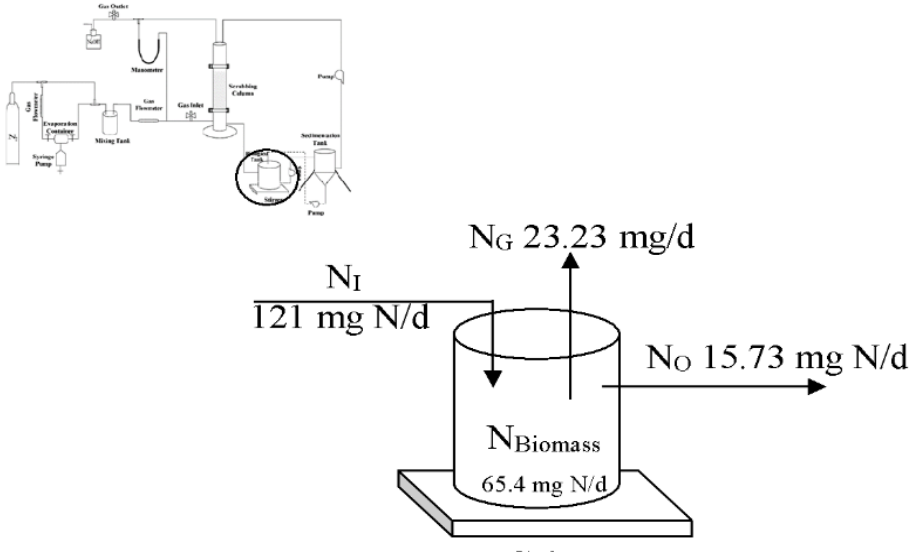

Figure 3.9: Nitrogen mass balance

The net growth of biomass in the denitrification reactor accounted for 54% (65.4 mg $N_{biomass}$/day) of the inlet nitrogen load. A total of 23.23 mg N/d of inlet N was converted to nitrogen gas through the denitrification process, which was approximately 19% of total incoming N. The difference between inlet and outgoing N was approximately 14%.

3.2.1.5 Biological ET oxidation stoichiometry and energetic reactions

Microorganisms oxidize inorganic and organic materials from oxidation reduction reactions to obtain energy for growth and maintenance (Sawyer et al, 2003). Oxidation reduction reactions always involve an electron exchange (food substrate for the organism), and the electron acceptor is oxygen (under aerobic conditions). Whether under anaerobic or anoxic conditions, some microorganisms can use other electron acceptors in energy metabolism, including NO_3^-, SO_4^{2-} and CO_2. Stoichiometry expresses quantitative relationships between reactants and products in a chemical equation. In this study, the reaction stoichiometry and the biomass yield ($Y_{X/D}$) for biological ET oxidation were calculated by taking a thermodynamic approach (Dogan et al, 2012). The following stoichiometric equations were derived from the thermodynamic analysis presented in the Appendix (A.1). When NO_3^- was used as an electron acceptor and the nitrogen source for biomass was NO_3^-, the end product was

sulfur,

$$CH_3CH_2SH + 1.35NO_3 + 1.35H^+ \rightarrow S^0 + 0.31C_5H_7NO_2 + 2.57H_2O + 0.52N_2 + 0.43CO_2, \quad (3.7)$$

or sulfate,

$$CH_3CH_2SH + 2.9NO_3 + 0.9H^+ \rightarrow SO_4^{2-} + 0.24C_5H_7NO_2 + 2.6H_2O + 1.33N_2 + 0.81CO_2. \quad (3.8)$$

Table 3.3: Thermodynamic calculated yield values ($Y_{ET/NO3^-}$) and free energies of ET oxidation when NO_3^- was used as the electron acceptor.

Thermodynamically calculated yield values and Gibbs free energies	Nitrogen source for biomass	Main product SO_4^{2-}	Main product S°
$Y_{ET/NO3}$ (mol/mol)	NO_3^-	0.34	0.74
Gibbs free energy kJ/e-mole		-1398.88	-675.73

Table 3.3 shows the theoretical yield molar ratios (Y_{ET/NO_3^-}) calculated from equation (3.7) and (3.8) and the Gibbs free energy at different end products when NO_3^- was used as the biomass source. As seen in Table 3.3, S^o is formed as a by-product when NO_3^- is limited. NO_3^- consumption and biomass production are lower when S^o is formed as the main end product in system than when SO_4^{2-} is formed as the main product. Energies of -1398.88 kJ/e-mole and -675.73 kJ/e-mole were obtained from the anoxic oxidation of ET to SO_4^{2-} and S^o, respectively; the negative signal refers to the reaction being exothermic and spontaneous (and product-favoured). Figure 3.10 shows the theoretical upper and lower limits of the yield molar ratios (Y_{ET/NO_3^-}) given in Table 3.3 and presents the experimental data for optimized operating conditions using a SD of 0.23 m^3/m^2 h and EBRT at 90 s under different ET concentrations.

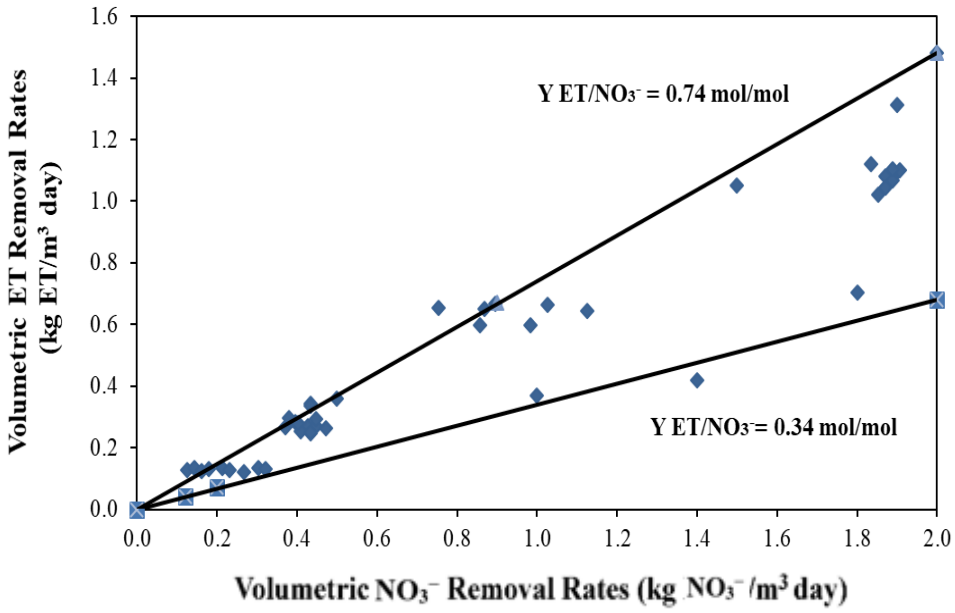

Figure 3.10: Volumetric ET removal versus volumetric NO_3^- removal

In a previous study, NO_3^- and NO_2^- were both present in the wastewater to de-sulphurate biogas (H_2S) in a continuous bio-scrubber, and as they were used as electron acceptors, the stoichiometric molar ratios (S^{2-}/ (NO_3^- + NO_2^-)) at different end products were 0.44–0.72 and 1.75–2.89 for SO_4^{2-} and S^o, respectively. Most of the experimental molar ratios were scattered within these limits, owing to the mixture of NO_3^- and NO_2^- and the variance of H_2S oxidation end products (Baspiner et al, 2011). In addition, Li et al. (2016) investigated the effect of the S/N ratio on the removal of H_2S from biogas in a bio-trickling filter (BTF) and a bubble column (BC). They found that the BTF was more stable in terms of H_2S removal performance than the BBC, which was attributed to their different gas–liquid mass transfers. For S/N ratios of 1–2.5, H_2S was almost completely removed by the bio-trickling filters, and the maximum EC of H_2S was 54.5 g H_2S/m^3 h. The S/N ratios did not significantly influence the biogas desulfurization efficiency, but the desulfurization products were obviously affected. With increasing S/N ratios from 1.0 to 2.5, the percentages of SO_4^{2-} decreased, and the denitrification performance clearly improved. In this study, the experimental data are distributed between two limits, and most data are scattered near the upper value of $Y_{ET/NO3}^-$ (0.74) when the end product is sulfur, as shown in Figure 3.10. This result refers to the presence of

S^o. In addition, Table 3.4 summarizes an average Y_{ET/NO_3^-} ratios obtained with different spray densities and ET loading rates; according to the Table, the average experimental yield values are closer to the Y_{ET/NO_3^-} theoretical value of 0.74 when the main product is S^o. This indicates that the partial formation of S^o and other forms of sulfur were produced rather than SO_4^{2-} and proves that S^o was produced in the bio-reactor tank. Therefore, to produce less biomass and consume less energy in biological ET oxidation systems, S^o needs to be the desired end product. It is known that a lower NO_3^- consumption can considerably reduce costs. Furthermore, S^o is non-toxic, stable and insoluble in water, and it can thus be recovered easily (Baspiner et al, 2011). The formation of S^o is preferred for the following reasons; first, S^o is non-soluble and thus can be removed easily; second, S^o can be recovered and used as a valuable raw material (e.g., in bioleaching processes in sulfuric acid production factories after purification from the water stream by gravity sedimentation, such as in a titled plate settler); and finally, as more oxygen is required to form SO_4^{2-}, a higher energy consumption is thus required for aeration (Janssen et al, 1997) and (Tichy et al, 1994). Methods used to separate S^o from the water stream include settlers, filter presses and sulfur melters, depending on the quality of S° required (Tichy et al, 1994) and (Pagella et al, 1996). Accordingly, in our practical system, S^o can be recovered from the bio-scrubber system by transferring the settled sludge from the sedimentation tank to the vacuum filter press.

Table 3.4: Comparison of thermodynamically and empirically calculated $Y_{ET/NO3^-}$ values when NO_3^- is used as the electron acceptor.

IL g/m^3h	SD m^3/m^2h	Average ET removal g/day	Average NO_3^- Consumption g/day	Experimental Y_{ET/NO_3^-} mol/mol	Thermodynamically calculated $Y_{ET/NO3^-}$ (mol/mol)	
					Main product S^o	Main product SO_4^{2-}
6±1.5	0.45	0.27	0.73	0.37	0.74	0.34
	0.3	0.28	0.43	0.65		
	0.23	0.27	0.44	0.61		
	0.18	0.26	0.37	0.7		
	0.12	0.25	0.31	0.8		
Average ± S.D.		**0.27 ± 0.01**	**0.46 ± 0.16**	**0.63 ± 0.16**		
14±0.6	0.45	0.64	1.93	0.33		
	0.3	0.61	1.18	0.52		
	0.23	0.59	0.90	0.66		
	0.18	0.60	0.80	0.75		
	0.12	0.54	0.75	0.72		
Average ± S.D.		**0.60 ± 0.04**	**1.11 ± 0.49**	**0.6 ± 0.17**		
34±1.5	0.45	1.38	2.4	0.58		
	0.3	1.36	1.87	0.72		

	0.23	1.34	1.94	0.69	
	0.18	1.33	1.91	0.70	
	0.12	1.27	1.9	0.67	
Average ± S.D.		**1.34 ± 0.04**	**2 ± 0.22**	**0.67 ± 0.05**	
58±1.35	0.45	2.22	5.22	0.43	
	0.3	2.2	4.75	0.46	
	0.23	2.19	3.9	0.56	
	0.18	2.14	3.54	0.60	
	0.12	2.1	3.4	0.62	
Average ± S.D.		**2.17 ± 0.05**	**4.16 ± 0.79**	**0.53 ± 0.08**	

The concentrations of NO_3^- consumed are shown in Table 3.4. They gradually and significantly increased with an increase in the ET loading rate, which occurred in relation to the need for more NO_3^- (electron acceptor) by microorganisms to oxidize the ET, and this subsequently promoted the denitrification process.

3.2.2 Removal of solo VOSC (DMS)

3.2.2.1 Startup and running performance

Prior the start-up of the experiments, the adaptation of the microbial community to waste gas in their environment was carried out to achieve greater improvements of pollutant removal. At this point Figure 3.11 (a) and (b) illustrate the performance of the lab-scale bio-scrubber for the removal of DMS over 38 days during the continuous adaptation period. The bio-scrubber operation was started and run for 38 days at a DMS inlet concentration of approximately 50-1450 mg/m^3 with SD of 0.12 m^3/m^2 h and a flow rate of 0.124 m^3/h. EBRT was maintained 60 s in scrubbing column and the hydraulic retention time (HRT) set in the bio-tank to 3.5 h until reaching stability in the output concentrations, which refer to the steady-state conditions being obtained in the bio-system and the occurrence of adaptation of a microorganism to DMS (E4 in Table 2.1).

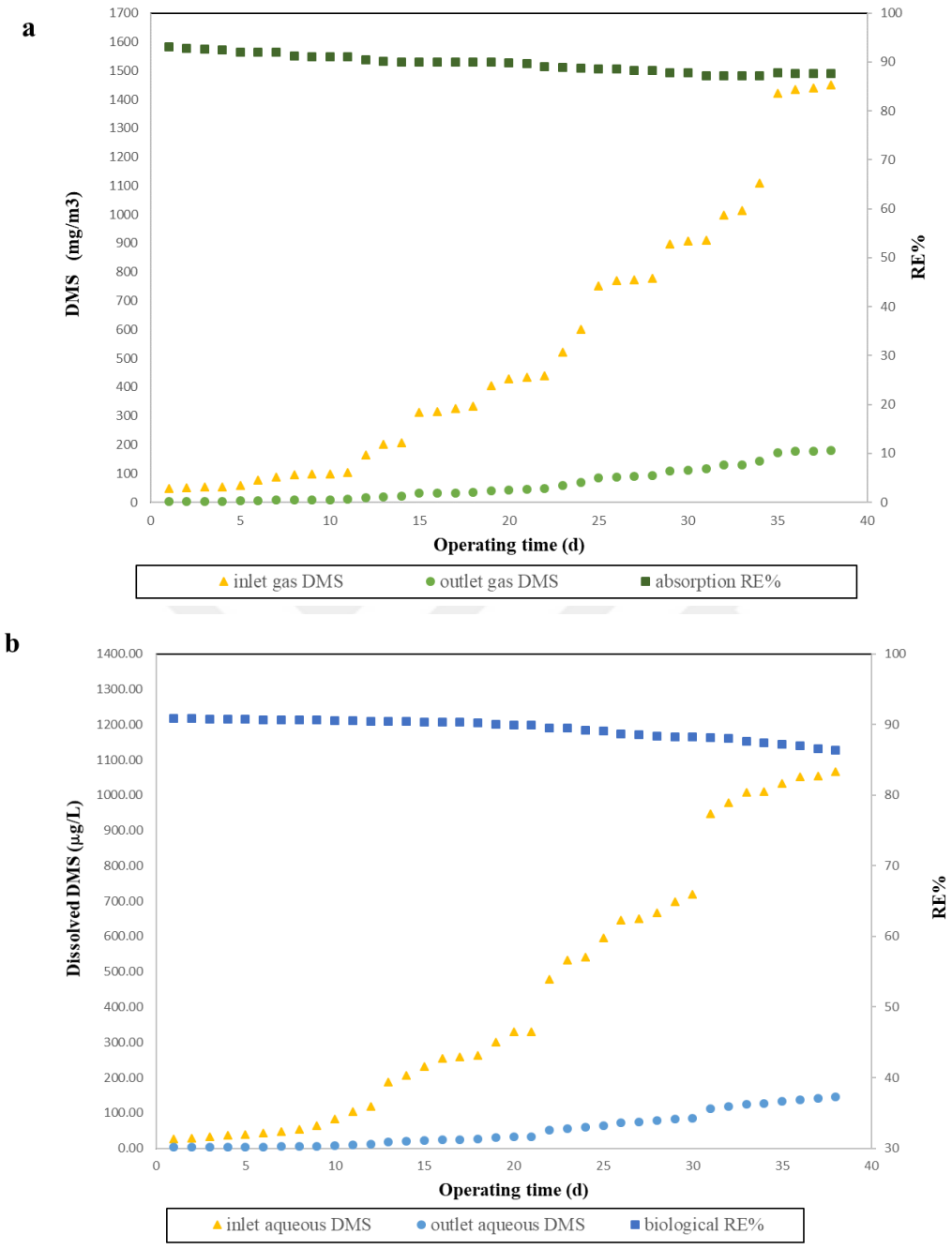

Figure 3.11: Performance of the DMS bio-scrubber during the start-up period (38 days); (a) gas phase and (b) aqueous phase.

To overcome nutrient limitation for microbial growth, 30% of the liquid media was exchanged weekly with fresh MSM. The DMS RE in the scrubbing column were recorded 93%, 91% and 90% at the 1st, 9th and 19th days of operation respectively,

under the operation of low inlet DMS concentrations less than 403 mg/m^3. The RE stabilized approximately at 88% after 22 days of operation in the system and increase inlet concentrations up to 1450 mg/m^3 didn't have significant effect on DMS RE. Furthermore, an 87% of RE was recorded at the 38th day as shown in Figure 3.11(a), whereas aqueous DMS (the DMS that transferred from gas to liquid phase after scrubbing DMS gas phase by irrigation liquid) bio-degradation efficiency through the bio-tank was reached to closer to the DMS gas RE of around 86% on the 38th day (Figure 3.11(b)). After reaching stabile RE in bio-scrubber column and bio-reactor tank acclimation period was completed at the 38th day of operation. Also, previous studies such as Wei et al. (2013) reported a 36-day period for the acclimation of microorganisms metabolizing DMS. During the operation period, 30% of the liquid media was exchanged with fresh (MSM) and resulted in an increase by activated sludge concentration (VSS) from 3000 to 8466 mg VSS/L.

In addition, the mineralization of DMS was determined by measuring COD in bio-tank. Results in Figure 3.12 illustrates that the COD degradation decreased while the DMS organic loading rate increased. The reactor stabilized after 38 days of the acclimation period and while the COD declined from 87% to 70% as DMS loading increased from 2.5 to 79 g/m^3 h, which corresponded to the high efficiency of organic substance removal.

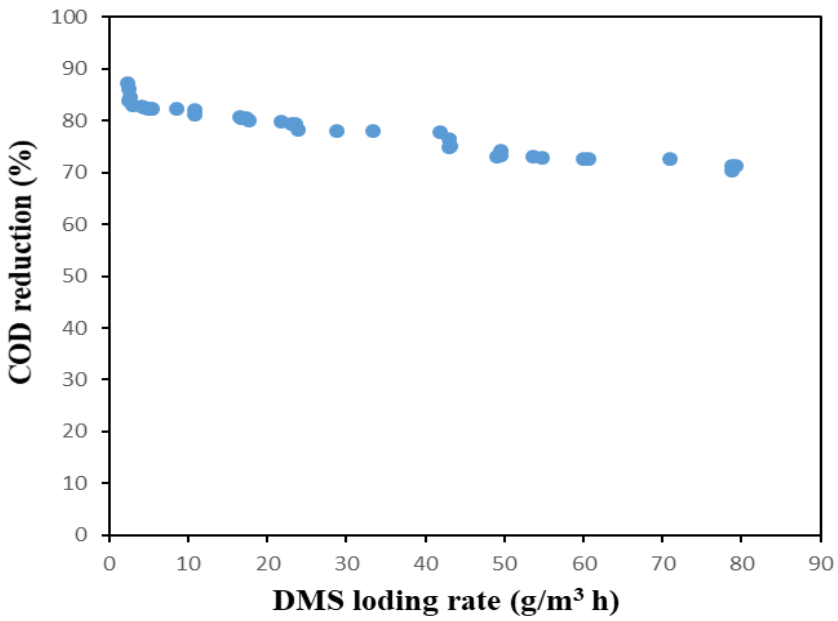

Figure 3.12: COD degradation for various organic loading rates

3.2.2.2 Effect of SD on DMS RE % and EC.

SD is considered a key indicator of bio-treatment for desulfurization process performance (Kim and Deshusses, 2005). Figure 3.13 (a) and (b) show the influence of SD on DMS absorption efficiency and EC. The effect of SD on RE was examined at EBRT 60 s and for four different inlet concentrations as shown in (E5 in Table 2.1). DMS absorption efficiency and EC were tested for different SD until reaching a steady state removal during the test assays. At this point, the optimum SD of absorption efficiency was determined. The increase by the liquid flow increased the mass transfer rate between the gas and liquid and hence, an increase in the DMS gas RE in the scrubbing column, and vice versa (Potivichayanon et al, 2006).

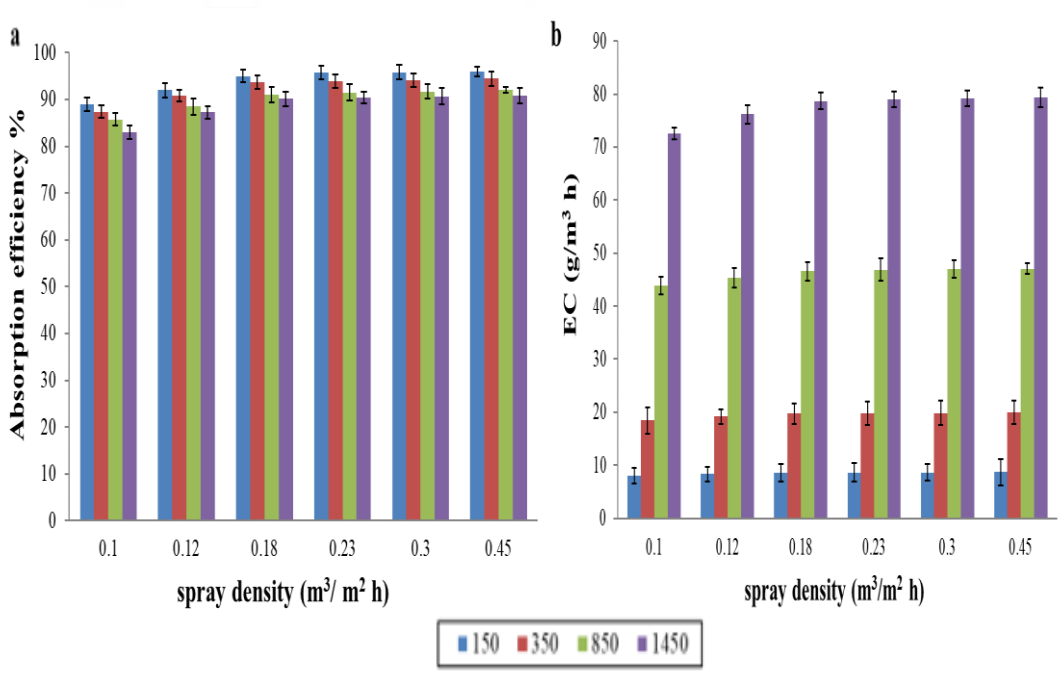

Figure 3.13: The effect of SD on gas absorption efficiency % and EC in scrubbing column at different inlet concentrations and fixed EBRT of 60 s. (a) absorption efficiency % (b) EC. Error bars indicate the S.D. of triplicate samples.

The results showed DMS ECs increased when the inlet concentration increased in all cases, in contrast to absorption efficiency. Furthermore, a slight improvement in the DMS removal of the four inlet concentration sets were observed between 0.18 and 0.45 m^3/m^2 h, where the gas REs were not significantly affected by the addition of SD. In addition, Figure 3.14, summarizes the impact of SD through the scrubbing column on the biological RE% in the bio-tank. Obviously, increase by irrigation

flow reduced the HRT in the bio-tank from 4 h at SD 0.1 m^3/m^2 h to 1 h at SD 0.45 m^3/m^2 h and resulted to the reduction in biological treatment time. However, higher than SD of 0.18 m^3/m^2 h, the biological RE% clearly decreased for all inlet gas concentrations with an increase in the SD and a decrease in the HRT. Moreover, the influence of HRT on COD RE was shown in Figure 3.15, the biological tank was operated with HRTs 1, 1.4, 2, 2.4, 3.5 and 4 h for SD 0.45, 0.3, 0.23, 0.18, 0.12 and 0.1 m^3/m^2 h respectively. Thus, the COD removal increased from 80% at HRT 1h for SD 0.45 m^3/m^2 h to 90.7% at HRT 4 h for SD 0.1 m^3/m^2 h respectively, at an inlet DMS concentration of 150 mg/m^3. In addition, it could be seen HRT higher than 2.4 h at SD 0.18 m^3/m^2 h, the COD RE% hardly increased.

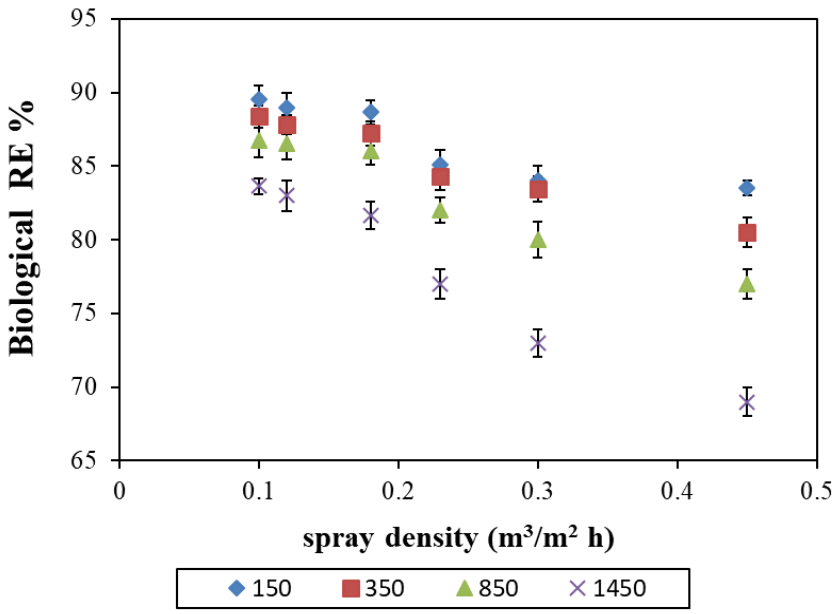

Figure 3.14: Effect of SD through scrubbing column on biological RE in the biological tank with different inlet concentrations. Error bars indicate the S.D. of triplicate samples.

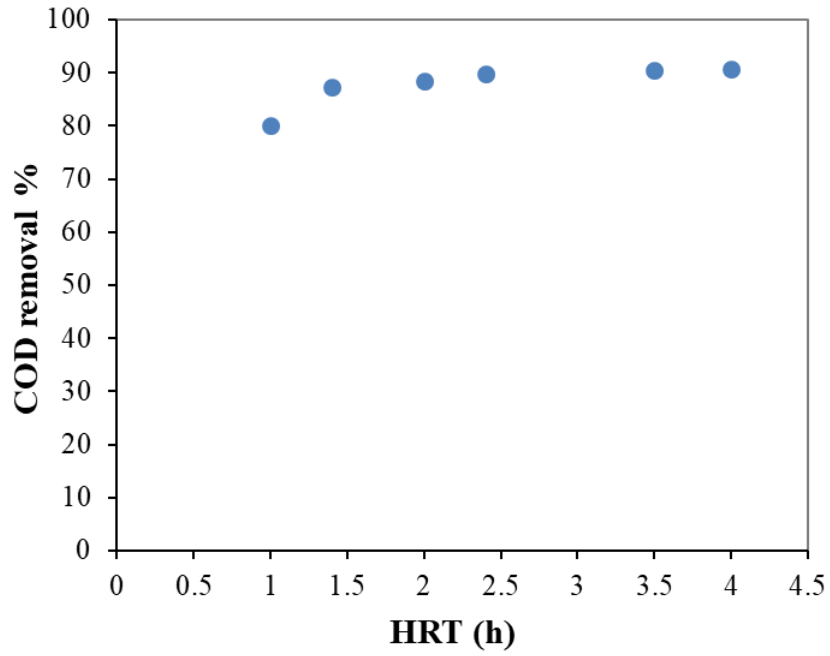

Figure 3.15: Influence HRT on COD RE at an inlet DMS concentration of 150 mg/m^3.

In summary, according to the results of the gas, aqueous and COD REs in Figure 3.13 (a) and (b), Figure 3.14 and Figure 3.15, the optimum SD and HRT in this study were determined as 0.18 m^3/m^2 h and 2.4 h respectively.

3.2.2.3 Influence of EBRT on DMS RE% and EC

The effect of EBRT in a lab-scale bio-scrubber system over DMS removal was investigated for different EBRTs (Pokorna et al, 2015). Figure 3.16 (a) and (b) represent the effect of EBRT on absorption efficiency and EC of DMS in gas phase at a permanent SD of 0.18 m^3/m^2 h and HRT 2.4 h as depicted in (E6 Table 2.1).

The results were obtained at steady-state, which were achieved when the DMS concentrations from the outlet of gas and liquid did not vary significantly for several consecutive observations. Four different inlet concentrations were tested in the bio-treatment system.

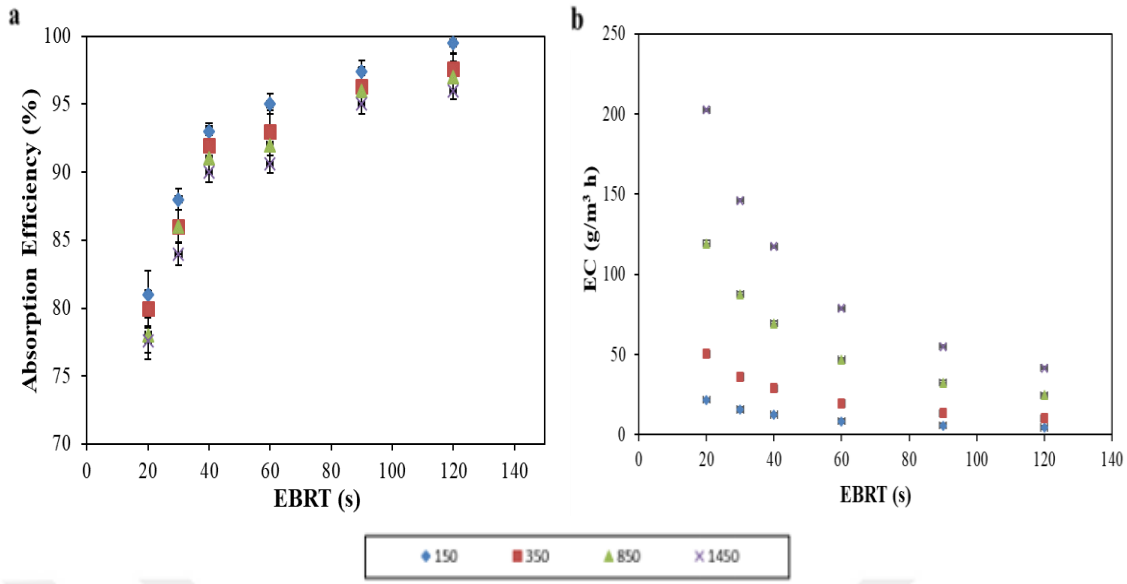

Figure 3.16: Influence of EBRT on DMS (a) absorption efficiency and (b) EC. Error bars indicate the S.D. of triplicate samples.

The results showed that the efficiency of removal was proportional to residence time and inversely proportional to DMS inlet concentration compared to the four groups of different inlet concentrations (150, 350, 850, 1450 mg/m^3), vice versa for gas EC. As it mentioned at Figure 3.16 (a) and (b), the higher DMS gas RE% increases and EC decreases from 81% (EC=25.38 g/m^3 h) to 99.5% (EC=3.42 g/m^3 h) with EBRT increasing and at minimum DMS concentration of 150 mg/m^3 rather than 350 mg/m^3, 850 mg/m^3 and 1450 mg/m^3. The reason for higher RE values in longer EBRTs is attributable to provide sufficient time for gas-liquid mass transfer followed by degrading the contaminant in the bio-reactor (Taicheng et al, 2010). In order to define best EBRT, the relationship between the REs of gas DMS, dissolved DMS and COD with EBRT from 20 s to 120 s at an inlet DMS gas concentration of 150 mg/m^3 and SD of 0.18 m^3/m^2 h were investaged and the results illustrated in Figure 3.17. The results showed a gradual increase of EBRT in the scrubbing column was leading to a boost in the DMS gas removal but in a declain of the biological RE of aqueous DMS and COD. At an EBRT of 20 s, the gas RE% of 81% were observed with dissolved DMS and COD removals were measured as 94% and 98% respectively. Meanwhile, the highest DMS removal (99%) was obtained at an EBRT of 120 s along with COD and aqueous DMS REs of 85% and 76% respectively. An EBRT of 40 s was resulted a 93% gas, 89% aqueous and 91.5% COD REs.

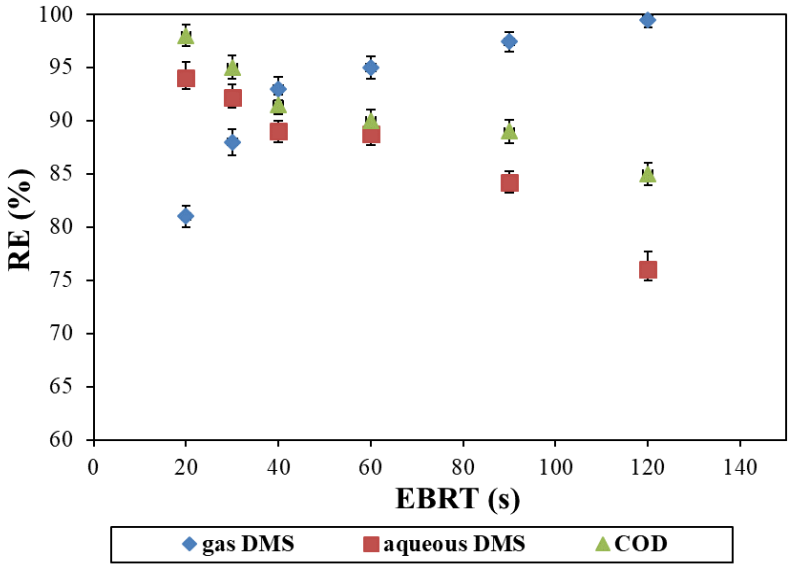

Figure 3.17: Influence of EBRT on REs of DMS gas, aqueous DMS and COD at an inlet concentration of 150 mg/m^3.

Additionally, the average gas RE and EC of all inlet DMS concentration (Figure 3.16) was 92% (EC= 54.03 g/m^3 h) at EBRT of 40 s. Therefore, it could be assumed that the best EBRT occurs in the 40 s in the system. Hence, the optimum gas to liquid ratio was 150 at EBRT of 40 s and SD of 0.18 m^3/m^2 h.

3.2.2.4 By-Product Analysis, Mass balance (Sulfur and Nitrogen) and pH Values

DMS can be metabolized to SO_4^{2-} and CO_2 using a bacterial culture during sulfur oxidation and carbon oxidation processes simultaneously in a bio-treatment system under aerobic conditions (Shu and Chen, 2009). Aerobic pathways for DMS degradation using the bacterium *Alcaligenes* sp. SY1 confirmed that high amounts of sulfur containing intermediates such as DMDS and Dimethyl trisulfide **(**DMTS) appeared during the DMS degradation process, after which they were completely converted into inorganic compounds ($SO_4^{2-}$and CO_2) (Sun et al, 2016). Interestingly, the amount of SO_4^{2-} could be considerable as the major metabolic products of DMS since no bio-conversion exists to consume SO_4^{2-} in bio-treatment processes (Wang et al, 2015). The SO_4^{2-} concentrations, pH, CO_2 and biomass growth in the bio-reactor were monitored daily at best operation conditions that defined previous steps of operation as SD 0.18 m^3/m^2 h and EBRT 40 s. An initial gas DMS injection from 150 to 1450 mg/m^3 through 28 days eventually were

converted in term of mmol to 10.85-104.9 mmol to simplify the comparison with the DMS stoichiometric equations. The dissolved DMS samples were entered to the bio-tank ranged from 0.9 to 20.43 mmol. Figure 3.18 illustrated, the aqueous DMS was biologically oxidized to one of CO_2, SO_4^{2-} or another intermediate, which was utilized by the cells to produce biomass. Stoichiometrically, 1 mmol of DMS converts to 0.42 mmol CO_2 and 0.32 bacterial growth according to Equations (A.5) and converts to 1 mmol of SO_4^{2-}, 0.8 mmol CO_2 and 0.24 bacterial growth according to (A.7) in the Appendix (A.1).

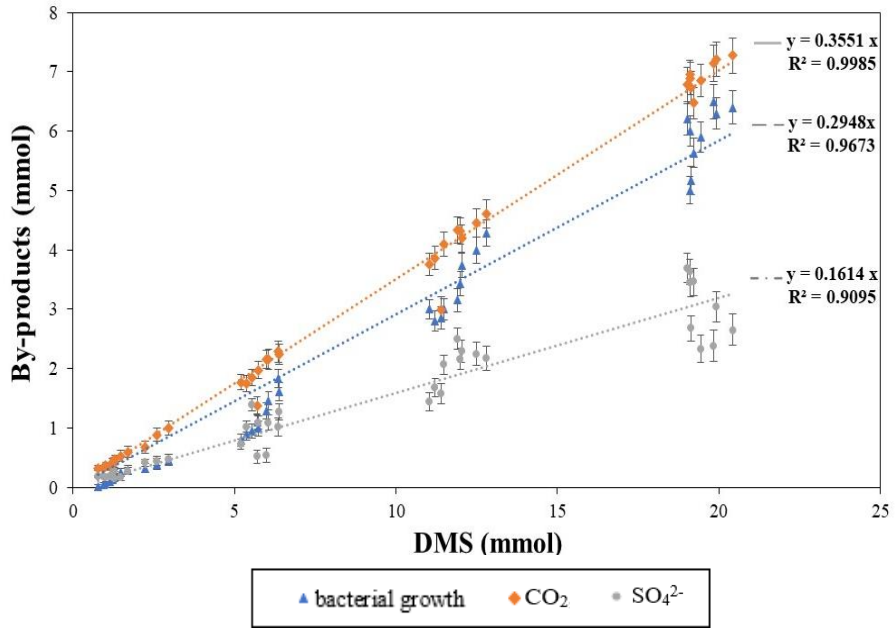

Figure 3.18: Relationship between SO_4^{2-}, CO_2 and bacterial growth with DMS degradation in the biological tank. Error bars indicate the S.D. of triplicate samples.

The results showed that the dissolved DMS amounts were from 0.9 mmol to 20.42 mmol eventually were converted into 0.32 mmol and 7.28 mmol CO_2, respectively. Furthermore, the bacterial growth ranged from 0.02 mmol to 6.5 mmol. The results made it clear substantially of the carbon source was eventually converted into CO_2 which was measured approximately 84 % of total carbon load and only less than 6 % of the carbon source was utilized by the cells to increase the biomass. Moreover, the empirical results showed that approximately 16% of the DMS was biologically converted into SO_4^{2-}. Consequently, this indicated that the desulfurization process in the bio-reactor of the DMS occurred in an aqueous phase and confirmed that the remaining DMS was converted into S^o and

other forms of sulfur rather than producing SO_4^{2-} via the desulfurization process (Sun et al, 2016; Baspinar et al, 2011). In order to calculate the S^o from the remaining DMS, the sulfur mass balance was applied by subtraction to calculate the S^o (Montebello et al, 2012) where the biological degradation of the DMS was mainly oxidized to either S^o or SO_4^{2-} as reported by stoichiometric equations (A.5) and (A.7). Figure 3.19 presents the relationship between the accumulated SO_4^{2-} production (expressed as SO_4^{2-}-S) and calculated S^o formation in the lab-scale bio-scrubber system from oxidation of aqueous accumulated DMS (suffixed as DMS-S). The results presented that the increase of DMS-S from 0.39 to 181.96 mmol led to increase of SO_4^{2-}-S production and S^o calculated from 0.18 to 57.2 mmol and from 0.33 to 157 mmol, respectively. Furthermore, the experimental results showed that approximately 11% and 89% of the DMS-S were biologically converted into SO_4^{2-}-S and S^o respectively.

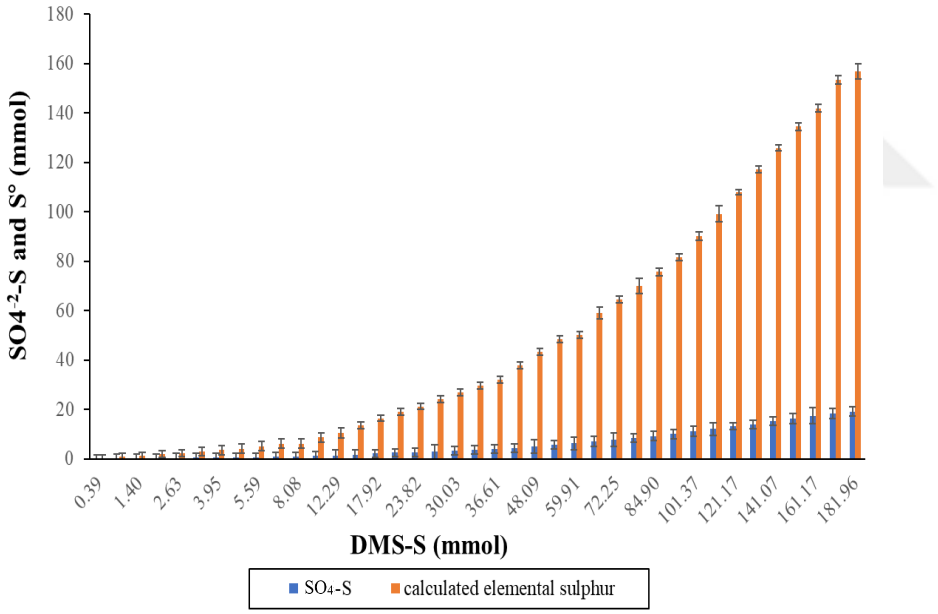

Figure 3.19. Sulfur mass balance. Error bars indicate the S.D. of triplicate samples.

The results that obtained during bio-scrubber operation, they matched with the Equation (A.5) when the end product was S^o. Furthermore, there were some pale-yellow color substances with some characteristics of the S^o material appearing within the biomass in the reactor using a microscope to take optical micrographs of

biomass (Figure 3.20), clearly referring to the fact that the S^o had become the main end product.

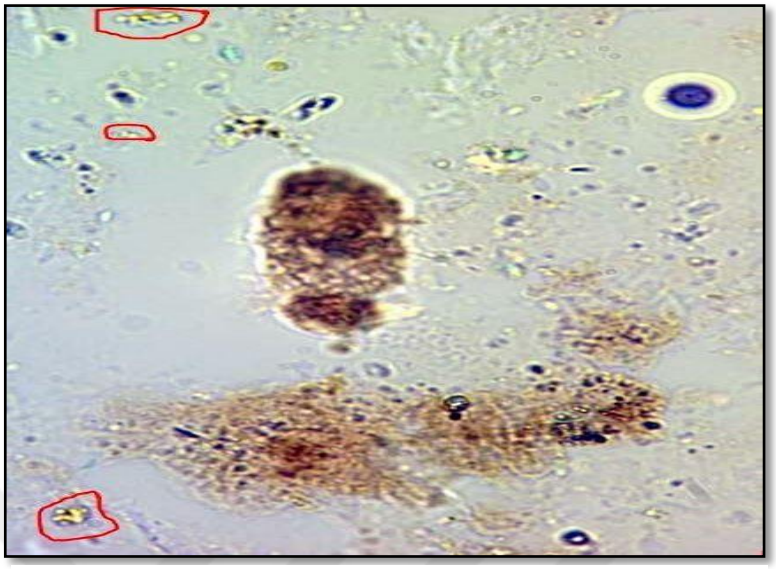

Figure 3.20. Microscope photo of DMS-degrading strain (1000× magnification).

In addition, the mass balance of nitrogen (expressed as NO_3^--N) was done depending on the actual inputs of feeding nitrogen and measured nitrogen concentrations in the system. Using the equation (3.9) below, the nitrogen balance was calculated for the whole system.

$$N_{input} = N_{output} + N_{biomass} + N_{gas} \tag{3.9}$$

where N_{input} represents the nitrogen amount that input to the system, N_{output} represents the output nitrogen from the biological tank, $N_{biomass}$ represents the consumption of nitrogen for biomass growth, and N_{gas} is the nitrogen gas produced during the denitrification process, which was estimated by calculating the average of N_2 from the stoichiometric equations (A.5) and (A.7), where it was difficult to measure since it was the same as the carrier gas. Figure 3.21 depicts the initial nitrogen amounts into the biological reactor at a rate of 1.528 mmol to 39.8 mmol N according to stoichiometric ratio DMS/NO_3^- of 0.54. The results showed that the N_{output} was approximately 30 % of the input nitrogen, 1% of the inlet nitrogen amount was represented the nitrogen of net growth of biomass in the biological

reactor ($N_{biomass}$) and N_2 gas production through the denitrification process was around 50 % of the total incoming N. It can be noticed that there is unbalanced N with about 19% difference between inlet and outlet N, which may be due to our assumptions and/or instrumental/experimental errors.

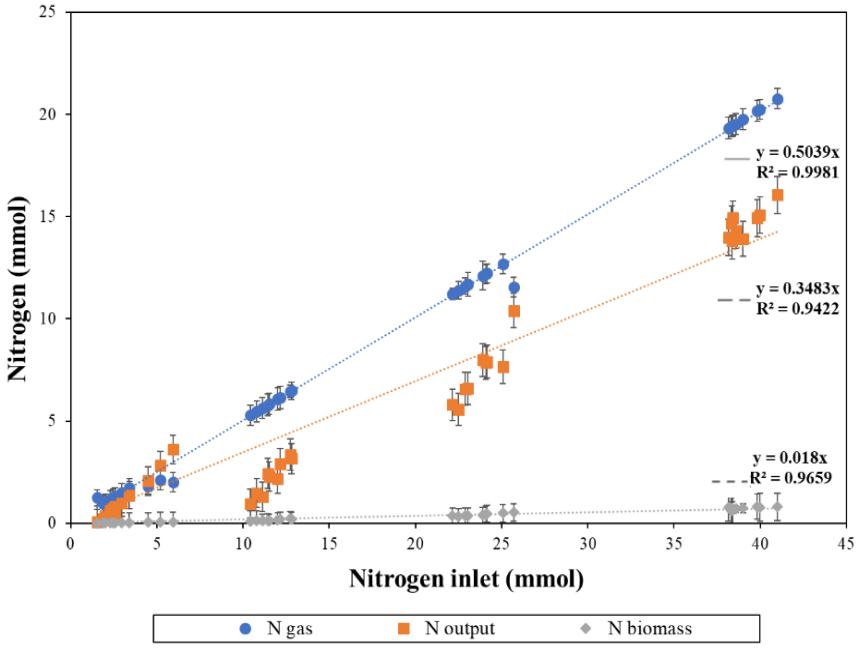

Figure 3.21. Nitrogen mass balance Error bars indicate the S.D. of triplicate samples.

As it shown in Figure 3.22, there was recognized a direct relationship between various DMS IL and pH values of the activated sludge. When the various DMS IL, g/m^3 h were added from 9.5 to 115, the pH value rapidly increased with during the operation time and loading from 7 to 8 probably due to the presence of an alkaline ion besides SO_4^{2-}, such as S^{2-} and SO_3^{2-} in the desulfurization process, also likely due to the denitrification process (Montebello et al, 2012). At this point, it is important to highlight that previous studies have reported that a pH of 6.0 promoted DMS removal, and moreover most DMS degrading bacteria presented optimum biological activity at a pH between 5.5 and 6.5 under aerobic conditions (Wei Z et al, 2013).

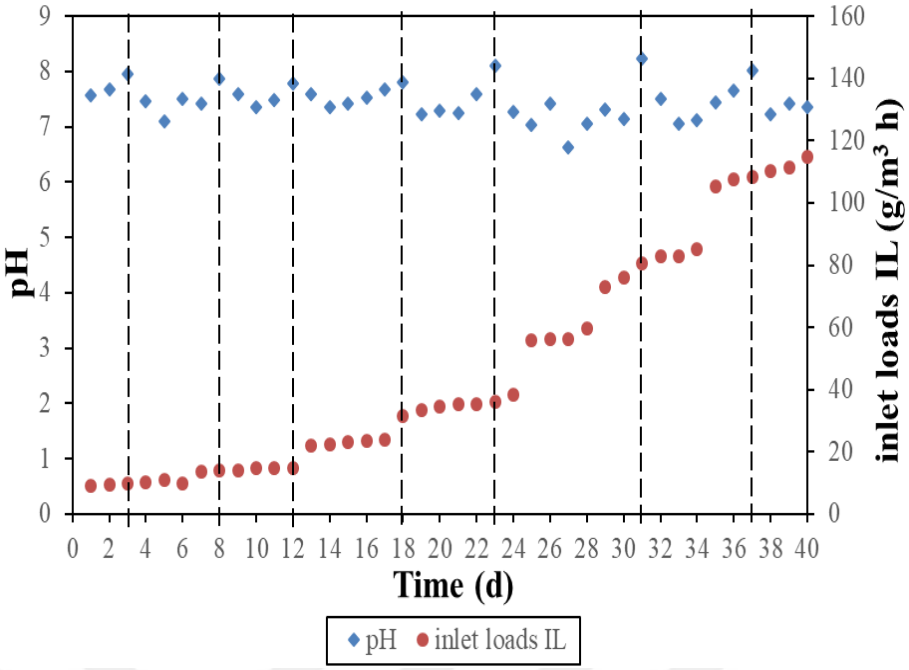

Figure 3.22. Effect of DMS loads on pH with operation times; dashed lines represent adjusted pH with 6N HCl.

The pH was adjusted by addition of 6N HCl into the sludge in the biological tank in order to maintain bacterial activities by adjusting the final pH to a value of 7.5 ± 0.1.

3.2.2.5 Biological DMS oxidation stoichiometry and energetics reactions

The energy required for the growth and maintenance from oxidation reduction reactions can be obtained from microorganisms by oxidizing inorganic and organic materials (Sawyer et al, 2003). The electron donor (food substrate for the organism) and the electron acceptor (oxygen) are playing an important role in oxidation-reduction reactions under aerobic conditions. Under anaerobic or anoxic conditions, other electron acceptors in energy metabolism can be used by some microorganisms including NO_3^-, SO_4^{2-} and CO_2. In a chemical equation, the quantitative relationships between reactants and products can be defined as stoichiometry. In this study, the thermodynamic approach (Dogan et al, 2012) was used to calculate the reaction stoichiometry and the biomass yield ($Y_{X/D}$) for biological DMS oxidation. The following stoichiometric equations were derived from the thermodynamic analysis presented in the Appendix (A.1).

When the NO_3^- was used as an electron acceptor and as a nitrogen source for the biomass, the end products were:

Sulfur:

$$CH_3SCH_3 + 1.35NO_3 + 1.34H^+ \rightarrow S^0 + 0.32C_5H_7NO_2 + 2.55H_2O + 0.52N_2 + 0.42CO_2 \quad (3.10)$$

Sulfate:

$$CH_3SCH_3 + 2.9NO_3 + 0.89H^+ \rightarrow SO_4^{2-} + 0.24C_5H_7NO_2 + 2.59H_2O + 1.33N_2 + 0.8CO_2 \quad (3.11)$$

Table 3.5: Thermodynamically calculated yield values (Y_{DMS/NO_3^-}) and free energies of DMS oxidation when NO_3^- was used as an electron acceptor

Thermodynamically calculated yield values and Gibbs free energies	**Nitrogen source for biomass**	**Main Product SO_4^{2-}**	**Main Product S^o**
Y_{DMS/NO_3^-} (mol/mol)	NO_3^-	0.35	0.74
Gibbs free energy kJ/e-mole		−1404.76	−682.65

Table 3.5 summarizes the results of the theoretical yield molar ratios (Y_{DMS/NO_3^-}) that were calculated using Equations (3.10) and (3.11) and Gibbs' free energy at different end products knowing that all of the results were obtained when the NO_3^- was used as the source for the biomass. As can be seen from Table 3.5, the anoxic oxidation of the DMS to SO_4^{2-} and S^o generated energies, as shown in the Appendix (A.1), are equal to −1404.76 kJ/e-mole and −682.65 kJ/e-mole respectively. The negative signal refers to the reaction being exothermic and spontaneous (and product-favored). From Table 3.5 and Equations (3.10) and (3.11), the NO_3^- is limited, S^o is formed as a by-product, and the NO_3^- and energy consumptions are comparatively lower compared to the systems where SO_4^{2-} is formed as the main product. Therefore, S^o would need to be the desired end product.

The upper and lower limits of the theoretical yield molar ratios (Y_{DMS/NO_3^-}) that were given in Table 3.5 can be seen in Figure 3.23, which also presents the experimental data for optimized operating conditions, with SD of 0.18 m^3/m^2 h and an EBRT of 40s under different DMS concentrations.

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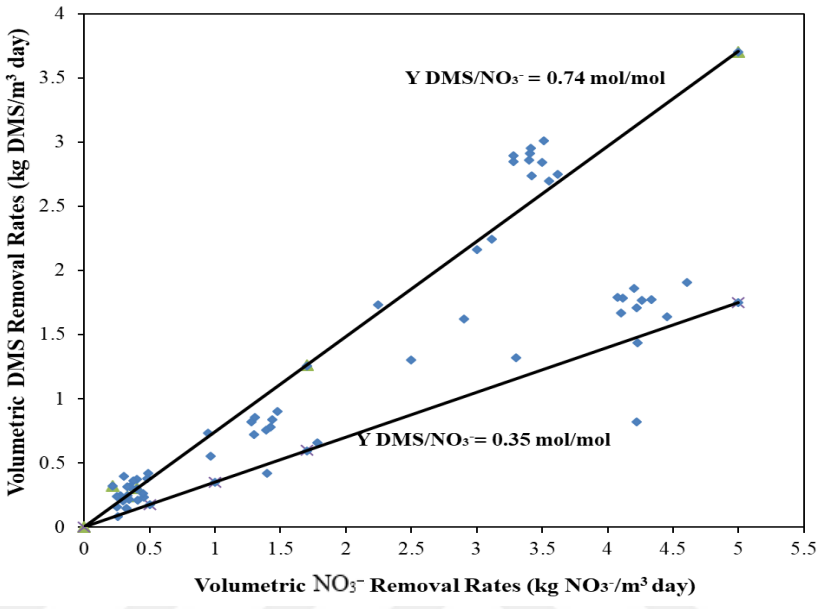

Figure 3.23. Volumetric DMS removal versus volumetric NO_3^- removal.

In a previous study, the desulfuration biogas (H_2S) in a continous bio-scrubber was done by using mixtures of NO_3^- and NO_2^- as electron acceptors. Most of the experimental molar ratios were scattered within two limits of stoichiometric molar ratio ($S^{2-}/(NO_3^- + NO_2^-)$) 0.75 and 2.89 of SO_4^{2-} and S^o, respectively. This is due to the mixture of NO_3^- and NO_2^- and the variance of H_2S oxidation end products (Baspinar AB et al, 2011). The removal of H_2S from the biogas under the effect of different sulfide/ NO_3^- (S/N) ratios in a bio-trickling filter (BTF) as Li et al. (2016) showed in the S/N ratios of 1:2.5, the H_2S was removed almost completely and the maximum EC of the H_2S was 54.5 g H_2S/m^3 h. The S/N ratios had no significant influence on biogas desulfurization efficiency, while the desulfurization products were obviously affected. The SO_4^{2-} percentages decreased and the denitrification performance clearly improved with the increase of the S/N ratios from 1.0 to 2.5. In this study, the experimental data was distributed between two limits and most of the data are scattered near the upper value of Y_{DMS/NO_3^-} (0.74) when the end product was S^o, as shown in Figure 3.23, which refers to the presence of S^o.

The average Y_{DMS/NO_3^-} ratios with different SD and DMS loading rates can be observed in Table 3.6. According to this Table, when the main end product was S^o, the average of the experimental yield values was closer to the Y_{DMS/NO_3^-} theoretical value of 0.74. This indicates that the partial formation of S^o and other forms of sulfur were produced rather than a SO_4^{2-}, thus proving the production of S^o in the bio-reactor tank. As a result, there is lower consumption of NO_3^- and energy. It is known that the cost reduction is related to lower NO_3^- consumption. Furthermore, S^o can be recovered easily due to its properties, such as its non-toxicity, stability and insolubility in water (Baspinar et al, 2011). Moreover, recovery of S^o can be used as a valuable raw material e.g., in bioleaching processes. Whereas, the SO_4^{2-} formation consumes higher energy for aeration, thus needs more oxygen supplied (Janssen et al, 1997), (Tichy et al, 1997). The quality of the required S^o is considered as a key factor in selecting the suitable method of separating S^o from the water stream include filter presses, sulfur melters and settlers (Tichy et al, 1997; Pagella et al, 1996). Accordingly, in this study, S^o can be recovered from the bio-scrubber system by transferring the settled sludge from the sedimentation tank to the vacuum filter press.

Table 3.6: Comparison of thermodynamically and empirically calculated Y_{DMS/NO_3^-} values when NO_3^- is used as the electron acceptor

IL (g/m^3 h)	SD (m^3/m^2 h)	Average DMS Removal (g/day)	Average NO_3^- Consumption (g/day)	Experimental $Y_{DMS/NO3^-}$ (mol/mol)	Thermodynamically Calculated $Y_{DMS/NO3^-}$ (mol/mol)	
					Main Product S^o	Main Product SO_4^{2-}
14 ± 0.10	0.45	0.66	1.50	0.44	0.35	0.74
	0.3	0.65	1.01	0.65		
	0.23	0.64	0.75	0.86		
	0.18	0.63	0.66	0.96		
	0.12	0.63	0.66	0.96		
	0.1	0.62	0.67	0.93		
	Average ± S.D.	**0.64 ± 0.01**	**0.88 ± 0.33**	**0.8 ± 0.21**		
31 ± 0.20	0.45	1.47	4.87	0.30		
	0.30	1.44	4.38	0.33		
	0.23	1.44	3.26	0.44		
	0.18	1.44	2.60	0.55		
	0.12	1.41	1.74	0.81		
	0.10	1.40	1.70	0.82		
	Average ± S.D.	**1.43 ± 0.03**	**3.09 ± 1.33**	**0.54 ± 0.23**		
77 ± 0.21	0.45	3.53	7.49	0.47		
	0.30	3.52	7.56	0.46		
	0.23	3.51	5.62	0.62		
	0.18	3.47	4.49	0.77		
	0.12	3.37	4.00	0.84		
	0.10	3.34	3.94	0.85		

	Average ± S.D.	**3.46 ± 0.08**	**5.52 ± 1.67**	**0.67 ± 0.18**	
132 ± 0.19	0.45	5.95	17.07	0.35	
	0.30	5.96	11.49	0.52	
	0.23	5.91	8.53	0.69	
	0.18	5.90	6.82	0.87	
	0.12	5.69	6.34	0.90	
	0.10	5.62	6.4	0.87	
	Average ± S.D.	**5.84 ± 0.15**	**9.44 ± 4.22**	**0.7 ± 0.22**	

The microorganisms consumed a greater amount of NO_3^- (electron acceptor) with an increase in the DMS loading rate to oxidize the DMS. This led to gradually and significantly increase by NO_3^- consumption, as seen in Table 3.6, which means that the denitrification process evidently occurred.

3.2.3 Removal of the binary mixture of ET and DMS

The bio-scrubber inoculated with mixed culture was used to treat waste gas containing ET or DMS separately as described above. The system was demonstrated that the same bio-scrubber inoculated with ET had the potentiality to biodegrade DMS as described above. Thus, to test the influence of a co-substrate with DMS on the performance of the bio-scrubber, the inlet concentration 150 ± 1.45 mg/m^3 ET was kept constant during the test with step-increases of DMS from 38 to 600 mg/m^3 as shown (E7 in Table 2.1). Outlet ET and DMS concentrations were measured after steady operation for 24 h. The REs for different ratios of the binary mixture of ET and DMS at fixed EBRT 90 s, SD 0.23 m^3/m^2 h and HRT 2 h of biological tank are illustrated in Figures 3.24 and 3.25

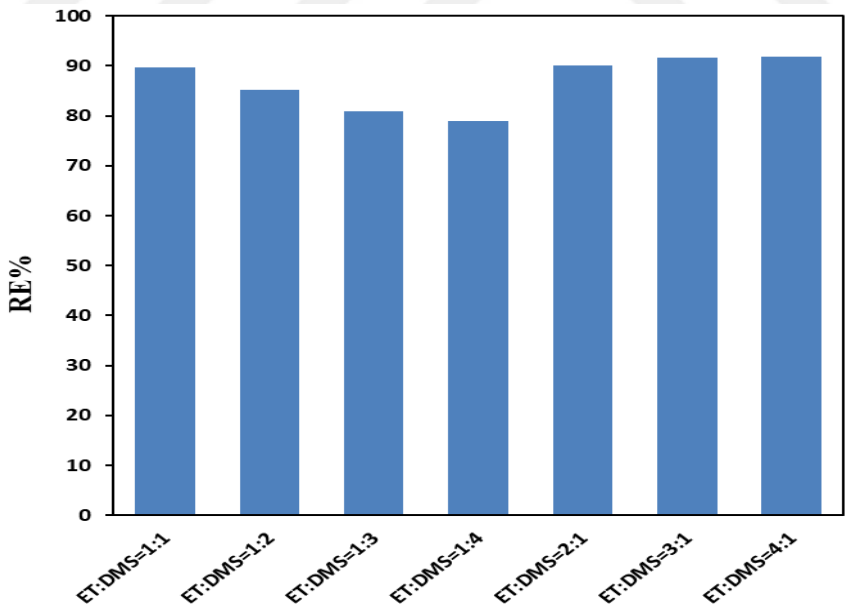

Figure 3.24: ET REs of binary gas mixture at different concentration ratios (ethanethiol: dimethyl sulfide = ET: DMS)

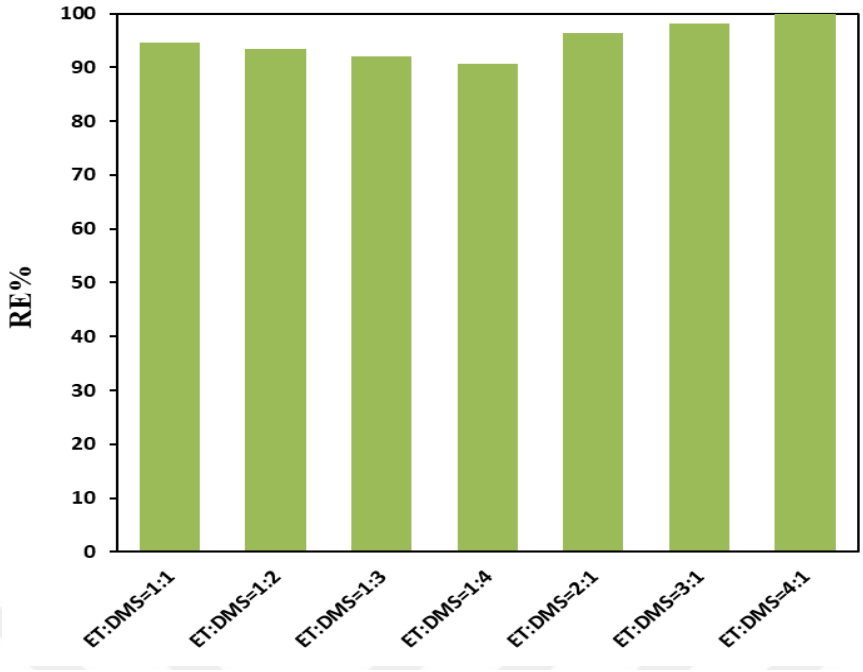

Figure 3.25: DMS REs of binary gas mixture at different concentration ratios (ethanethiol: dimethyl sulfide = ET:DMS)

Overall, higher ET and DMS REs were obtained at lower concentrations of DMS, while higher concentrations of DMS led to lower REs. For DMS, the highest RE 100% of DMS-RE was obtained at DMS concentration 38 mg/m^3 and ratio was 4:1. As inlet DMS concentration was increased to 75 mg/m^3 and ratio 2:1, RE slightly decreased to 96.8%. With further increase of DMS concentration, an abrupt drop in total RE was observed; for example, RE decreased from 100% at DMS concentration 38 mg/m^3 (ratio 4:1) to 90.6% at DMS concentration 600 mg/m^3 (ratio 1:4). The RE of ET was stable around 91 %, as DMS concentration was increased from 38 to 150 mg/m^3. Thus, ET REs% in the presence of DMS were not significantly affected by addition of DMS below 150 mg/m^3. While, with increasing the DMS concentrations above 150 mg/m^3 the RE of ET abrupt drop from 92 to 79 %, which is due to the bacterial activity becoming the rate-limiting step for pollutant removal when DMS concentrations more than 150 mg/m^3. Interestingly, the REs of DMS are higher compared with ET, this indicates that DMS is more absorbed than ET. Because DMS solubility is higher than ET in water with DMS Henry's coefficient of 0.07 while ET Henry's coefficient of 0.15. The total ECs, which reflect the ability of the bio-scrubber to purge the mixed gas containing ET and DMS, are listed in Table 3.7.

Table 3.7: Total REs and ECs of binary mixture of ET and DMS with different ratios at fixed EBRT 90 s, SD. 0.23 m^3/m^2 h and HRT 2 h of biological tank.

Inlet concentration (mg/m^3)	IL (mg/m^3 h)	EC (mg/m^3 h)	RE (%)
Ethanethiol			
150 (ET:DMS=4:1)	20	19	91.7
150 (ET:DMS=3:1)	23	22	91.5
150 (ET:DMS=2:1)	22	21	90.46
150 (ET:DMS=1:1)	18	17	89.58
150 (ET:DMS=1:4)	16	13	78.87
150 (ET:DMS=1:3)	15	13	80.87
150 (ET:DMS=1:2)	17	15	84.9
Dimethyl Sulfide			
38 (ET:DMS=4:1)	3.1	3.1	100
50 (ET:DMS=3:1)	5.1	5.0	98
75 (ET:DMS=2:1)	7.4	6.9	96.8
150 (ET:DMS=1:1)	18	15	94.69
600 (ET:DMS=1:4)	52	41	90.6
450 (ET:DMS=1:3)	52	42	92
300 (ET:DMS=1:2)	23	19	93.45

In Table 3.7 the results show, ET elimination capacities remained constant around 20 mg/m^3 h as the inlet ET concentration was about 150 mg/m^3 and DMS increased from 38 to 150 mg/m^3. With more increase of the DMS concentrations from 300 to 600 mg/m^3, the ECs of ET then drop slightly to 13 mg/m^3h. At the same time, for DMS ECs rose from 3.1 mg/m^3 h to 42 mg/m^3 h as DMS concentrations increased from 38 to 600 mg/m^3, the increase of the DMS inlet concentration boosted the transfer rate of DMS from gas phase to liquid phase in biological tank as ET concentrations were maintained constant thus, the ECs of DMS were gradually increased with rising amount of DMS transferred to biological tank, while ECs to ET were constant with low DMS concentrations from 38 to 150 mg/m^3 and were reduced with high DMS concentrations from 300 to 600 mg/m^3. In summary,

depending on REs and ECs, the optimal ratio was 3:1 for binary mixture of ET and DMS at constant EBRT 90 s, SD 0.23 m^3/m^2 h and HRT 2 h of biological tank.

3.3 acterial community analysis in the bio-scrubber system by next generation sequence (NGS):

Figure (3.26) shows the percentage of microbial community inoculated in the bio-scrubber system of raw sludge at (0 Day) and the microbial community after gas (DMS or ET) bio-degradation at the end of the system operation (at 300 Day). Based on 16S™ NGS data, the percentage of dominant microbial distributions in raw sludge were found to be 38% *Proteobacteria,* 15% *Bacteroidetes* and 13% *Actinobacteria*, which *Proteobacteria* and *Bacteroidetes* represent the predominate heterotrophic bacterial phylum within the raw sludge and they capable of degradation a high molecular weight of carbohydrates, mercaptans and inorganic sulfide (Stevens et al., 2005; Thomas et al., 2011 and Wei et al., 2013). *Actinobacteria* (Ho et al, 2008) can be metabolized the volatile sulfur compounds (VSCs) to S^o and SO_4^{2-}. Whereas, the ratios of their distributions at the end of DMS degradation experiments were slightly increased to 53% *Proteobacteria,* 21% *Bacteroidetes* and 16% *Actinobacteria*. In addition, new types of microbial community were found 1 % *Antibacterial* and 1.4 % *Firmicutes*. *Antibacterial* (Stephanie et al., 2015), responsible of carbon oxidation processes and have potentially producing fermentation products such as acetate, ethanol, and CO_2 under anoxic condition. *Firmicutes* was able to degrade (VSCs) (Ho et al, 2008). The percentage of bacterial communities in sludge bio-tank of the bio-scrubber system at the end of ET degradation experiments were 50% *Proteobacteria*, 23% *Bacteroidetes* and 17% *Actinobacteria*, 1.8 % *Antibacterial* and 1.5 % *Firmicutes*. These predominate bacteria may be due to the capability for sulfur compounds oxidation and carbon oxidation processes to occur together in bio-scrubber system.

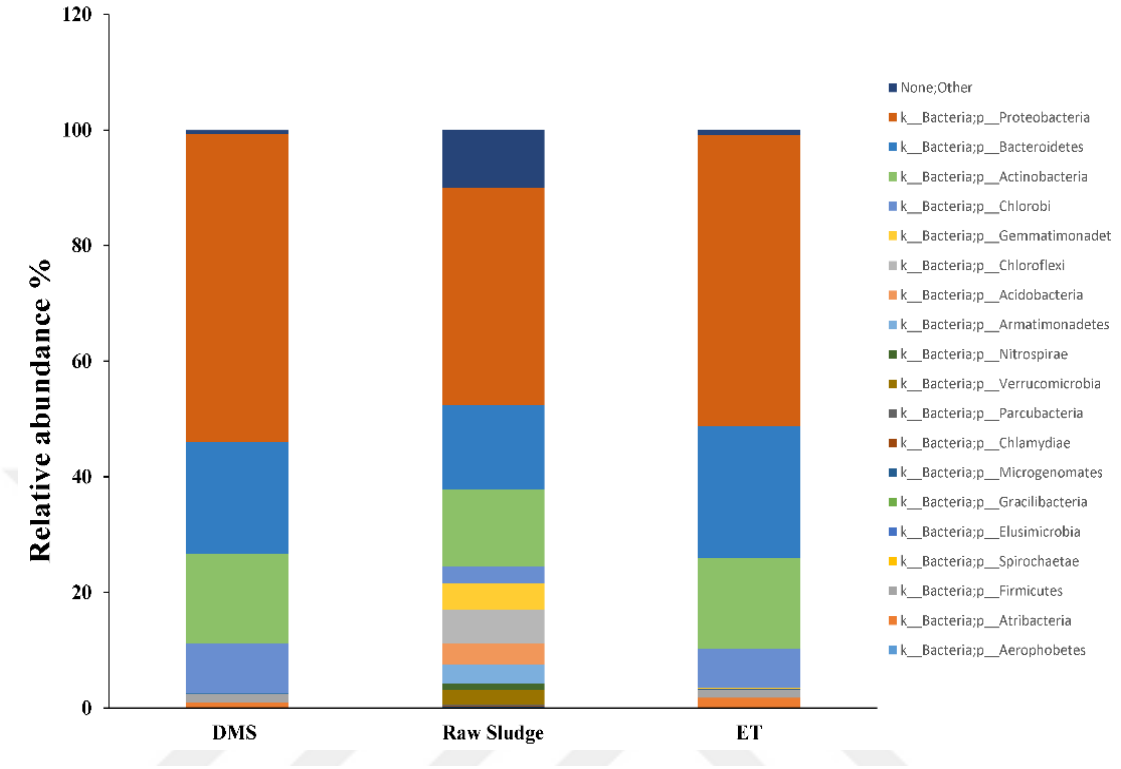

Figure 3.26: Profiles of bacterial community composition at phylum level of raw sludge (at 0 day) and the microbial community of each of ET and DMS after (300 day) at the end of the system operation.

The identification of the dominant bacterial communities at class level is depicted in Figure (3.27). The more percentage class in the bio-scrubber system for ET or DMS biodegradation was *Betaproteobacterial* and *alphaproteobacterial*, relative abundance ratios of these two classes from Day 0 to Day 300 (around from 10% to 26% *Betaproteobacterial* and from 6% to 20% *alphaproteobacterial*, respectively), where it belongs to the phylum of *proteobacteria*. Ho et al. (2008) identified that some from the predominate bacterium for degradation sulfur compounds were 1.2% *Betaproteobacteria* and 1.4% *alphaproteobacterial.* The third abundant class was *Sphingobacteriia* (i.e., belongs to *Bacteroidetes* phylum) approximately was from 12% on Day 0 to 19% on Day 300, which this organism lives in anoxic environments or organic rich environments and capable of oxidizing reduced inorganic sulfur compounds other than H_2S gas (Cayford et al, 2012). A slight increase was also observed in the bacterial class namely, *Chlorobea* (i.e., from

3% on Day 0 to about 8% on Day 300) under the phylum of *Chlorobi*, where it is called green sulfur bacteria (*Chlorobiaceae*) and capable of anoxygenic photosynthesis. Bacterial anoxygenic photosynthesis is using inorganic sulfur rather than water and, in the by-product, generated (e.g. S° instead of molecular oxygen) (Hiras et al, 2015). *Chlorobi* is classified based on the properties of the green sulfur bacteria (GSB), the class *Chlorobea* within the *chlorobi* family, is able to metabolize the reduced sulfur compounds to S° as reported by (Chan et al, 2008). This also confirms that one of the end products was S°. Finally, relative abundance ratios of calss *Bacilli* within the *Firmicutes* familly was around 0% on Day 0 to 1% on Day 300.

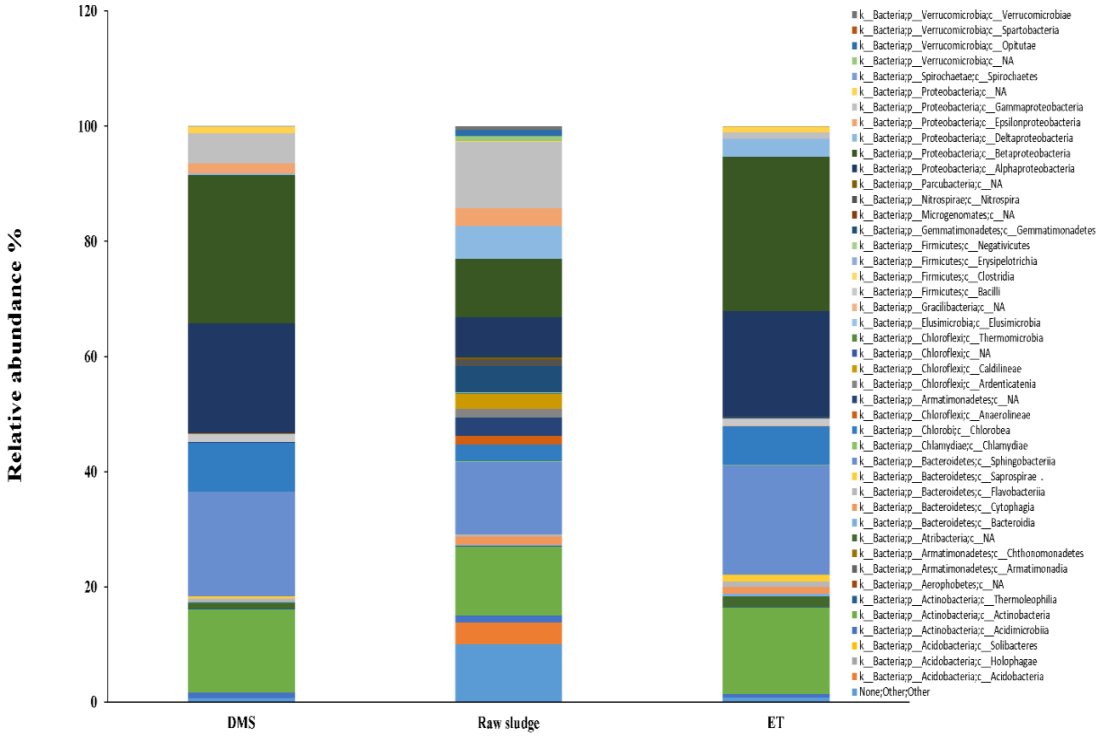

Figure 3.27: Profiles of bacterial community composition at class level of raw sludge (at 0 day) and the microbial community of each of ET and DMS after (300 day) at the end of the system operation.

As well, the high bacterial diversity of mixed culture presisted in the bio-scrubber system confirmed the easy implementation for purifying of ET or/and DMS.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

4.1 Conclusion

In this study, a lab-scale bio-scrubber under anoxic conditions acclimated with mixed culture is confirmed to have a high capacity and long-term stability to treat ET and DMS as sole or dual substrates in the bio-scrubber. The results obtained from this study revealed that the better running conditions of ET degradation were at inlet concentration of 150 mg/m^3, a SD of 0.23 m^3/m^2 h, a gas/liquid ratio of 53 and an EBRT of 90 s.91% average RE of all inlet concentrations was obtained. Whereas, the results show that the best running conditions of DMS degradation were at the inlet concentration of 150 mg/m^3, SD of 0.18 m^3/m^2 h, a gas/liquid ratio of 150 and EBRT of 40 s with an average RE of 92% at four sets of inlet concentrations. This bio-scrubber system can also successfully eliminate binary (ET and DMS) mixture. The optimal ratio was 3:1 for dual mixture at an EBRT of 90s and a SD of 0.23 m^3/m^2 h. Therefore, based on the results, bio-scrubber has significant potential for ET or DMS and even ET: DMS mixture removal under anoxic conditions.

The biodegradation of ET or/and DMS had been investigated by batch experiments using mixed culture and the results of batch experiments showed that ET or DMS was a readily biodegradable compound under anoxic conditions than as mixture compounds ET:DMS because the supplementation of an additional carbon and energy source always led to lower contaminate biodegradation rates, which proven the appearance of a competitive inhibition and an increased biodegradation time compared to the biodegradation of VOSCs as individual. The Monod model was used to indicate the values for μ_{max}, K_s, Y_{XS}, and q_{max} and their biodegradation were characterized by μ_{max} values of ET or/and DMS. The average experimental yield value was closer to the theoretical value (Y_{ET/NO_3^-}) of 0.74 when the main product was S°. This indicates that S° and other sulfur forms were produced rather than SO_4^{2-} as the end product.

The 16S™ NGS was performed to study the microbial community profile of sample taken from bio-tank in the bio-scrubber system for removal ET or/and DMS. Based on 16S™ NGS results revealed the predominate microbial phylum *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* were distinguished by increased relative abundance around 1.33 times of its relative abundance in the biomass samples taken

from the bio-scrubber system as the operation continued, they capable of degradation of carbohydrates, mercaptans groups and inorganic sulfide. The microbial profile indicated also that other microorganisms (non-inoculated) namely *Antibacterial* and *Firmicutes* were generated during the operation of the bio-scrubber. The class *Chlorobea* within the *chlorobi* family, is capable of oxidation the reduced sulfur compounds to S°. The strains of mixed biomass persisted in the bio-scrubber system have been successfully applied for bio-degradation of ET or/and DMS.

4.2 Future Perspectives

Based on the study conducted which discussed in this thesis, several aspects need to be covered in the future. These aspects are presented below:

- The study of batch biodegradation can be used for the other VOSCs such as DMDS, MT, etc. and it can be extended to the inorganic compounds such as H_2S, carbonyl sulphide (COS), carbon disulphide (CS_2), ect.
- The effect of other influencing parameters such as pH, concentration of nutrients, concentration of pollutant, VSS concentration, electron acceptor, etc can be conducted using the batch biodegradation studies.
- The bio-scrubber experiments can be examined to study the effect of other important parameters such as pH, concentration of sludge, higher loading rate of pollutant, particle size or type of packing materials, etc.
- Studying the effect of the non-aqueous phase liquid (NAPL) addition i.e. silicone oil, large branched alkane, and a plastic polymer to the aqueous phase to improve the mass transfer process especially for poorly soluble (hydrophobic) gaseous pollutants.
- Using specific microorganisms rather than mixed culture are required to be examined deeply.

REFERENCES

Akmirza, I., Carvajal, A., Muñoz, R., Lebrero, R. (2016). Interactions between BTEX Compounds during their Anoxic Degradation. *Chemical engineering transactions*, 54, 115-120.

Akmirza, I., Pascual, C., Carvajal, A., Pérez, R., Muñoz, R., Lebrero, R. (2017). Anoxic biodegradation of BTEX in a biotrickling filter. *Science of the Total Environment*, 587–588, 457–465.

Albert, J.H.J, Bosch, P.V.D., Leerdam, R.C.V., Graaff, M.D. (2013). Bioprocesses for the Removal of Volatile Sulfur Compounds from Gas Streams. *Air Pollution Prevention and Control: Bioreactors and Bioenergy*. John Wiley & Sons, Ltd. Retrieved from https://doi.org/10.1002/9781118523360.ch11.

An, T., Shungang, W., Guiying, L, Sun, L., Bin, G. (2010). Comparison of the removal of ethanethiol in twin-biotrickling filters inoculated with strain RG-1 and B350 mixed microorganisms. *Journal of Hazardous Materials.* 183, 372–380.

Anderson, G.M. (2005). Thermodynamics of Natural Systems, (2nd ed., pp. 91-100), *Cambridge University Press*, New York, USA.

APHA, AWWA and WEF (1999). Standard Methods for the Examination of Water and Wastewater, (20nd ed., Chapter 5, pp. 15-16) Washington: *American Public Health Association, American Water Works Association, Water Environment Federation*. Retrieved from, http://www.standardmethods.org/.

Bajpai, P. (2014). *Biological Odour Treatment*: NewYork,Springer.

Barbusinski, K., Kalemba, K., Kasperczyk, D., Urbaniec, K., and Kozik, V. (2017). Biological methods for odor treatment – A review. *Journal of Cleaner Production, 152*, 223-241.

Baspinar, A.B., Turker, M., Hocalar, A., Ozturk, I. (2011). Biogas desulfurization at technical scale by lithotrophic denitrification: Integration of sulfide and nitrogen removal. *Proc. Biochem*. 46, 916-922.

Benschop, A., Janssen, A., Hoksberg, M., Seriwala, R., Abry, R., Ngai, C., (2002). The Shell-Paques/THIOPAQ Gas Desulphurization Process: Successful Start Up First Commercial Unit. http://www.paques.nl.

Burgess, J.E., Parsons, S.A., Stuetz, R.M. (2001). Developments in odour control and waste gas treatment biotechnology: a review, *Biotechnol. Adv*., 19, 35-63.

Cai, J., Zheng, P., and Mahmood, Q. (2008). Effect of sulfide to nitrate ratios on the simultaneous anaerobic sulfide and nitrate removal. *Bioresource Technology, 99*(13), 5520-5527.

Callahan, B.J., McMurdie, P.J., Rosen ,M.J., Han, A.W., Johnson, A.J., Holmes, S.P. (2016). DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods, 13, 581-3. DOI: 10.1038/nmeth.3869.

Campos, J.L., Carvalho, S., Portela, R., Mosquera-Corral, A., Mendez, R. (2008). Kinetics of denitrification using sulphur compounds: Effects of S/N ratio, endogenous and exogenous compounds, *Bioresource Technology*, 99, 1293–1299.

Cano, P. I., Brito, J., Almenglo, F., Ramírez, M., Gómez, J. M., and Cantero, D. (2016). *Simultaneous Ammoniun removal from Ammoniun-Rich Water and Biogas Desulfurization.* Paper presented at the 1st International Conference on Bioenergy and Climate Change-towards as Sustainable Development. June 6–7. Soria, Spain.

Cano-Santana, P. (2015). *Optimization of nitrate dosage in anoxic biotrickling filters for biogas desulfurization*. Paper presented at the 6th International Conference on Biotechniques for air pollution control. September 2–4. Ghent University, Belgium.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K. et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*, 7, 335-336. DOI: [10.1038/nmeth.f.303]

Carvajal, A., Akmirza, I., Navia, D., Perez, R., Muñoza, R., Lebrero, R. (2018) Anoxic denitrification of BTEX: Biodegradation kinetics and pollutant interactions. *Journal of Environmental Management*. 214, 125-136.

Cayford, B.I., Dennis, P.G., Keller, J., Tyson, G.W., Bond, P.L. (2012). High-Throughput Amplicon Sequencing Reveals Distinct Communities within a Corroding Concrete Sewer System, *Appl Environ Microbiol*, 78, 7160–7162.

Chan, L.K., Weber, T.S., Morgan-Kiss, R.M., Hanson, T.E. (2008). A genomic region required for phototrophic thiosulfate oxidation in the green sulfur bacterium Chlorobiumtepidum (syn. Chlorobaculum tepidum), *Microbiology*, 154, 818–829.

Cho K.S., Hirai M., Shoda M., (1991). Degradation characteristics of hydrogen sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide by Thiobacillus thioparus DW44 isolated from peat biofilter. *Journal of Fermentation and Bioengineering*, 6, 384-389.

Christian, K. and Fre, D. T. (1998). Waste Gas Biotreatment Technology. *J. Chem. Technol. Biotechnol.*, 72, 303-319.

Demeestere, K., Dewulf, J., Witte, B.D., Langenhove, H.V. (2005). Titanium dioxide mediated heterogeneous photocatalytic degradation of gaseous dimethyl sulfide: Parameter study and reaction pathways, *Applied Catalysis B: Environmental*. 60,93- 106.

Devinny, J. S., Deshusses, M. A., and Webster, T. S. (1999). *Biofiltration for air pollution control*: CRC press.

Dogan, E.C., Türker, M., Dağaşan, L., Arslan, A. (2012) Simultaneous sulfide and nitrite removal from industrial wastewaters under denitrifying conditions, *Biotechnology and Bioprocess Engineering*, 17, 661-668.

Dolejs, P., Paclík, L., Maca, J., Pokorna, D., Zabranska, J., Bartacek, J. (2015). Effect of S/N ratio on sulfide removal by autotrophic denitrification, Applied Microbiology and Biotechnology, 99, 2383–2392.

Dumont E. (2015). H_2S removal from biogas using bioreactors: a review. *International journal of energy and environment*. 6, 479-498.

Fernandez, M., Ramirez, M., Gomez, J. M., Cantero, D. (2014). Biogas biodesulfurization in an anoxic biotrickling filter packed with open-pore polyurethane foam. *Journal of Hazardous Materials,* 264, 529–535.

Fernández, M., Ramírez, M., Pérez, R. M., Gómez, J. M., and Cantero, D. (2013a). Hydrogen sulphide removal from biogas by an anoxic biotrickling filter packed with Pall rings. *Chemical Engineering Journal, 225*, 456-463.

Fortuny, M., Gamisans, X., Deshusses, M.A., Lafuente, J., Casas, C. Gabriel, D. (2011). Operational aspects of the desulphurization process of energy gases mimics in biotrickling filters, *Water Research*, 45, 5665-5674.

Frederick, A., Brown, W.H., Campbell, M.K., Farrel, S.O. (2013). Introduction to organic and biochemistry. (7^{th} ed., pp. 106-107). *Brooks/ Cole, Cengage learning* , USA.

Garcia, L.A., Sanchez, A.G., Baquerizo, G., Jimenez, S.H., Revah S. (2010). Treatment of carbon disulfide and ethanethiol vapors in alkaline biotrickling filters using an alkaliphilic sulfo-oxidizing bacterial consortium. *J Chem. Technol*. Biotechnol. 85: 328–335.

Giri B.S., Kim KH, Pandey RA, Cho, J., Song, H., Kim, Y.S. (2014). Review of biotreatment techniques for volatile sulfur compounds with an emphasis on dimethyl sulfide, *Process Biochemistry*, 49, 543–1554.

Giri, B.S. and Pandey, R.A. (2013). Biological treatment of gaseous emissions containing dimethyl sulphide generated from pulp and paper industry, *Bioresource Technology*, 142, 420–427.

Giri, B.S., Goswamia, M., Pandeyc, R.A., Kimb, K.H. (2015). Kinetics and biofiltration of dimethyl sulfide emitted from P&P industry. *Biochemical Engineering Journal*, 102: 108–114.

Giri, B.S., Juwarkar, A., Mudliar, S.N., Pandey R.A. (2013). Treatment of waste gas containing low concentration of dimethyl sulfide in a high performance biotrickling filter, *Indian Journal of Experimental Biology*. 51, 1018-1023.

Gómez, M. E. L. (2016). *Biofiltration of volatile compound mixtures from pulp and paper industries.* Universidade da Coruña.

Gypens, N. and Borges, A.V. (2014). Increase in dimethyl sulfide (DMS) emissions due to eutrophication of coastal waters offsets their reduction due to ocean acidification, *Frontiers in Marine Science- Marine Ecosystem Ecology*, 1, 1-6.

Heijnen, J.J. (1999). Bioenergetics of microbial growth. (pp. 267-290), *In M.C. Flickinger and S.W. Drew, Eds., Bioprocess Technology; Fermentation Biocatalysis and Bioseperation*. New York: Wiley

Hiras, J., Wu, Y.W., Eichorst, S.A., Simmons, B.A., Singer, S.W. (2015). Refining the phylum Chlorobi by resolving the phylogeny and metabolic potential of the representative of a deeply branching, uncultivated lineage. *The International Society for Microbial Ecology Journal*. 10, 833–845.

HO, K.L., Chung, T.C., Lin, Y.H., Tseng, C.P. (2008). microbial populations analysis and field application of biofilter for the removal of volatile-sulfur compounds from swine wastewater treatment system. *Journal of Hazardous Materials*, 152, 580-588.

Hort, C., Gracy, S., Platel, V., and Moynault, L. (2009). Evaluation of sewage sludge and yard waste compost as a biofilter media for the removal of ammonia and volatile organic sulfur compounds (VOSCs). *Chemical Engineering Journal, 152*(1), 44-53.

Jaber, M. B., Couvert, A., Amrane, A., Le Cloirec, P. and Dumont, E. (2017). Hydrogen sulfide removal from a biogas mimic by biofiltration under anoxic conditions. *Journal of Environmental Chemical Engineering, 5*(6), 5617-5623.

Janssen, A., van den Bosch, P. L., van Leerdam, R. C., and de Graaff, M. (2013). Bioprocesses for the removal of volatile sulfur compounds from gas streams. In C. K. a. M. C. Veiga (Ed.), *Air Pollution Prevention and Control: Bioreactors and Bioenergy* (pp. 247-274). John Wiley and Sons, Inc., New York USA: John Wiley and Sons, Ltd, Chichester, UK.

Janssen, A.J.H., Ma, S.C., Lens, P., Letting, G. (1997). Performance of a Sulfide-Oxidizing Expanded-Bed Reactor Supplied with Dissolved Oxygen. *Biotechnology and Bioengineering*, 53, 32–40.

Jennings, P.A., Snoeyink, V.L., Chian, E.S.K. (1976). Theoretical model for a submerged biological filter, *Biotechnol. Bioeng*. 18, 1249-1273.

Joback, K.G. and Reid, **R.C.** (1987) Estimation of Pure-Component Properties from Group-Contributions, *Chem. Eng. Common*., 57, 233–243.

Jones, C. E., Hatcher, N. A, Weiland, R. H. (2014) Mercaptans Removal from Gases by Absorption into Amines and Caustic, Retrieved from, http://www.digitalrefining.com/article/1000903, Mercaptans_removal_from_gases_by_absorption_into_amines_and_caustic.htmL,Optimized Gas Treating, Inc., Buda.

Kachina, A., Preis, S., Kallas, J. (2006). Catalytic TiO2 oxidation of ethanethiol for environmentally begnin air pollution control of sulphur compounds, *Environ Chem Lett.*, 4, 107–110.

Kastner, J.R. and Das, K.C. (2002). Wet scrubber analysis of volatile organic compounds removal in the rendering industry. *J. Air Waste Manage. Assoc*., 52, 459–469.

Kennes, C. and Thalasso, F. (1998). Waste gas biotreatment technology. *J. Chem. Technol. Biotechnol*., 72, 303-319

Kennes, C. V., María C. (2013). *Bioreactors for waste gas treatment* (Vol. 4): Springer Science and Business Media.

Kiene, R.P., Oremland, R.S., Catena, A., Miller L.G., Capone, D.G. (1986). Metabolism of reduced methylated sulfur compounds in anaerobic sediments and by a pure culture of an estuarine methanogen. *Applied and Environmental Microbiology*, 52, 1037–45.

Kim, H., Kim, J.Y., Chung, S.J., Xie, Q., (2002). Long-term operation of a biofilter for simultaneous removal of H2S and NH3. *Journal of the Air and Waste Management Association*, 52, 1389-1398

Kim, S. and Deshusses, MA. (2005). Understanding the limits of H2S degrading biotrickling filters using a differential biotrickling filter, *Chemical Engineering Journal*, 113, 119 126.

Lebrero, R., L., Cervantes, A. T., Muñoz, R., Nery, V., Forestib, E. (2015). Biogas upgrading from vinasse digesters: a comparison between an anoxic biotrickling filter and an algal-bacterial photobioreactor. *J. Chem. Technol. Biotechnol.* 91, 2488–2495.

Li, L., Zhang, J., Lin, J., Liu, J. (2015). Biological technologies for the removal of sulphur containing compounds from waste streams: bioreactors and microbial characteristics, *World J. Microbiol. Biotechnol.*, 31, 1501–1515.

Li, X., Jiang, X., Zhou, Q., Jiang, W. (2016). Effect of S/N Ratio on the Removal of Hydrogen Sulfide from Biogas in Anoxic Bioreactors. *Appl. Biochem. Biotechnol*. 180, 930–944.

Lomans, B. P., den Camp, H. J. O., Pol, A., and Vogels, G. D. (1999). Anaerobic versus aerobic degradation of dimethyl sulfide and methanethiol in anoxic freshwater sediments. *Applied and Environmental Microbiology, 65*(2), 438-443.

Lomans, B.P., Drift, C.V.D., Pol, A. Camp, H.J.M.O.D. (2002). Microbial cycling of volatile organic sulfur compounds, *Cellular and Molecular Life Sciences, CMLS*. 59, 575-88.

Lomans, B.P., Smolders, A., Intven, L. M., Pol, A., Op, D., Van Der Drift, C. (1997). Formation of dimethyl sulfide and methanethiol in anoxic freshwater sediments. *Applied and environmental microbiology*. 63, 4741-4747.

López, M. E., Rene, E. R., Veiga, M. C., and Kennes, C. (2012). Biogas technologies and cleaning techniques. In *Environmental Chemistry for a Sustainable World* (pp. 347-377): Springer.

Luvsanjamba, M., Kumar, A., and Van Langenhove, H. (2008). Removal of dimethyl sulfide in a thermophilic membrane bioreactor. *Journal of Chemical Technology and Biotechnology: International Research in Process, Environmental and Clean Technology, 83*(9), 1218-1225.

Mahmood Q., Zheng, P., Cai, J., Hayat, Y., Hassan, M.J., Wu, D.L., Hu, B.L. (2007). Sources of sulfide in waste streams and current biotechnologies for its removal. *Journal of Zhejiang University - Science A: Applied Physics & Engineering*. 8, 1126-1140.

Mahmood, Q., Zheng, P., Hayat, Y., Islam, E., Wu, D., and Ren-Cun, J. (2008). Effect of pH on anoxic sulfide oxidizing reactor performance. *Bioresource Technology, 99*, 3291-3296.

Mesa, M.M., Macías, M., Cantero, D. (2002). Biological iron oxidation by Acidithiobacillus ferrooxidans. *Chemical and Biochemical Engineering Quarterly*, 16, 69-73.

Montebello, A. M., Fernandez, M., Almenglo, F., Ramírez, M., Cantero, D., Baeza, M., Gabriel, D. (2012) Simultaneous methylmercaptan and hydrogen sulfide removal in the desulfurization of biogas in aerobic and anoxic biotrickling filters, *Chemical Engineering Journal.* 200–202, 237–246.

Mundy, A.P. (1991). The determination of dimethyl-sulfide in beer by headspace gas chromatography-a collaborative investigation of precision, *Journal of the Institute of Brewing*, 97, 45-46.

Muñoz, R., Souza, T.S., Glittmann, L., Pérez, R., Quijano, G. (2013). Biological anoxic treatment of O2 free VOC emissions from the petrochemical industry: A proof of concept study. *Journal of Hazardous Materials*. 260, 442 450.

Nishimura, S. and Yoda, M. (1997). Removal of hydrogen sulfide from anaerobic biogas using a bio-scrubber. *Wat. Sci. Tech*., 36(6-7), 349-356.

Okpokwasili, G.C. and Nweke, C.O. (2005). Microbial growth and substrate utilization kinetics. *Journal of Biotechnology,* 5, 305-317.

Orhon, D., and N. Artan (1994). Modeling of Activated Sludge Systems. pp. 101-110. Lancaster, PA, Technomic.

Ottengraf, S.P.P. and Van den Oever. A.H.C. (1983). Kinetics of organic compound removal from waste gases with a biological filter. *Biotechnol. Bioeng*. 25, 3089-102.

Pagella, C., Silvestri, P., De Faveri, D.M. (1996). H2S treatment with Thiobacillus ferrooxidans: overall process performance and chemical step. *Trans IchemE*, 74, 123-132.

Pokorna, D., Carceller, J. M., Paclik, L., Zabranska, J. (2015). Biogas cleaning by hydrogen sulfide scrubbing and bio-oxidation of captured sulfides, *Energy & Fuels*, 29, 4058-4065

Potivichayanon, S., Pokethitiyook, P., Kruatrachue M. (2006). Hydrogen sulfide removal by a novel fixed-film bio-scrubber system. *Process Biochemistry*, 41,708-715.

Potivichayanon, S.,Pokethitiyook, P., Kruatrachue, M. (2006). Hydrogen sulfide removal by a novel fixed-film bioscrubber system, *Process Biochemistry*, 41, 708–715.

Rafson, H.J. (1998) Odor and VOC control handbook, (pp.101-102), *The McGraw-Hill*, New York.

Rappert, S., Müller, R. (2005). Microbial degradation of selected odorous substances. *Waste Management*.;25, 940-954.

Rittmann, B.E., and McCarty, P.L. (2001) Environmental Biotechnology: Principles and Applications, (pp. 130-172). *McGraw-Hill*, New York, USA.

Sawyer, C.N., McCarty, P.L., Parkin, G.F. (2003). Chemistry for Environmental Engineering and Science. 5th ed., pp. 336-338. McGraw-Hill, New York, USA.

Sedighi, M., Vahabzadeh, F., Zamir, S. M., Naderifar, A. (2013). Ethanethiol degradation by Ralstonia eutropha. *Biotechnology and Bioprocess Engineering*, 18, 827-833.

Sedighi, M., Zamir, S.M., Vahabzadeh, F. (2016). Cometabolic degradation of ethyl mercaptan by phenol-utilizing Ralstonia eutropha in suspended growth and gas-recycling trickle-bed reactor, *Journal of Environmental Management*, 165, 53-61.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., and Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12: R60. DOI: 10.1186/gb-2011-12-6-r60.

Shu, C.H. and Chen, C.K. (2009). Enhanced removal of dimethyl sulfide from a synthetic waste gas stream using a bioreactor inoculated with Microbacteriumsp. NTUT26 and Pseudomonas putida. *Journal of Industrial Microbiology & Biotechnology*. 36, 95-104.

Soreanu, G., Beland, M., Falletta, P., Edmonson, K., Seto, P. (2008). Laboratory pilot scale study for H_2S removal from biogas in an anoxic biotrickling filter. *Water Sci Technol*., 57, 201-207.

Sun, Y., Qiu, J., Chen, D., Ye, J., Chin, J. (2016). Characterization of the novel dimethyl sulfide-degrading bacterium Alcaligenes sp. SY1 and its biochemical degradation pathway. *Journal of Hazardous Materials*. 304, 543–552.

Tangerman, A. (2009) Measurement and biological significance of the volatile sulfur compounds hydrogen sulfide, methanethiol and dimethyl sulfide in various biological matrices, *J Chromatogr B Analyt Technol Biomed Life Sci*.;877, 3366-3377.

Tchobanoglous, G., Burton, F. L. and Stensel H.D. (2003) Wastewater Engineering, Treatment and Reuse, (4th ed, pp. 571-579). *Metcalf & Eddy, Inc. Mc Graw-Hill Companies*, NY, USA.

Tichy, R., Janssen, A., Grotenhuis, J.T.C., Lettinga, G., Rulkens, W.H. (1994) Possibilities for using biologically-produced sulfur particles for cultivation of Thiobacilli with respect to bioleaching processes. *Biores.Technol,* 48, 221–227.

Türker, M., Baspinar, A.B., Hocalar, A. (2012) Monitoring and control of biogas desulphurization using oxidation reduction potential under denitrifiying conditions. *Journal of Chemical Technology & Biotechnology*, 87, 682-688.

Vesilind, P.A., Morgan, S.M., Heine, L.G. (2010). Introduction to environmental engineering. (3rd ed), *Singapore: Cengage Learning*, Asia.

Visscher, P. T. and Taylor, B. F. (1993). Aerobic and anaerobic degradation of a range of alkyl sulfides by a denitrifying marine bacterium. *Appl Environ Microbiol.* 59, 4083–4089.

Wan, S., Li, G., An, T. (2011) Treatment performance of volatile organic sulfide compounds by the immobilized microorganisms of B350 group in a bio-trickling filter, *J Chem. Technol Biotechnol*., 86, 1166–1176.

Wan, S., Li, G., An, T., Guo, B. (2011). Co-treatment of single, binary and ternary mixture gas of ethanethiol, dimethyl disulfide and thioanisole in a biotrickling filter seeded with Lysinibacillussphaericus RG-1, *Journal of Hazardous Materials*, 186 ,1050–1057.

Wan, S., Li, G., An, T., Guo, B., Sun, L., Zu, L., Ren, A. (2010) Biodegradation of ethanethiol in aqueous medium by a new Lysinibacillus sphaericus strain RG-1 isolated from activated sludge. *Biodegradation*, 21, 1057–1066.

Wang, P., Peng, S., Zhu, C., Zhang, X., Teng, F. (2015) Removal of ethanethiol gas by iron oxide porous ceramsite biotrickling filter, *Journal of Chemistry*, 1-9.

Wei, Z., Liu, X., Ma, C., He, J., Huang, Q. (2013). Gas Dimethyl Sulfide Removal in Biotrickling Filtration. *Open Access Scientific Reports*. 4, 2-4.

Yavuz, B., Türker, M., Engin, G.Ö. (2007) Autotrophic removal of sulphide from industrial wastewaters using oxygen and nitrate as electron acceptors. *Environmental engineering science*, 24, 457-470.

APENDICES

APPENDIX A.1: Stoichiometry equations

APPENDIX A.2: ZymoBIOMICS® Services for Molecular biological analysis

APPENDIX A.1: Stoichiometry equations

Calculation of total reaction stoichiometry, biomass yield and free energy for ET and DMS oxidation systems.

The reaction stoichiometry of biological ET and DMS oxidation can be mathematically calculated from the biomass yield, (YX/D), which is calculated using the following equation (Dogan et al, 2012),

$$Y_{X/D} = \frac{\gamma_D}{\gamma_X} = \frac{\Delta G_{eD} - \Delta G_{eA}}{(\Delta G_{eD} - \Delta G_{eA}) + [\frac{Y_{G/X}}{\gamma_X} + (\Delta G_{eX} - \Delta G_{eD})} \qquad \text{(A. 1)}$$

where

$Y_{X/D}$: Biomass yield (C-mole biomass/C-mole electron donor)

$\triangle G_{eD}$, $\triangle G_{eA}$: Gibbs free energy for electron donor and acceptor, respectively (kJ/e mole)

$\triangle G_{eX}$: Gibbs free energy for biomass (kJ/e-mole)

γ_D, γ_x : Degree of reduction of electron donor and biomass (e-mole/C mole)

$Y_{G/X}$: Gibbs free energy released per C-mole of biomass (kJ/C-mole)

The values of $Y_{G/X}$ for electron donors are equal to 434 and 1176 kJ/C-mole of S^o and SO_4^{2-}, respectively, based on (Heijnen, 1999). When the biomass yield is known, the reaction stoichiometry can be obtained using the half-reactions (see Table A.1) as given in equation (A. 2) (Orhon et al, 1994),

$$R = R_d - Y_{X/D}R_c - (1 - Y_{X/D})R_a, \qquad \text{(A. 2)}$$

where

R : The total reaction

Rd : The half-reaction for the electron donor

Ra : The half-reaction for the electron acceptor

Rc : The half-reaction for biomass synthesis

Table A. 1: Reactions for ET and DMS oxidation and Gibbs standard free energies at pH 7 (Rittmann and McCarty, 2001; Tchobanoglous and Stensel, 2003)

Reduced–oxidized Compounds	Half-reaction	ΔG^o (kJ/e-mol)
S^o /CH_3CH_2SH*	$\frac{1}{7}CO_2 + H^+ + \frac{1}{14}S^\circ + e^- \rightarrow \frac{1}{14}CH_3CH_2SH + \frac{2}{7}H_2O$	+28.097 **
SO_4^{2-}/CH_3CH_2SH*	$\frac{1}{10}CO_2 + \frac{11}{10}H^+ + \frac{1}{20}SO_4 + e^- \rightarrow \frac{1}{20}CH_3CH_2SH + \frac{2}{5}H_2O$	+25.39**
S^o/CH_3SCH_3*	$\frac{1}{7}CO_2 + H^+ + \frac{1}{14}S^\circ + e^- \rightarrow \frac{1}{14}CH_3SCH_3 + \frac{2}{7}H_2O$	+28.94**
SO_4^{2-}/CH_3SCH_3*	$\frac{1}{10}CO_2 + \frac{11}{10}H^+ + \frac{1}{20}SO_4^{2-} + e^- \rightarrow \frac{1}{20}CH_3SCH_3 + \frac{2}{5}H_2O$	+25.99**
NO_3^-/N_2	$\frac{1}{5}NO_3 + \frac{6}{5}H^+ + e^- \rightarrow \frac{1}{10}N_2 + \frac{3}{5}H_2O$	−72.2
NO_3^- (Biomass)	$\frac{5}{28}CO_2 + \frac{1}{28}NO_3 + \frac{29}{28}H^+ + e^- \rightarrow \frac{1}{28}C_5H_7NO_2$	+10.36** *

* Half-reactions of ET and DMS were written according to the steps mentioned by (Rittman and McCarty, 2001); **Calculated using Table A.2; ***Calculated based on (Heijnen, 1999).

The standard free energies of half-reactions (S^o /CH_3CH_2SH), (SO_4^{2-}/CH_3CH_2SH), (S^o /CH_3SCH_3) and (SO_4^{2-}/CH_3SCH_3) can be calculated using values of free energy of formation for individual constituents, as listed in (Table A.2 and substitution in the equation (A.3)).

Table A. 2: Free energies of formation for various chemical species, 25°C (Rittmann and McCarty, 2001; Joback and Reid, 1987; Yavuz and Engin, 2007).

Compounds	Gibbs free energy kJ/e-mole
CH_3CH_2SH	-4.8
CH_3SCH_3	7.0
H_2O	-237.2
CO_2	-394.359
H^+	-39.87
S^o	ZERO
SO_4^{2-}	-744.63
NO_3^-	-111.34
N_2	0
$C_5H_7NO_2$ (Biomass)	-340.28

The free energy of the reaction (ΔG_r^o) is equal to the sum of the free energies of formation of the products minus the sum of the free energies of the reactants at standard state (Anderson, 2005),

$$\Delta G_r^o = \sum \Delta G_{f\,(products)}^o - \sum \Delta G_{f\,(reactants)}^o, \qquad (A.3)$$

ΔG_r^o = (S^o /CH_3CH_2SH) = +28.097 kJ/e-mole,

ΔG_r^o = (SO_4^{2-}/CH_3CH_2SH) = +25.39 kJ/e-mole.

ΔG_r^o =(S^o/CH_3SCH_3) = +28.94 kJ/e-mole

ΔG_r^o =(SO_4^{2-}/CH_3SCH_3) = +25.99 kJ/e-mole

The biomass yield and reaction stoichiometry were determined for the system where NO_3^- was used as the electron acceptor and a nitrogen source of biomass, while ET or DMS was used as the electron donor.

To calculate the reaction stoichiometry of the system where the main product was sulphur, the degree of reduction S^o to ET or DMS was γ_D = +4.7, and the Gibbs free energy for biomass ($C_5H_7NO_2$) production was $\triangle G_{eX}$ = +10.36 kJ/e-mol. The

degree of reduction of 1 C mol ($CH_{1.4}$ N $_{0.2}$ $O_{0.4}$) of biomass, γ_x = 28/5 = 5.6. Gibbs free energy, was taken for 1 C mol of biomass, $Y_{G/X}$ = 434 kJ/C-mole.

For instant calculation the yield biomass of ethanethiol when the end product was S^o.

$$Y_{X/D} = \frac{4.7}{5.6} \frac{(28.09 - (-72.2))}{(28.09 - (-72.2)) + \left[\frac{434}{5.6} + (10.36 - 28.09)\right]}$$

$$Y_{X/D} = 0.52 \ \frac{C - molbiomass}{molET}$$

$$Y_{X/D} = \ 0.52 \ \frac{C - molbiomass}{molET} . \frac{1molET}{4.7e - mol} . \frac{5.6e - molbiomass}{1C - molbiomass}$$

and was found to be

$$Y_{X/D} = 0.63 \ \frac{e - molbiomass}{e - mol}$$

The *R*d, *R*c and *R*a values were as follows for the ET and DMS oxidation systems using NO_3^- as the electron acceptor. By using equation A.2, the total reaction stoichiometry where the main product was sulphur was found to be,

For ethanethiol:

$$R: CH_3CH_2SH + 1.35NO_3 + 1.35H^+ \rightarrow S^0 + 0.31C_5H_7NO_2 + 2.57H_2O + 0.52N_2 + 0.43CO_2 . \quad (A.4)$$

For dimethyl sulfide

$$R: CH_3SCH_3 + 1.35NO_3 + 1.34H^+ \rightarrow S^0 + 0.32C_5H_7NO_2 + 2.55H_2O + 0.52N_2 + 0.42CO_2 \quad (A.5)$$

For the reaction stoichiometry of the system where the main product was SO_4^{2-}, the biomass yield was calculated according to equation A.1.

The degree SO_4^{2-} reduced to ET or DMS was γ_D = +6.7, and the Gibbs free energy for biomass ($C_5H_7NO_2$) production was $\triangle G_{eX}$= +10.36 kJ/e-mol. The degree of reduction of 1 C mol ($CH_{1.4}$ N $_{0.2}$ $O_{0.4}$) of biomass was γ_x = 28/5 = 5.6, and the Gibbs free energy was taken for 1 C mol of biomass, $Y_{G/X}$= 1176 kJ/C-mole.

For instant calculation the yield biomass of ethanethiol when the end product was SO_4^{2-}:

$$Y_{X/D} = \frac{6.7}{5.6}\frac{(25.39-(-72.2))}{(25.39-(-72.2))+\left[\frac{1176}{5.6}+(10.36-25.39)\right]}$$

$$Y_{X/D}=0.4\ \frac{C-molbiomass}{molET}$$

$$Y_{X/D}=0.4\ \frac{C-molbiomass}{molET}.\frac{1molET}{6.7e-mol}.\frac{5.6e-molbiomass}{1C-molbiomass}$$

and was found to be

$$Y_{X/D}=0.33\ \frac{e-molbiomass}{e-mol}$$

In the systems where the main product was SO_4^{2-}, only the electron donor reaction changes. Therefore, new *R*d, *R*c and *R*a values were as follows, and the total reaction stoichiometry was found to be as follows, using equation A. 2,

For ethanethiol:

R: $CH_3CH_2SH + 2.9NO_3 + 0.9H^+ \rightarrow SO_4^{2-} + 0.24C_5H_7NO_2 + 2.6H_2O + 1.33N_2 + 0.81CO_2.$ (A.6)

For dimethyl sufide

R: $CH_3SCH_3 + 2.9NO_3 + 0.89H^+ \rightarrow SO_4^{2-} + 0.24C_5H_7NO_2 + 2.59H_2O + 1.33N_2 + 0.8CO_2$ (A.7)

To obtain the free energies for the full-reaction stoichiometry for ET and DMS oxidation for each of equations A. 4, A.5, A.6 and A.7, the free energy of the reaction (ΔG_r^o) was found to be as shown in Table A.3 using equation A.3, and the values of Gibbs free energy is shown in Table A. 2.

Table A.3: Free energies of total-reaction stoichiometry for (A.4), (A.5), (A.6) and (A.7) when the main product was S° or SO_4^{2-}.

Free energy of reaction (ΔG_r^o) (kJ/e-mole)	Main product S^o	Main product SO_4^{2-}
Ethanethiol	-675.73	-1398.88
Dimethyl sulfide	-682.65	-1404.7

APPENDIX A.2: ZymoBIOMICS® Services for Molecular biological analysis

A.2.1. Materials and Methods

The samples were processed and analyzed with the ZymoBIOMICS® Service - Targeted Metagenomic Sequencing (Zymo Research, Irvine, CA).

- **DNA Extraction:** One of three different DNA extraction kits was used depending on the sample type and sample volume. In most cases, the ZymoBIOMICS® DNA Miniprep Kit (Zymo Research, Irvine, CA) was used. For low biomass samples, such as skin swabs, the ZymoBIOMICS® DNA Microprep Kit (Zymo Research, Irvine, CA) was used as it permits for a lower elution volume, resulting in more concentrated DNA samples. For a large sample volume, the ZymoBIOMICS®-96 MagBead DNA Kit (Zymo Research, Irvine, CA) was used to extract DNA using an automated platform.

- **Targeted Library Preparation:** Bacterial 16S ribosomal RNA gene targeted sequencing was performed using the Quick-16S™ NGS Library Prep Kit (Zymo Research, Irvine, CA). The bacterial 16S primers amplified the V1-V2 or V3-V4 region of the 16S rRNA gene. These primers have been custom-designed by Zymo Research to provide the best coverage of the 16S gene while maintaining high sensitivity. Fungal ITS gene targeted sequencing was performed using the Quick-16S™ NGS Library Prep Kit with custom ITS2 primers substituted for 16S primers.

The sequencing library was prepared using an innovative library preparation process in which PCR reactions were performed in real-time PCR machines to control cycles and therefore prevent limit PCR chimera formation. The final PCR products are were quantified with qPCR fluorescence readings and pooled together based on equal molarity. The final pooled library was cleaned up with the Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA), then quantified with TapeStation® and Qubit®.

- **Sequencing:** The final library was sequenced on Illumina® MiSeq™ with a v3 reagent kit (600 cycles). The sequencing was performed with >10% PhiX spike-in.

- **Bioinformatics Analysis:** Unique amplicon sequences were inferred from raw reads using the Dada2 pipeline (Callahan et al, 2016). Chimeric sequences were also removed with the Dada2 pipeline. We normally generated two bioinformatics reports that differ in the way taxonomy assignment was performed. In the report named with .zymo, taxonomy was assigned using an internal script with our own curated 16S database as reference. In the report named with greengene, taxonomy was assigned using Uclust from Qiime v.1.9.1 with Greengenes 16S database as reference. Composition visualization, alpha-diversity and beta-diversity analyses were performed with Qiime v.1.9.1 (Caporaso et al, 2010). If applicable, taxonomy that have significant abundance among different groups were identified by LEfSe (Segata et al, 2011) using default settings. Other analyses such as heatmaps, Taxa2SV_deomposer and PCoA plots were performed with internal scripts.

A.2.2. QC Data

Zymo's microbiomics workflows include sufficient quality controls. The ZymoBIOMICS® Microbial Community Standards (both cellular standard and DNA standard) were used as positive controls in each run. Negative controls (e.g. blank extraction control) were included to assess the level of bioburden carried by the wet-lab process. The following barplot shows the microbial composition of the ZymoBIOMICS® Microbial Community Standards measured in this run in Figure (A.1). Theoretical composition of the ZymoBIOMICS® Microbial Community Standards as shown in Figure (A.2).

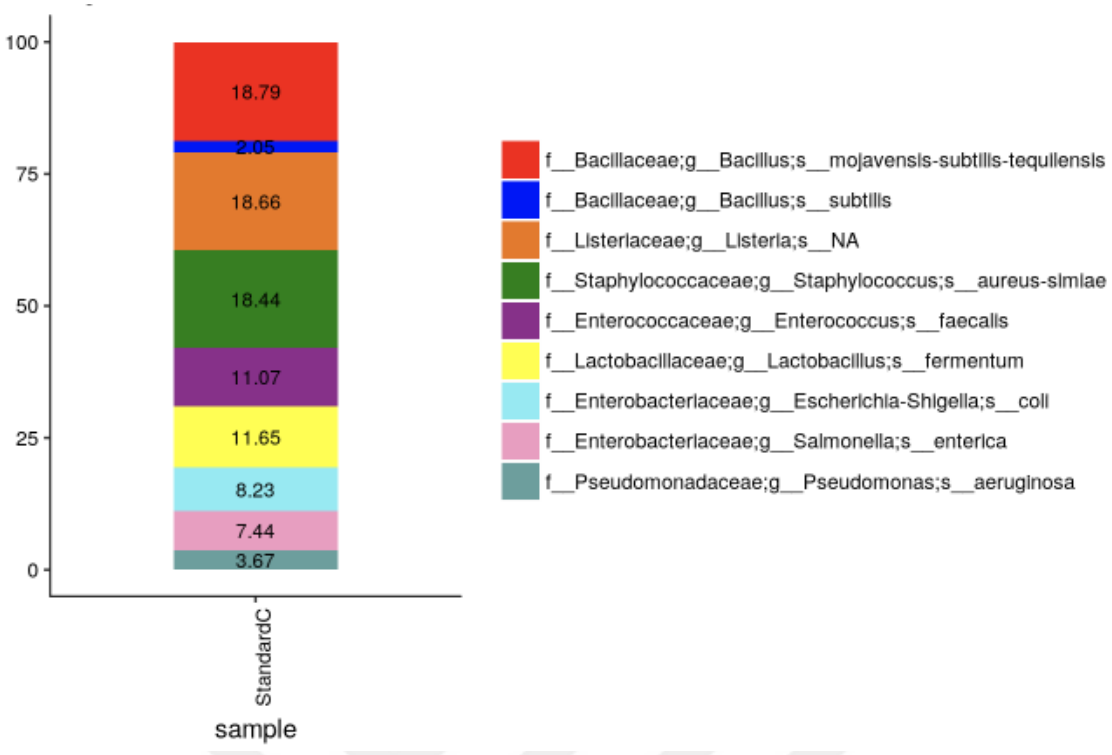

Figure A.1: Composition of microbial standards measured (species level)

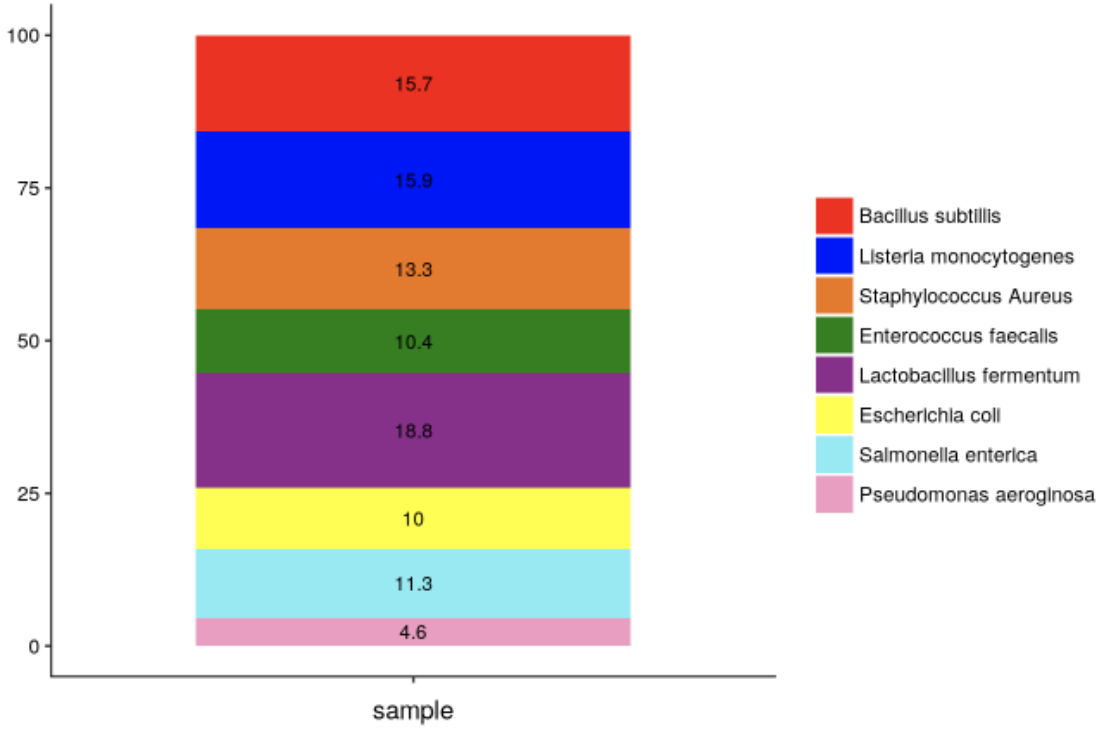

Figure A.2: Theoretical composition of the ZymoBIOMICS^{TM} Microbial community standard

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PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

- **MHEMID, R.K., ALP, K., TURKER, M., AKMIRAZ, I., SHIHAB, M.S.** 2018. Removal of Dimethyl Sulfide Via Bio-Scrubber Under Anoxic Condition, *environmental technology Journal,* DOI: 10. 1080/09593330. 2018. 1545801.
- **MHEMID, R.K., SHIHAB, M.S., ALP, K., TURKER, M., AKMIRAZ, I.** 2019. Ethanethiol gas removal in an anoxic bio-scrubber, *Journal of Environmental Management*, 233: 612–625. https://doi.org/10.1016/j.jenvman.2018.12.017

OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS:

- B.Sc. Research Project (2005): Structure analysis using Stad pro. Software.

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- **YONUS, R.M., MHEMID, R.K., QASIM, A.F.** 2011: Effect Of Local Generator On Heavy Metals Concentrations In Some Soils Of Mosul City. Conference-Environmental and Pollution Control Research Center, November 10-15, 2011 Mosul, Iraq.

- **MHEMID, R.K., YONUS, R.M., QASIM, A.F.** 2012. Effect of Air Pollutants on Rain Water Characteristics in Hammam Al-Aleel District/ Al-Erej Village South of Mosul City, *Tikrit Journal of Engineering Sciences*, 19(2), 1-12.

- **MHEMID, R.K.** 2013. Biological Treatment of Grey Water Using Sequencing BatchReactor, *Environmental Science & Technology College/university of Mosul/ Mosul*, 31(4), 539-550.

- **SHIHAB, M.S., ALP, K., TURKER, M., AKMIRAZ, I., MHEMID, R.K.,** 2018. Removal of Ethanethiol using a Biotrickling Filter with Nitrate as an Electron Acceptor, *environmental technology Journal,* DOI: 10.1080/09593330.2018.1545804