UNIVERSITI PUTRA MALAYSIA

BIOREMEDIATION OF SULFOLANE DERIVED FROM GAS INDUSTRY USING UYAMA ENZYME

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By

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THIS BOOK IS SPECIAL DEDICATED TO:

Musa Jusoh, far but always in my heart.

Zaimah Mohamad, silent but strong.

My siblings, big and happy.

And my friends,

Thank you for all of your infinite and unfading love, sacrifice, moral support and encouragement

ABSTRACT

Sulfolane (Tetrahydrothiophene 1,1-dioxide) is used as a solvent extraction of aromatic hydrocarbon from natural oil refinery streams and acid gas purification. Because of its high water solubility, sulfolane had migrated offsite and impacted the groundwater. In this study, sample of sulfolane derived from gas industry from MLNG Bintulu, Sarawak was used. This study was conducted to test the ability of Uyama enzyme to degrade sulfolane. Sulfolane was treated with combination of 10 % sulfolane + 40 % Uyama enzyme, 40 % sulfolane + 40 % Uyama enzyme and 60 % sulfolane + 40 % Uyama enzyme under aerobic conditions at 28°C ± 2°C for one week. Results obtain after 7 days showed that all of the treatments gave significant difference on the degradation of sulfolane. The effective treatments were the combination of 10 % sulfolane + 40 % Uyama enzyme. Detail study should be conducted in the future on treating sulfolane derived from industry using organic based product.

ABSTRAK

Sulfolan (Tetrahidrotiofin 1,1-dioksida) digunakan sebagai pelarut untuk menyerap aromatik hidrokarbon semasa pemprosesan minyak asli dan penulenan gas berasid. Oleh kerana ia adalah satu bahan yang sangat larut di dalam air, ia boleh meresap ke dalam tanah dan mencemari air bawah tanah. Dalam kajian ini, sampel diambil daripada MLNG Bintulu, Sarawak yang telah digunakan di dalam pemprosesan gas. Kajian ini dibuat untuk mengkaji keupayaan enzim Uyama untuk menguraikan sulfolan. Sulfolan dirawat dengan tiga kombinasi rawatan iaitu 10 % sulfolan + 40 % enzim Uyama, 40 % sulfolan + 40 % enzim Uyama dan 60 % sulfolan + 40 % enzim Uyama di dalam keadaan aerobik selama seminggu pada suhu 28 °C ± 2°C. Hasil daripada eksperimen yang dijalankan, kesemua rawatan menunjukkan perbezaan yang jelas terhadap penguraian sulfolan. Kesan rawatan yang paling berkesan didapati daripada rawatan 10 % sulfolan + 40 % enzim Uyama. Kajian yang lebih terperinci perlu dijalankan pada masa hadapan bagi merawat sulfolan yang digunakan di dalam industri menggunakan produk organik.

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APPROVAL

I certify that this research project report entitled "Biodegradation of Sulfolane Derived from Gas Industry Using Uyama Enzyme" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor Of Science Bioindustry in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS

mL - milliliter

COD - Chemical Oxygen Demand

mg/L - milligram per liter

M - Mol

LNG - Liquefied Natural Gas

°C - Degree Celsius

% - Percentage

et al. - And all

CHAPTER 1

1.0 INTRODUCTION

Crude oils and high-boiling crude oil fractions are composed of many members of relatively few homologous series of hydrocarbon. Petroleum is essentially a mixture of hydrocarbon, and even the nonhydrocarbon elements are generally present as components of complex molecules, predominantly hydrocarbon in character, but containing small amount of oxygen, sulfur, nitrogen, vanadium, nickel and chromium. The hydrocarbons present in crude petroleum are classified into three general groups, namely paraffin, naphthenes and aromatics (Nelson, 1958).

Aromatic series of hydrocarbons is chemically and physically very different from the paraffin and cycloparaffin (naphthenes) (Nelson *et al.,* 1955). Aromatic hydrocarbons contain a benzene ring, which is unsaturated but very stable and frequently behave as saturated compounds. Some typical aromatic compounds found in petroleum are benzene, toluene, ethylbenzene, ortho-xylene, meta-xylene, para-xylene, cumene and naphthalene. Therefore various physical and chemical methods are used in the refining processes (James *et al.,* 2007).

One of the chemical used for purifying the crude oil is sulfolane. Sulfolane has been used in the extraction of aromatics from hydrocarbon mixtures and in sour gas sweetening. In the extraction processes, sulfolane is used for the absorption of aromatic hydrocarbon such as benzene, toluene and naphthalene. In sweetening process, sulfolane will absorb hydrochloride acid, H_2S and other contaminants. Due

to its combination of physical and chemical properties, sulfolane also has been used in a variety of new applications including as an extraction distillation, solvent, polymer solvent and others.

Over many years of operation, these chemical have been contaminated the groundwater resulting from improper waste disposal and landfill practices (James and Miles, 2003). Because of its high water solubility, sulfolane had migrated offsite and impacted the groundwater (Fedorak and Coy, 1996).

Biodegradation of sulfolane under aquifer conditions can be very slow as reported by Gieg *et al.* (1998). Most studies have demonstrated that sulfolane biodegrades in nutrient-enriched and enzymatic aerobic microcosms from a variety of sulfolane contaminated environment samples (Chou and Swatloski, 1983).

Uyama enzyme was created after 30 years of research by Mr. Shizuo Uyama in Japan. This unique solution is a combination of 39 different kinds of enzyme extracts from fruit and plants. According to Ministry of Environment of Quebec (2002), they had certified that Uyama enzyme can be used for biological degradation of petroleum hydrocarbon in soil and groundwater. It is non poisonous and harmless to human and animals.

Hence, the objectives of this study are to determine the organic approach of treating sulfolane effluent using Uyama Enzyme and its combination of treatment.

CHAPTER 2

2.0 LITERITURE REVIEW

2.1. Sulfolane

Sulfolane, $C_4H_8O_2S$, is known under a variety of synonyms and trade names (Table 1). It was first described in the chemical literature in 1916 (Kirk-Othmer, 1999). Based on its chemical, thermal stability and solvent properties, research into commercial processes of sulfolane production commenced in approximately 1940. In 2003, sulfolane production was 1100 t/year in Japan. Global production in 2003 was approximately 13,300 t/year. Geographically, production of sulfolane was divided between sites in Americas (35-45 %), Asia (20-30 %) and Europe/ Africa (35-45 %).

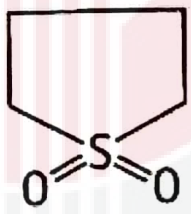

Figure 1: Structural formula of sulfolane

Table 1: Common synonyms and trade names for sulfolane

Synonyms	
Bondelane A	Sulfoxaline
Bondolane A	Sulpholane
Cyclic Tetramethylene Sulfone	Tetrahydrothiofen-1,1 Dioxide
Cyclotetramethylene Sulfone	2,3,4,5-Tetrahydrothiophene-1,1-Dioxide
Dihydrobutadiene Sulfone	Tetramethylene Sulfone
Dioxothiolan	Thiocyclopentane Dioxide
1,1-Dioxothiolan	Thiolane-1,1-Dioxide
Sulfalone	Thiophane Sulfone

2.2. Physical and Chemical Properties

Sulfolane is a colourless, highly polar, water soluble compound with exceptional chemical and thermal stability and unusual solvent properties (Noorhalieza, 1991). Published physical and chemical properties are summarized in Table 2.

Table 2: Physical and chemical properties of sulfolane

Property	Value	Units	Reference
CAS registry number	126-33-0		
Molecular formula	$C_4H_8O_2S$		Kirk-Othmer (1999)
Molecular weight	120.17	g/mol	Lide (1996)
Melting point	28.5	°C	Kirk-Othmer (1999)
Boiling point	287.3	°C	Kirk-Othmer (1999)
Specific gravity			
30 °C sulfolane/ 30 °C water	1.266	-	Kirk-Othmer (1999)
100 °C sulfolane/ 4 °C water	1.201	-	Kirk-Othmer (1999)
Flashpoint	165-175	°C	Kirk-Othmer (1999)
Density at 15 °C	1.276	g/cm^3	Kirk-Othmer (1999)
Vapour density (air = 1)	4.2	g/L	Shell (1976)
Solubility in water			
20 °C	1,266	g/L	Shell Chemicals
25 °C	379,miscible	g/L	(1994)
30 °C	Miscible	g/L	Witzaney *et al.* (1996)
PKa	12.9	-log K	Windholz *et al.* (1983)
			Coetzee (1977)

2.3. Uses of Sulfolane

The major use of sulfolane is as a solvent extraction of aromatic hydrocarbon from oil refinery streams and acid gas purification. These uses account for approximately 80 % of production. A number of minor uses (accounting for 20 % of production) include fractionation of wood tars, tall oil and other fatty acids, electronic applications and others.

2.3.1. Extractive solvent

Sulfolane is dominantly used as an extraction solvent of benzene, toluene, and xylene from aliphatic hydrocarbon mixtures (Kirk-Othmer, 1999). This process is referred to as BTX processing and was introduced in 1959 by the Shell Development Company. The BTX process is licensed by Universal Oil Products. The BTX processing involves sulfolane extraction of aromatic and some light non-aromatic hydrocarbons from hydrocarbon feed. In addition, sulfolane is used as extraction solvent for normal and branched aliphatic hydrocarbons.

In the latter process, sulfolane is used to enrich the saturation level of animal and vegetation fatty oil for use in paint, synthetic resins, food products, plastics and soaps.

Further extractive solvent applications include removing mercaptans and sulfides from sour petroleum, removing t-butylstyrene from t-buthyethylbenzene, removing

mixtures of cleso-boiling chlorosilanes and removing aromatics from kerosene, naptha and aviation fuel (Jain and Chopra, 1991).

2.3.2. Gas treatment

Sulfolane also used as solvent in the Sulfinol process to remove acid gases from natural gas. Acid gases including hydrogen sulphide (H_2S), carbon dioxide (CO_2), carbonyl sulphide (COS), carbon disulphide (CS_2) and mercaptans are physically absorbed by Sulfolane in the Sulfinol process thereby 'sweetening' the gas stream.

Other used of sulfolane in gas treatment processes including hydrogen selenide removal from gasification of coal, shale or tarsands and H_2S and SO_2 removal from gas mixtures, which differs from the Sulfinol process in that H_2S and SO_2 are converted directly to elemental sulphur (Witzaney and Fedorak, 1996).

2.3.3. Polymer solvent and plasticizer

Sulfolane is used as a solvent in a variety of polymers including polyvinyl chloride (PVC), polyacrylonitrile (PAN), polyvinylide cyanide and polysulfones. It also used to plasticize nylon, cellulose, and cellulose esters to improve flexibility and increase elongation of the polymer.

2.3.4. Other applications

Sulfolane has been used in electronic and electrical applications and as a polymerization solvent to increased polymerization rate, ease of polymer purification and improved thermal stability (Kirk-Othmer, 1999).

2.4. Water Quality Guideline for Sulfolane

In fresh water aquatic life the recommended guidelines was 50 mg/L. For source water for drinking, guidelines were calculated for children were 0.26 mg/L and adult were 0.46 mg/L (Komex International Ltd, 2001).

2.5. Biodegradation of Sulfolane

The biodegradation of sulfolane has been investigated in activated sludge system, in wastewater treatment, in laboratory microcosms studies and as part of a natural attenuation study in wetlands. Published sulfolane biodegradation rates and lag times are highly variable. Biodegradation rates range from 0 to 330 mg/L per day. Lag times range from < 1 to 220 days.

The ability to degrade sulfolane in refinery sulfolane wastewater and groundwater using activated sludge or biologically activated carbon was investigated by Bridie *et al.* (1979), Chou and Swatloski (1983), Bagnall *et al.* (1984), Juhl and Clark (1990)

and Tian (1992). The complete oxidation of sulfolane was given by Greene *et al.*, (1999) as:

$$C_4H_8O_2S + 6.5CO_2 \rightarrow 4CO_2 + 3H_2O + 2H^+ + SO_4^{2-}$$

2.6. Uyama Enzyme

Created after 30 years of study by Shizou Uyama, Uyama enzyme consists of 39 different kinds of enzyme extracts from fruit and plant such as alcohol dehydrate, lactose dehydrate, sugar glucose dehydrate, aldehydrate, amino acid and non-pathogenic microbe. It is no chemical, artificial color or scene and it is non poisonous and non-toxic enzyme to environmental, animal and human. Because it is a patterned product, there is not so much information available. According to Ministry of Environment of Quebec (2002), they had certified that Uyama enzyme can be used for biological degradation of petroleum hydrocarbon in soil and groundwater.

It also can dissolves and eliminates the major noxious odors from ammonia, trimethylamine, methyl mercaptan and hydroden sulfide as well as the odors from the decomposition of proteins and other complex odors. This enzyme stimulates bacterial activities to remove water and purify the water and improves the water quality (Greenfield Biotech 2007).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1. Sample Collection

Sulfolane was taken from LNG plant at Malaysia LNG Sdn. Bhd, Tanjung Kidurung, Bintulu, Sarawak. The sample was stored at 4°C in refrigerator.

Figure 2: Sulfolane derived from natural gas industry

3.2. Experimental Set-up

The experiments were conducted in Completely Randomized Design (CRD) with three replications.

3.2.1. Effect of Uyama enzyme concentration on Chemical Oxygen Demand (COD) and pH value of sulfolane

Uyama enzyme was diluted into 20 %, 40 % and 60% with distilled water in separated volumetric flask while sulfolane also was diluted into to 40 % in other volumetric flask with distilled water. 50 ml of 20 % Uyama enzyme then was filled in a plastic vial added with 50 ml of 40 % sulfolane. The step was repeated for other 40% and 60 % of Uyama enzyme.

3.2.2. Effect of dilution of sulfolane and aeration on COD and pH of sulfolane

Based on the result from the earlier experiment, 40 % of Uyama enzyme had been chosen for next experiment.

Sulfolane was diluted into 10 %, 40 % and 60 % concentration with distilled water. From each concentration of sulfolane, 150 ml was transferred into 1 L bottle, filled with 400 ml distilled water in three replications. 300 grams of sago compost was then transferred into each bottle and were aerated for a days under 28±2°C. On the second day, each bottle was filled with 150 ml of 40 % Uyama enzyme. The bottles were aerated for 144 hours. For the control treatment, 150 ml of 100 % sulfolane was filled in a 1 L bottle with 550 ml distilled water and 300 grams of sago compost. The bottle was left for 144 hours without aeration.

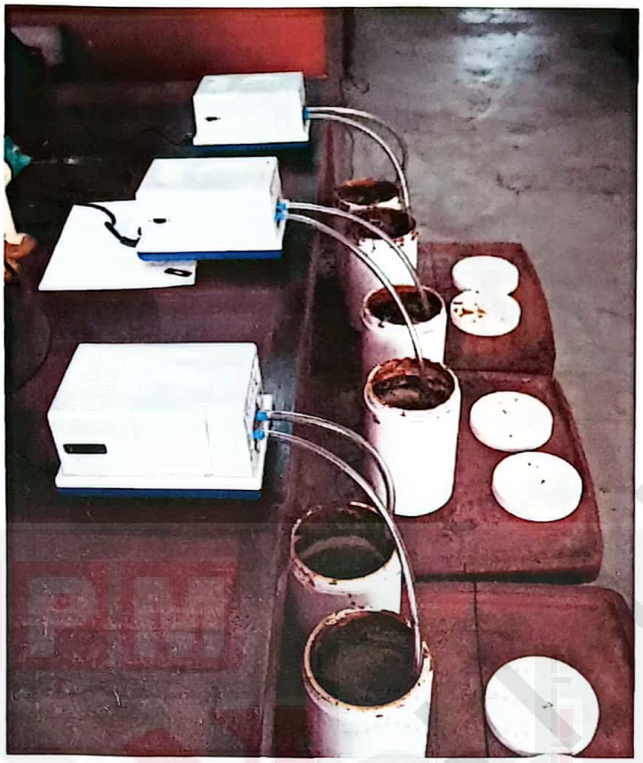

Figure 3: Experimental set-up with aerator

Figure 4: Solution of sulfolane, Uyama enzyme and sago compost

3.3. Data Collection

3.3.1. Effect of Uyama enzyme concentration on COD and pH value of sulfolane

Initial COD and pH of the solutions was taken and after 144 hours, the COD and pH value of the solution was taken again. The pH values were taken using bench top pH meter METTLER TOLEDO SevenEasy.

COD analysis was conducted by using open reflux with titration method. The experiment was carried out by transferred 10 ml of sample and 6.0 ml digestion solution into Erlenmeyer flask. Handle with care, 14 ml of sulfuric acid reagent was poured into the flask. The flask then was preheated and refluxed for 2 hours. After the flask cooled, the solution was titrated with 0.1 M Ferrous Ammonium Sulfate (FAS). The end point was a sharp color change from blue green to reddish brown. In the same manner, a blank containing the reagents and distilled water equal to the sample was refluxed and titrated.

COD calculation:

$$\text{COD as mg } O_2/L = \frac{(A - B) \times M \times 8000}{\text{ml sample}}$$

Where:

A = ml FAS used for blank

B = ml FAS used for sample

M = molarity of FAS

3.3.2. Effect of dilution of sulfolane and aeration on COD and pH of sulfolane.

The pH value and COD of every bottle were taken every 0 hour, 48 hours, 96 hours and 144 hours respectively.

3.4. Statistical Analysis

All data were subjected to Analysis of Variance (ANOVA) and treatment means separated by the use of the Tukey's test at 5% probability.

CHAPTER 4

4.0 RESULTS

4.1. Result on The Effect of Uyama Enzyme Concentration on COD and pH Value of 40 % Sulfolane

4.1.1. pH value

According to the result in Table 3, pH of the mixture of 40 % sulfolane and Uyama enzyme reduced. The mixture of 40 % sulfolane and 40 % Uyama enzyme gave the biggest reduction of pH value from 10.47 to 10.39 compared to mixture of 40 % sulfolane and 20 % Uyama enzyme (from 10.51 to 10.46) and the mixture of 40 % sulfolane and 60 % Uyama enzyme after 144 hours.

Table 3: pH value of 40 % sulfolane after 144 hours

Treatment (% Uyama enzyme)	pH value	
	Initial (0 hour)	Final (144 hours)
20	10.51	10.46
40	10.47	10.39
60	10.45	10.38

4.1.2. COD analysis

From Table 4, it was clearly showed that the treatments have significant different in reducing the COD of 40 % sulfolane. Form the result obtain, treatment of 40 % sulfolane with 40 % Uyama enzyme gave the highest reduction in COD value compared to other treatment. It reduced the COD up to 10 % of the initial COD after 144 hours while for treatment of 40 % sulfolane with 60 % Uyama enzyme and 20 % Uyama enzyme, they can only reduce 6 % and 5 % of initial COD respectively.

Based on this data, 40 % of Uyama enzyme was selected to undergo future experiment.

Table 4: COD analysis of 40 % sulfolane after 144 hours

Treatment (% Uyama Enzyme)	**COD value**		**Percentage of COD Reduction (%)**
	Initial (0 hour)	**Final (144 hours)**	
20	52850 [a]	49500 [a]	6
40	60350 [b]	53950 [b]	10
60	79300 [bc]	75000 [bc]	5

The mean with the same alphabet do not show significantly different at $P< 0.05$

4.2. Result on The Effect of Concentration of Sulfolane and Aeration on COD and pH of Sulfolane

4.2.1. pH of sulfolane berofe treatment

The pH value of sulfolane in different concentration before treatment was obtained as in Table 5. It showed that when sulfolane was diluted or added with distilled water, the pH of sulfolane will reduced.

Table 5: pH of different concentration of sulfolane before treatments

Concentration	pH value
10 % Sulfolane (10 ml sulfolane + 90 ml distilled water)	10.48
40 % Sulfolane (40 ml sulfolane + 60 ml distilled water)	10.56
60 % Sulfolane (60 ml sulfolane + 40 ml distilled water)	10.62
100 % Sulfolane (100 ml sulfolane)	10.74

4.2.2. pH of treatments after 144 hours of aeration

Upon treatment using the stated treatment method, the pH value of the treatments showed some reduction as recorded in Table 6. From statistical analysis, it was clearly shown that each treatment have significant different as compared to control in reducing the pH of sulfolane.

From the result obtained, ph of T1 (10 % sulfolane + 40 % Uyama + sago compost) decreased from 9.78 to 9.27. The treatment reduced about 5 % of the initial pH value of the solution. The pH value of T2 (40 % sulfolane + 40 % Uyama + sago compost) decreased from 10.13 to 9.58 and it reduced about 4 % of the initial pH. In T3 that is the treatment of 60 % sulfolane + 40 % Uyama + sago compost, the pH value dropped from 10.15 to 9.53 and it reduced 6 % of the pH from the initial value recorded.

Table 6: pH of treatments after 144 hours of aeration

Treatment	Duration of treatment (hours)			
	0	48	96	144
Control	10.51 ^{c}	10.49 ^{c}	10.49 ^{c}	10.46 ^{c}
T1 10 % sulfolane + 40 % Uyama + sago compost	9.78 ^{b}	9.44 ^{b}	9.29 ^{b}	9.27 ^{b}
T2 40 % sulfolane + 40 % Uyama + sago compost	10.13 ^{a}	9.88 ^{a}	9.75 ^{a}	9.68 ^{a}
T3 60 % sulfolane + 40 % Uyama + sago compost	10.15 ^{a}	9.92 ^{a}	9.82 ^{d}	9.53 ^{a}
Std Err	± 0.188	± 0.238	± 0. 251	± 0.261

The mean with the same alphabet do not show significantly different at $P< 0.05$

4.2.3. COD Analysis

Table 7 showed the result of COD analysis obtained after 144 hours of aeration. Results of this study showed that all of the treatments were significantly different ($P<0.05$) as compared to control treatment.

Table 7: COD analysis after 144 hours of aeration

Treatment	Duration of Treatment (hours)			
	0	48	96	144
Control	567000c	566000 c	565000 c	565000 c
T1 10 % sulfolane + 40 % Uyama + sago compost	258000 b	212000 b	180000 b	164000 b
T2 40 % sulfolane + 40 % Uyama + sago compost	302000 ab	314000 a	308000 a	286000 a
T3 60 % sulfolane + 40 % Uyama + sago compost	340000 a	306000 a	310000 a	315000 a

The mean with the same alphabet do not show significantly different at $P< 0.05$

From Figure 4, T2 (40 % sulfolane + 40 % Uyama + sago compost) and T3 (60 % sulfolane + 40 % Uyama + sago compost) only showed a little reduction of COD after 144 hours of aeration while for T1 (10 % sulfolane + 40 % Uyama + sago compost) showed the largest reduction in the COD value of sulfolane.

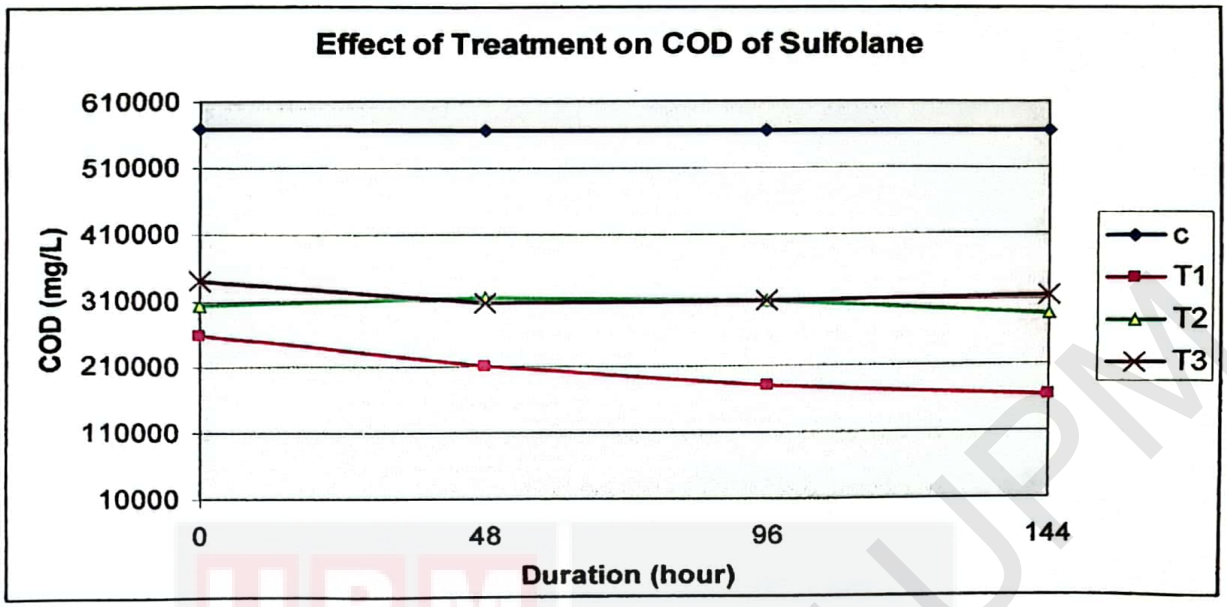

Figure 4: Effect of treatment on COD of sulfolane

CHAPTER 5

5.0 DISCUSSION

5.1. Effect of Aeration in Sulfolane Degradation

In the present study, Uyama enzyme was able to reduce pH of sulfolane under aerobic condition and comply with Chou and Swatloski (1983). According to their result, the aerobic degradation of sulfolane by an activated sludge was associated significant drop in pH. In an unbuffered system, a pH decrease from 7 to 4.5 and 5 occurred after degradation of approximately 10 % of sulfolane present. The complete oxidation of sulfolane was given by Greene *et al.* (1999) as followed:

$$C_4H_8O_2S + 6.5CO_2 \rightarrow 4CO_2 + 3H_2O + 2H^+ + SO_4^{2-}$$

Thus, pH reading reduced due to the release of H_2SO_4 a strong acid when the degradation of sulfolane occurred after 144 hours of aeration (Table 6).

Under aerobic condition, according to Greene *et al.* (1998), nearly complete sulfolane removal occurred within 2 to 4 days at 28°C and within 8 to 12 days at 8°C. Under anaerobic condition, no evidence of sulfolane biodegradation was observed at 28°C. Greene *et al.* (1998) concluded that sulfolane degradation would not be significant in anaerobic aquifers.

According to Chou and Swatloski (1983), uncontaminated samples were able to degrade sulfolane but the lag time before degradation started was typically longer and the degradation rate was slower contaminated samples. This suggests that soil bacteria exposed to sulfolane adapt over time to be able to degrade sulfolane.

5.2. COD Analysis

Analysis of COD was used in this study because COD gave more consistent and accurate results rather than 5-day Biological Oxygen Demand (BOD_5) analysis (Shelton, 1991). It is possible that under certain conditions BOD_5 may underestimate the amount of organics in wastewater and this is when differences between BOD_5 and COD based design methodologies may occur (Albertson, 1995).

COD analyses obtained from this experiment (Table 4 and 7) were high exceed the allowed level provided by the Malaysia Ministry of Environmental. According to Environmental Quality Act and Regulations 1979, the maximum level of COD for sewage and industrial effluents were only 100 mg/L. High level of COD showed that the effluent released contain a lot of substances that will effect the environment especially the aquatic ecosystem. This effluent should be treated before released into the environment.

The result of COD analysis in Table 4 and 7 showed reductions of COD and it comply with Sermin *et al.* (1996). According to the study, decrease of COD indicated that the

degradation is increased with increasing ozone dose. The study also stated that result of the decrease in COD depending on the organic fragments.

5.3. Enzymatic Treatment

In this study, Uyama enzyme was used. It contains a lot of dehydrate enzyme from plant and fruits. The dioxygenase was purified and shown to display exceptionally broad substrate specificity: it can oxidize PAHs up to 5 rings although its best substrate is naphthalene (Demaneche *et al.*, 2004).

There are some important advantages of enzymatic processes over biological systems. Enzymes can be applied to transform targeted contaminations including many of those that may resist biodegradation. This catalytic action can be carried out on or in the present of substances that are toxic to microbes (Vieth et al., 1973).

According to (Nicell et al., 1993), some enzymes can operate over restively wide temperature, pH and salinity ranges compared to cultures of microorganisms. They can also be used to treat contaminants at high and low concentrations and are not susceptible to shock loading effects associated with changes in contaminant concentrations that can often irreversible damage or metabolically inactivate microbial cells. In addition, because bacterial growth is not required to accomplish waste transformations, sludge production is reduced because no biomass is generated.

5.4. Sago compost

Although there was no analysis done for sago compost, the ability of sago waste as an adsorption for the removal of copper and lead was investigated by Quek *et al.* (1998), Maheswari *et al.* (2007) and Christine (2003). It can adsorbs copper and lead up to 700 mg/L.

According to Quek *et al.* (1998), the removal of metal ions from aqueous solution by adsorption is related to the pH of solution. The removal by sago waste decreased as the pH of the solution decreased. The critical pH values were 4.5 for lead and 5 for copper. Solution with high pH will cause precipitation of lead. Therefore for this present study, the pH solution should be decreased in the range of pH 5 to obtain optimum adsorption of copper and lead from the solution.

CHAPTER 6

6.0 CONCLUTION

Experiments were conducted in this study in order to assess the ability of Uyama enzyme to degrade sulfolane. The effect of Uyama enzyme to pH and COD of sulfolane was observed. It showed that every treatment gave significant different on the degradation of sulfolane. 10 % sulfolane + 40 % Uyama enzyme gave the best result in degrading sulfolane rather than treatment of 10 % and 40 % sulfolane. The COD obtained in the present study exceeding the limit allowed by the Ministry of Environmental. Therefore, future study should be done in longer period of time for the optimum degradation of sulfolane.

REFERENCES

Albertson, O.E. 1995 "Is $CBOD_5$ test viable for raw and settled wastewater?" *Journal of Environmental Engineering* **121**:515-520.

Bagnall, E.A., Gurvitch, M.M and Horner, R.L. 1984. Biological decomposition of cyanuric acid. UK patent Application.

Bridie, A.L., Wolff, C.J.M. and Winter, M. 1979. BOD and COD of some petrochemicals. *Water resources* **13**:627-630.

Chou, C.C. and Swatloski, R.A. 1983. Biodegradation of Sulfolane in *Refinery Wastewater*. Proceeding 27th Purdue Industrial Waste Conference. p. 559-566.

Christine J. 2003. Potensi Tanaman Sagu dan Pemanfaatannya. Nasional. Universitas Riau, Pekanbaru.

Demaneche, S., Meyer, C., Micoud, J., Louwagie, M., Willison, J.C. and Jouanneau, Y. 2004. Identification and functional analysis of two aromatic ring-hydroxylating dioxygenases from a Sphingomonas strain degrading various polycyclic aromatic hydrocarbons. *Applied and Environmental Microbiology* **70**: 6714-6725.

Fedorak, P.M. and Coy, D.L. 1996. Biodegradation of sulfolane in soil and groundwater samples from a sour gas plant. *Environmental. Tech*nology **17:** 1093-1102.

Gieg, L.M, Greene, E.A, Coy, D.L., and Pedorak, P.M. 1998. Diisopropanolamine biodegradation potential at sour gas plant sites. *Groundwater Monitering and Remediation* **18**:158-173.

Greenfield Biotech Sdn. Bhd, 2007. Environmental Bioremediation with Uyama Enzyme.

Greene, E.A, Coy, D.L. and Fedorak, P.M. 1999. Laboratory evaluations of factors affecting biodegradation of sulfolane and diisopropanolamine (DIPA). *Bioremediation Journal* **3**:299-313.

Greene, E.A., Gieg, L.M., Coy, D.L. and Fedorak, P.M. 1998. Sulfolane biodegradation potential in aquifer sediments at sour natural gas plant sites. *Water Resources* **32**:3680-3688.

Jain, A.K. and Chopra, S.J. 1991. *Proceeding of an International Conference on Petroleum Refining and Petrochemical Processing* p. 368-374.

James, H.G., Glenn, E.H. and Mark, J.K. 2007. Introduction in Petroleum Refining: Technology and Economics, 5th ed. p. 2- 36. Florida: CRC press.

James, S. and Miles, T. 2003. Soil and Water Quality Guidelines for Sulfolane and Diisopropanolamine (DIPA). Komex Risk Assessment Group.

Juhl, M.J and Clark, D.P. 1990. Thiophene-degrading *Escherichia coli* mutants possess sulfolane oxidase activity and show altered resistance to sulphur-containing antibiotics. *Application Environmental Microbial* **56**:3179-3185.

Kirk-Othmer, 1999. Encyclopedia of Chemical Technology. 4th Edition. US: John Wiley & Sons.

Komex International Ltd. 2001. Water Quality Guidelines for Sulfolane. Report prepared for British Colombia Ministry of Water, Land and Air Protection.

Mahesware, P., Venilamani, N., Madhavakrishnan, S., Syed Shabudeen, P.S., Venckatech R. and Pattabhi, S. 2007. Utilization of Sago Waste as an Adsorbent for the Removal of Copper ion fron Aqueous Solution. Kumaraguru College of Technology.

Ministry of Environment of Quebec, 2002. Clean Technologies: The Other Industrial Wastewater Purification Method.

Nicell, J.A, Al-Kassim, L. 1993. Wastewater treatment by enzyme catalyzed polymerization and precipitation. *Journal of Applied Biochemistry* **2**:414-421.

Nelson, W.L. Fombona, G.T. and Cordero, L.J. 1955. *Proceeding 4th Petroleum Congress.* p. 13-23.

Nelson, W.L. 1958. Petroleum Refinery Engineering, 4th ed. p. 232. New York: McGraw Hill Book Co.

Noorhalieza, A. 1991. Application Of Solvent Extraction: A summary, Department of Chemical Engineering, University Technology of Malaysia.

Quek. S.Y., Wase, D.A.J. and Forster C.F. 1998. The Use of Sago Waste for the Sorption of Lead and Copper. Birmingham University.

Sermin G., Osman S.G. and Hamit, B. 1996. Effects of Ozonation on COD Elimination of Substituted Aromatic Compounds in Aqueous Solution. University of Cukurova, Turkey.

Shelton, T. 1991. Interpreting Drinking Water Quality Analysis—What Do the Numbers Mean? New Jersey:Rutgers Cooperative Extension.

Tian, G. 1992. Preliminary study on the biochemical treatment of wastewater containing sulfolane. *Journal of Chemistry* **5**:54-57.

Van Der Linden, A. C. and Van Ravenswaay Claasen, J. C. 2006. Hydrophobic enzymes in hydrocarbon degradation. *Journal of Lipid* **6**:437-443.

Vieth, W.R., Venkatasubramanian, K. 1973. Enzyme engineering: The utility of supported enzyme systems. *Chemtech* **2**:677-684.

Witzaney, A.M. and Fedorak, P.M. 1996. A Review of The Characteristics, Analyses and Biodegradability of Sulfolane and Alkanolamines Used in Sour Gas Processing. Shell Canada Limited.

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Bioremediation of Sulfolane Derived from Gas Industry Using Enzyme" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.

(_____)

Nurul Sa`adah binti Musa

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