

**DETERMINATION OF VARIOUS SULFUR COMPOUNDS IN
INDIGENOUS CRUDE OILS AND THE DEVELOPMENT OF
BIOCHEMICAL DESULFURIZATION TECHNIQUES**

BY

MOHAMMAD NAEEM



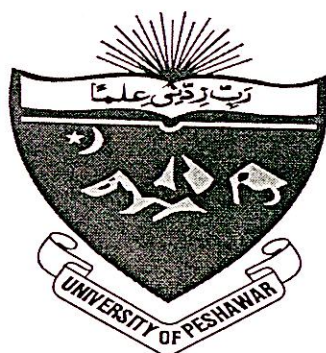
**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR PAKISTAN**

2008

**DETERMINATION OF VARIOUS SULFUR COMPOUNDS IN
INDIGENOUS CRUDE OILS AND THE DEVELOPMENT OF
BIOCHEMICAL DESULFURIZATION TECHNIQUES**

BY

MOHAMMAD NAEEM



*This Dissertation is submitted to the University of Peshawar
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Chemistry.*

**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR PAKISTAN**

2008

Dr. 13/12/2012
Controller of Exams
COP

INSTITUTE OF CHEMICAL SCIENCES UNIVERSITY OF PESHAWAR PAKISTAN

It is recommended that this dissertation prepared by Mr. Mohammad Naeem entitled
“Determination of Various Sulfur Compounds In Indigenous Crude Oils and The
Development of Biochemical Desulfurization Techniques” be accepted as fulfilling
this part of the requirements for the degree of
“DOCTOR OF PHILOSOPHY IN CHEMISTRY”



SUPERVISOR

DR. MOHAMMAD ISHAQ

Associate Professor
Institute of Chemical Sciences
University of Peshawar

INTERNAL EXAMINER



EXTERNAL EXAMINER



**PROF.DR. MOHAMMAD NISAR
DIRECTOR**

Institute of Chemical Sciences
University of Peshawar

DEDICATED

To

My Parents, Teachers,
Brother and Whole
Humanity

ACKNOWLEDGEMENTS

With the name of almighty Allah, I feel great honor and privilege to record my deep sense of gratitude to my learned and kind hearted supervisor, **Dr. Mohammad Ishaq**, Associate professor, Institute of Chemical Sciences, University of Peshawar for his valuable guidance, inspiring and ever encouraging attitude throughout this research work.

I extend my gratitude to **Dr. Imtiaz Ahamad**, Assistant professor, Institute Of Chemical Sciences, University of Peshawar for his wonder full support and positive criticism, who make me able to complete this research work.

I am much thankful to **Prof. Dr. M. Rasool Jan**, Director Institute of Chemical Sciences, University of Peshawar for providing all the research facilities during this work.

I am obliged to **Dr. M. Shakirullah, Dr. Habib-u-Rahaman and Dr. Yousaf Iqbal** for their encouragement and positive suggestions.

I am grateful to **DR. Murad, Dr. Iqbal, Mr. Nazeer khan, Mr. Thamur Ahmad, Mr. Mehtab Faisal**, senior scientific officers (SSO), PCSIR laboratories, Peshawar, especially microbiology section for providing all the available research facilities during this work.

I am obliged to the fuel laboratory staff, **Mr. Rahman-u-din, Mr. Haroon Gul, Mr. Falaknaz and Mr. Sabaz Ali khan** for their cordial cooperation.

I am also much thankful to my friends like **Mr Imram, Mr Fazal Mabood, MR Rasool Khan , Mr Noor Zada and Mr Fazal Akbar** for their cooperations.

A lot of thanks to the library staff; namely **Mr. Aziz-u-Rahaman** and **Mr. Ashfaq Khan** for providing library facilities.

I am extremely thankful to all my friends whose help and good wishes made this research work a success.

I feel obliged to mention the **directorates of colleges**, who provided an opportunity to complete my Ph.D theses.

Last but not least, I extend special thanks to my **parents, brothers** and the whole **family** who supported me financially and morally throughout my study and prayed for my success and bright future. I can never compensate their unlimited love and kindness.

Syed Mohammad Naeem

ABSTRACT

Sulfur in crude oil and in the products derived from it, is perhaps the most talked about subject in public because it has a pivotal bearing on the environment on one side and nuisance to the refiner on the other side. During combustion, sulfur and its analogues are oxidized to SO_x which are thrown into the environment with several implications. The public awareness in term of pollutants has grown up tremendously, hence the refiners have to resort to desulfurize even residues before they are put to use.

The present study was focused on to desulfurize the distillate fractions chemically as well as biologically to make such products sweet for environmentally friendly utilization and to help the refiners to remove even the least traces of sulfur and to enable them to adhere strictly with environmental codes and regulations in vogue.

Crude oil sample was collected from Attock oil refinery (Pakistan) and was fractionated into three distillate fractions i.e. light fraction; b.pt 30-100⁰C, middle fraction; b.pt 101-200⁰C and heavy fraction; b.pt 201-300⁰C. In the first set of experiments, each of the fractions was scrubbed with four different chemical reagents e.g. HCl, NaOH, Na₂HAsO₄ and NH₄VO₃, separately. The various sulphur moieties like elemental sulfur, total sulfides, aliphatic sulfides, mercaptan sulfur, hydrogen sulfides, dibenzothiophene and benzothiophene were determined in original oil fractions and then in residual oil fractions from each chemical scrubbing. The percent reduction in sulphur analogues was also calculated to decide about the suitability of each scrubbing agent understudy for removal of a particular sulphur moiety.

In the second set of experiments, oil fractions under study were cultivated with two bacterial strains cultured in coal mine water collected from Much coal, mine, Pakistan. The bacterial strains identified were *Pseudomonas putida* and *Thiobacillus thiooxidans*. The inoculations were performed for time duration of 12 hr, 24 hr, 48 hr, one week, and one month.

Sulfur and its analogues in the original and residual samples from chemical and biological treatments were investigated by using various analytical protocols. Elemental sulfur, total sulfides, aliphatic sulfides, mercaptan sulfur, & hydrogen sulfides were determined by gravimetric, potentiometric, titrimetric, and UV spectrophotometric methods. GC Mass spectrophotometry was used for identification of dibenzothiophene and benzothiophene.

From the results obtained with chemical desulphurization, it is inferred that ammonium vanadate (NH_4VO_3) is proposed to be the best desulphurization reagent among the four reagents studied which caused significant sulfur depletion.

Among the bacterial strains, *Thiobacillus thiooxidans* was found to be the best than *Pseudomonas putida* in term of sulfur removal capability. *T. thiooxidans* caused 72% sulfur depletion compared to *Ps. putida* which reduced nearly 63 % sulphur in one month inoculation time.

The percent reduction data obtained for all inoculations performed reveals that desulphurization was enroute when the time was extended beyond 12 hr even up to one month. However, from the reduction data calculated between 00 hr-12 hr, 12 hr-24 hr, 24 hr-48 hr, 48 hr-one week, and one week- one month inoculations, it is suggested that 12 hr is the optimum time for significant removal of sulphur moieties.

From the comparative study of both chemical and biochemical desulphurization attempted, it is note worthy that biodesulfurization is more easy, economical, less tedious, more environmentally friendly method than chemical desulphurization.

TABLE OF CONTENTS

S. No	Title	Page
	ACKNOWLEDGEMENT	01
	ABSTRACT	03
1.0	CHAPTER—1 INTRODUCTION	12
1.1	Crude petroleum oil	14
1.2	Naturally occurring hydrocarbon groups in crude oils	14
1.2.1	Paraffins	14
1.2.2	Aromatics	15
1.2.3	Naphthenes	15
1.3	Other hydrocarbons	16
1.3.1	Alkenes	16
1.3.2	Dienes and alkynes	16
1.4	Non hydrocarbons compounds	17
1.4.1	Sulfur compounds	17
1.4.2	Oxygen compounds	18
1.4.3	Nitrogen compounds	18
1.4.4	Trace metals	18
1.4.5	Salts	19
1.5	Major refinery products	19
1.5.1	Gasoline	19
1.5.2	Kerosene	19
1.5.3	Liquefied petroleum gas (LPG)	20
1.5.4	Distillate fuels	20
1.5.5	Residual fuels	20
1.5.6	Coke and asphalt	21
1.5.7	Solvents	21
1.5.8	Lubricants	21
1.6	Common refinery chemicals	21
1.6.1	Leaded gasoline additives	21
1.6.2	Oxygenates	21
1.6.3	Caustics	22
1.6.4	Sulfuric acid and hydrofluoric acid	22
1.7	Sulfur distribution in crude oil	22
1.7.1	Elemental sulfur	23
1.7.2	Hydrogen sulfide	24
1.7.3	Mercaptan	24
1.7.4	Sulfide	26
1.7.5	Disulfides	26
1.7.6	Non-Reactive Sulfur	27
1.8	Desulphurization of crude oil	27
1.8.1	Conventional or chemical desulphurization	27
1.8.1.1	hydrodesulphurization (HDS)	28

1.8.1.1(a)	Hydrodesulphurization catalysts	28
1.8.1.1(b)	Chemistry of catalytic hydrodesulphurization	29
1.8.1.2	Solvent extraction method	29
1.8.1.3	Absorption method	30
1.8.1.4	Caustic treating method	31
1.8.1.5	Adsorption method	32
1.9	Biodesulfurization (BDS)	33
1.9.1	Model systems for desulphurization	34
1.9.2	Sulfur-specific PASHs metabolism	34
1.9.3	Conversion to the sulfuone	35
1.9.4	Cleavage of the first C-S linkage	35
1.9.5	Liberation of inorganic sulfur	35
1.9.6	The supply of reducing equivalents	36
1.9.7	Regulation of the activity in the cell.	36
1.9.8	Developing the technology	37
1.9.9	Biodesulfurization processes	38
2.0	CHAPTER—2 EXPERIMENTAL	39
2.1	Crude oil samples	39
2.1.1	Distillation study of crude oil	39
2.1.2	Chemicals treatment	40
2.4	Biological desulphurization of crude oil sample	40
2.4.1	Collection of the microorganism (bacteria).	40
2.4.2	Confirmatory study of bacteria	41
2.4.3	Microorganism, media, and culture conditions	41
2.4.4	determination of desulphurization rate	42
2.5.	Physicothermal properties	43
2.5.1	Specific gravity	43
2.5.2	Viscosity:	43
2.5.3.	Aniline point	44
2.5.4	Pour point:	44
2.5.5	Cloud point	45
2.5.6	Flash point	45
2.5.7	Ash content	45
2.5.8	Conradson carbon residue	46
2.6.	Sulfur (reactive to copper) in cutting oil sample	47
2.6.1	Determination of total sulfur by flask combustion method	51
2.6.2	Determination of total sulfur by bomb combustion method.	55
2.6.3	Determination of elemental sulfur Spectrophotometrically	56
2.6.4	Determination of total sulfides by potentiometric method	58
2.6.5	Determination of aliphatic sulfides by Spectrophotometric method	59
2.6.6	Determination of mercaptan sulfur potentiometric method	60
2.6.7	Determination of hydrogen sulfide by volumetric method	62
2.6.8	Determination of dibenzothiophene Spectrophotometrically	63
2.6.9	Determination of benzothiophene Spectrophotometrically	64
3	CHAPTER—3 RESULTS AND DISCUSSION	65

3.1	Physicochemical properties	66
3.1.1	Chemically treated crude oil and its distillates	66
3.1.2	Biologically treated crude oil and its distillate fractions	67
3.1.2.1	Removal with <i>P. putida</i> and T.Thiooxidane	67
3.2.	Desulphurization study of Crude and its distillate fractions	74
3.2.1	Removal of sulfur (reactive to copper) in fraction distillates by volumetric method	75
3.2.2.	Removal of total sulfur in crude oil and its distillates by flask combustion method	90
3.2.3	Determination of total sulfur in petroleum products by bomb combustion method.	102
3.2.5	Determination of elemental sulfur in crude oil and its distillates Spectrophotometrically	113
3.2.6	Determination of total sulfides in crude oil and its distillates by potentiometric method.	128
3.2.7	Determination of Aliphatic sulfide in crude oil distillates Spectrophotometrically	140
3.2.8.	Determination of Mercaptan sulfur potentiometrically	155
3.2.9	Determination of hydrogen sulfide volumetrically	166
3.2.10	Determination of dibenzothiophene Spectrophotometrically	178
3.2.11.	Determination of benzothiophene Spectrophotometrically	199
3.4	CONCLUSION	222
4.0	CHAPTER –4. REFERENCES	223
	TABLES AND FIGURES.	
Table-1(a)	Physicothermal properties of virgin crude oil and its distillation fractions.	70
Table-1(b)	Physicothermal properties of the chemically treated crude oil and its distillation fractions.	71
Table-1(c)	Physicothermal properties of the pseudomonas treated crude oil and its distillation fractions.	72
Table-1(d)	Physicothermal properties of the thiobacillus thiooxidans treated crude oil and its distillation fractions	73
Figure-1	Level of copper reactive sulfur determined (%) in chemically treated samples	79
Figure-2	Reduction percentage (%) of copper reactive sulfur in chemically treated samples.	80
Figure-3	Copper reactive sulfur (%) determined in pseudomonas putida treated samples.	82
Figure-4	Reduction percentage (%) of copper reactive sulfur in pseudomonas putida treated sample as function of inoculation time.	83

Figure-5	Level of copper reactive sulfur (%) determined in thiobacillus thiooxidans treated samples as function of inoculation times.	88
Figure-6	Reduction percentage (%) of copper reactive sulfur in thiobacillus thiooxidans treated sample as function of inoculation time	89
Figure-7	Level of total sulfur determined (%) by flask combustion method in chemically treated samples.	92
Figure-8	Reduction percentage (%) of total sulfur flask by combustion method in chemically treated samples.	93
Figure-9	Level of total sulfur determined (%) by flask combustion in pseudomonas treated samples as a function of inoculation time.	96
Figure-10	Reduction percentage (%) of total sulfur by flask combustion method in pseudomonas treated sample as function of inoculation time.	97
Figure-11	Level of total sulfur determined (%) by flask combustion method in thiobacillus thiooxidans treated sample as function of inoculation time.	100
Figure-12	Reduction percentage of total sulfur flask combustion method in thiobacillus thiooxidans treated samples as function of inoculation time	101
Figure-13	Level of total sulfur determined (%) by bomb method in chemically treated samples.	104
Figure-14	Reduction percentage (%) of total sulfur bomb method in chemically treated samples.	105
Figure-15	Level of total sulfur (%) determined by bomb method in pseudomonas treated as function of inoculation time.	108
Figure-16	Reduction percentage (%) of total sulfur by bomb method in pseudomonas treated samples as a function of inoculation time.	109
Figure-17	Level of total sulfur (%) determined by bomb method in thiobacillus thiooxidans treated sample as function of inoculation times.	111
Figure-18	Reduction percentage (%) of total sulfur by bomb method in thiobacillus thiooxidans samples as function of inoculation times.	112
Figure-19	Level of elemental sulfur (ppm) in chemically treated samples.	115
Figure-20	Reduction percentage (%) of elemental sulfur in chemically treated samples	116
Figure-21	Level of elemental sulfur (ppm) in pseudomonas treated sample as function of inoculation times.	120
Figure-22	Reduction percentage (%) of elemental sulfur in pseudomonas putida treated samples as function of inoculation times.	121
Figure-23	Level of elemental sulfur (ppm) in thiobacillus thiooxidans treated sample as function of inoculation times.	125
Figure-24	Reduction percentages (%) of elemental sulfur in thiobacillus thiooxidans treated samples as function of inoculation times	126
Figure-25	Level of total sulfides (%) in chemically treated samples.	130
Figure-26	Reduction percentage (%) of total sulfides in chemically treated samples.	131
Figure-27	Level of total sulfides (%) in pseudomonas treated samples as function of inoculation times.	133

Figure-28	Red. %age of the total sulfide in pseudomonas treated samples	134
Figure-29	Level of total sulfides (%) in thiobacillus thiooxidans treated samples as function of inoculation times.	138
Figure-30	Reduction percentages (%) of total sulfides in thiobacillus thiooxidans treated samples as function of inoculation times.	139
Figure-31	Level of aliphatic sulfide (ppm) in chemically treated sample	142
Figure-32	Reduction percentage (%) of aliphatic sulfide in chemically treated samples.	143
Figure-33	Level of aliphatic sulfide (ppm) in pseudomonas treated sample as a function of inoculation time	147
Figure-34	Reduction percentage (%) of aliphatic sulfide in pseudomonas treated samples as function of inoculation times.	148
Figure-35	Level of aliphatic sulfide (ppm) in thiobacillus thiooxidans treated sample as a function of inoculation time	152
Figure-36	Reduction percentage (%) of aliphatic sulfide in thiobacillus thiooxidans treated samples as function of inoculation times.	153
Figure-37	Level of mercaptan sulfur (%) in chemically treated sample	157
Figure-38	Reduction percentage of mercaptan sulfur (%) in chemically treated samples	158
Figure-39	Level of mercaptan (%) sulfur in pseudomonas treated sample as a function of inoculation times	161
Figure-40	Reduction percentage (%) of mercaptan sulfur in pseudomonas treated samples as a function of inoculation times.	162
Figure-41	Level of mercaptan (%) sulfur in thiobacillus thiooxidans treated sample as a function of inoculation times	164
Figure-42	Reduction percentage (%) of mercaptan sulfur in thiobacillus thiooxidans treated samples as a function of inoculation times.	165
Figure-43	Level of hydrogen sulfide (%) in chemically treated sample.	168
Figure-44	Reduction percentage (%) of hydrogen sulfide in chemically treated samples.	169
Figure-45	Level of hydrogen sulfide (%) in pseudomonas treated samples as a function of inoculation times.	172
Figure-46	Reduction percentage (%) of hydrogen sulfide in pseudomonas treated samples as function of inoculation times.	173
Figure-47	Level of hydrogen sulfide (%) in thiobacillus thiooxidans treated samples as a function of inoculation times.	176
Figure-48	Reduction percentage (%) of hydrogen sulfide in thiobacillus thiooxidans treated samples as function of inoculation times	177
Figure-49	Level of dibenzothiophene (ppm) in chemically treated sample	180
Figure-50	Reduction percentage (%) of dibenzothiophene in chemically treated samples	181
Figure-51	Level of dibenzothiophene (ppm) sulfur in pseudomonas treated as a function on of inoculation times.	187

Figure-52	Reduction percentage (%) of dibenzothiophene in pseudomonas treated samples as a function of inoculation	188
Figure-53	Level of dibenzothiophene (ppm) sulfur in thiobacillus thioxidans treated as a function on of inoculation times.	194
Figure-54	Reduction percentage (%) of dibenzothiophene in thiobacillus thioxidans treated samples as a function of inoculation.	195
Figure-55	Level of benzothiophene (ppm) in chemically treated sample	201
Figure-56	Reduction percentage (%) of benzothiophene in chemically treated samples.	202
Figure-57	Level of benzothiophene (ppm) sulfur in pseudomonas treated as a function on of inoculation times	210
Figure-58	Reduction percentage (%) of benzothiophene in pseudomonas treated samples as a function of inoculation.	211
Figure-59	Level of benzothiophene (ppm) sulfur in thiobacillus thioxidans treated as a function on of inoculation times	217
Figure-60	Reduction percentage (%) of benzothiophene in thiobacillus thioxidans treated samples as a function of inoculation.	218
	SPECTRUM PROFILES	202
	Spectrum profiles (1-9). UV spectra for determination of elemental sulfur	117, 122, 127
	Spectrum profiles (10-18). UV spectra for determination of aliphatic sulfides.	144, 149, 154
	Spectrum profiles(19-27).GC-MS spectra for determination of dibenzothiophene	182-198
	Spectrum profiles (28-36) GC-MS spectra for determination of benzothiophene	203-221

INTRODUCTION

The current study was carried out with the objectives to remove various sulphur moieties from the distillate fractions obtained from Pakistani crude collected from Morgah Oil Refinery, Rawalpind, Pakistan. Morgah Oil Refinery provides gasoline, diesel, kerosene and other marketable products to premium market of all the country particularly to the northern parts. The concern of the population regarding the sulfurous emissions particularly from traffic fuels which lead to public nuisance and discomfort as well as environment degradation is growing. This study was planned to mitigate such problems by pre removal of such obnoxious constituents from the oil samples. The study will be helpful to provide practical information data, which will be helpful to the oil companies, refineries and large scale user of commercial petroleum based products. Moreover, this study will be helpful to identify fuel adulterations and in the selection of fuel having less hazardous effects on human health as well as on the environment.

The focus has been kept on the removal of sulfur and its various analogous in three different distillation fractions i.e. light fraction; b.pt 30-100⁰C, middle fraction; b.pt 101-200⁰C and heavy fraction; b.pt 201-300⁰C chemically as well microbially.

In the first set of experiments, each of the fractions was scrubbed with four different chemical reagents e.g. HCl, NaOH, Na₂HAsO₄ and NH₄VO₃, separately. The various sulphur moieties like elemental sulfur, total sulfides, aliphatic sulfides, mercaptan sulfur, hydrogen sulfides, dibenzothiophene and benzothiophene were determined in original oil fractions and then in residual oil fractions from each chemical scrubbing. The percent reduction in sulphur analogues was also calculated to decide about the suitability of each scrubbing agent under study for removal of a particular sulphur moiety.

In the second set of experiments, oil fractions under study were cultivated with two bacterial strains cultured in coal mine water collected from Much coal, mine, Pakistan. The bacterial strains identified were *pseudomonas putida* and *thiobacillus thiooxidans*. The inoculations were performed for time duration of 12 hr, 24 hr, 48 hr, one week, and one month.

Sulfur and its analogues in the original and residual samples from chemical and biological treatments were investigated by using various analytical protocols. Elemental sulfur, total sulfides, aliphatic sulfides, mercaptan sulfur, & hydrogen sulfides were determined by gravimetric, potentiometric, titrimetric, and UV spectrophotometric methods. GC Mass spectrophotometry was used for identification of dibenzothiophene and benzothiophene.

From the results obtained with chemical desulphurization, it is inferred that ammonium vendate (NH_4VO_3) is proposed to be the best desulphurization reagent among the four reagents studied which caused significant sulfur depletion.

Among the bacterial strains, *thiobacillus thiooxidans* was found to be the best than *pseudomonas putida* in term of sulfur removal capability. *T. thiooxidans* caused 72% sulfur depletion compared to *Ps. putida* which reduced nearly 63 % sulphur in one month inoculation time.

The percent reduction data obtained for all inoculations performed reveals that desulphurization was enroute when the time was extended beyond 12 hr even up to one month. However, from the reduction data calculated between 00 hr-12 hr, 12 hr-24 hr, 24 hr-48 hr, 48 hr-one week, and one week- one month inoculations, it is suggested that 12 hr is the optimum time for significant removal of sulphur moieties.

1.1. Crude petroleum

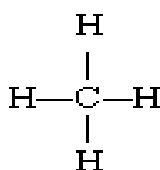
Crude oil is complex mixture of various hydrocarbon species which have different chemical composition based on the locality of the oil field. Crude oils have a variety of colors ranging from transparent clear to black and its nature is from water like to tar like solids. An ordinary crude oil is composed of 85-89% C, 10-13% H, 0.25-4% S, and less than 1% N, O, metals, and salts [1].

1.2. Naturally occurring hydrocarbon groups in crude oil

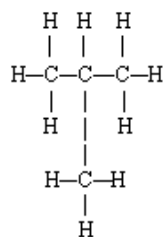
1.2.1 Paraffins

Paraffins are the saturated hydrocarbons with a formula C_nH_{2n+2} found in crude oils. In light distillate fractions of crude oils they are mostly gaseous straight chain paraffines like CH_4 , C_2H_6 , C_3H_8 and C_4H_{10} , whereas C_5H_{12} , C_6H_{14} and C_7H_{16} are the liquid paraffines in light crudes. Heavy crude oil contains branched paraffines, which increase its octane number and modify its quality during combustion in engine.

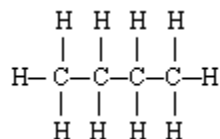
Methane (CH_4)



Isobutane (C_4H_{10})

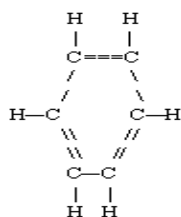
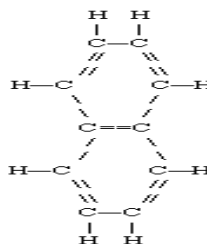


n-Butane (C_4H_{10})

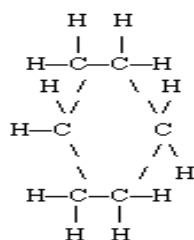
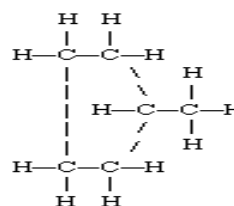


1.2.2. Aromatics

Aromatics are the ring structured unsaturated hydrocarbon molecules. Benzene is a mononuclear aromatic compound, whereas naphthalene and anthracene are the di and trinuclear hydrocarbons respectively. The more complicated aromatics are called polynuclear aromatic hydrocarbons.

Benzene (C₆H₆)**Naphthalene (C₁₀H₈)****1.2.3. Naphthenes**

These are the cyclic saturated compounds with a general formula of C_nH_{2n}, which are scattered nearly in all the distillation fractions of crude oil. Monocyclic naphthenes are composed of five carbons (Cyclopentane) or six carbons (Cyclohexane). In heavier fractions di, tri and poly cyclic naphthenic compounds are present.

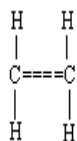
Cyclohexane (C₆H₁₂)**Methyl Cyclopentane (C₆H₁₂)****1.3. Other hydrocarbons****1.3.1. Alkenes**

Alkenes are the doubly bonded hydrocarbons with a general formula C_nH_{2n}. They are formed the catalytic or thermal cracking of untreated / unrefined crude oils. Ethylene (CH₂ = CH₂) is the simplest example of alkenes.

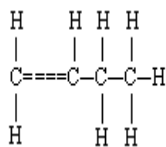
1.3.2. Dienes and alkynes

Those compounds who have two carbon- carbon double bonds ($C=C=C$) are called dienes, where as the triply bonded carbon ($C \equiv C$) are the alkynes. Alkynes and dienes have a general formula (C_nH_{2n}). These compounds are more reactive due to their unsaturated nature.

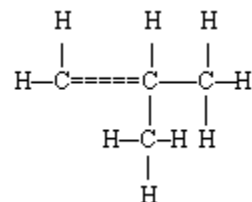
Ethylene (C_2H_4)



1-Butene (C_4H_8)



Isobutene (C_4H_8)



1.4. Nonhydrocarbon compounds

Beside the above mentioned compounds crude oil also contains a trace amount of other compounds as given below.

1.4.1 Sulfur compounds

sulfur is available in various forms like hydrogen sulfide (H_2S), mercaptans ($R-SH$), sulfides ($R-S-R$), disulfides ($R-S-S-R$), thiophenes (C_5H_5-S), benzothiophene ($C_6H_6-C_5H_5-S$) and elemental sulfur (S) etc. Hydrogen sulfide is present in light fraction where as other complicated sulfurous compounds are concentrated in heavy fractions because its maximum stability. Sulfur compounds like hydrogen sulfide (H_2S), mercaptans ($R-SH$) and elemental sulfur have bad smell and mainly causes the corrosion of refinery processing cell, which forms pyretic sulfides (FeS). On combustion Sulfurous compounds in crude oil produce environmentally unfriendly gases like H_2SO_4 and SO_2 .

sulfur content may be either remove by catalytic hydrodesulphurization or by treating with NaOH or AgNO₃, which convert to an easily removable H₂S, **sodium mercaptide** (NaSH) and silver mercaptid (AgSH) respectively as the chemical reactions have been shown below.



1.4.2. Oxygen compounds

variety of oxygen containing compound like carboxylic acid (RCOOH), ether (ROR), acetone (RCOR), phenol (PhOH) can be found in crude oil, which depends upon the nature and locality of the respective crude.

1.4.3. Nitrogen compounds

Nitrogen is present in a small amount in all the distillation fractions of crude oil, which converts to their respective oxides (NO_x) upon thermal and catalytic decomposition process. On hydrotreating it also converts to ammonia (NH₃) and cyanide (CN⁻). the chemical reaction is as under:



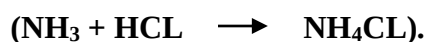
1.4.4. Trace metals

small amounts of vanadium (V), iron (Fe) and nickel (Ni) are present in heavy crude oil. Trace metals has to be remove before treatment they because their oxides like

Ni-oxide, V-oxides and Fe-oxide leaves deposits on boilers, ducts and pipes during refining process and also poisoned catalysts and deactivate it.

1.4.5. Salts

Deferent forms of inorganic salts like NaCl, MgCl₂ and CaCl₂ are present in the entrained water, they must be remove or neutralized before their input to the refinery because they deactivate the catalysts and also causes refinery plant corrosion. On hydrolyses HCl forms which combine with ammonia to produce NH₄Cl which causes fouling and refinery processing unit corrosion.



1.5. Major refinery products

1.5.1. Gasoline

It is the important component of refined crude oil, which is used as vehical fuel. It quality is based on octane number, volatility and vapor presser which evolves it uniform oxidation and to minimizes its knocking character. Its boiling point is up to 400⁰F. Special chemical additives like toluene (C₅H₆-CH₃), xylene (CH₃-C₆H₄- CH₃), TML pb(CH₃)₄ and TEL pb(CH₃- CH₂)₄ are used for enhancement of gasoline quality

1.5.2kerosene

Kerosene is a common fuel distillate, which is mainly using for cooking, space heating and aircrafts. It boiling point range is from 130 to 550 ⁰F. Kerosene quality is based on its flash point, freezing point etc.

1.5.3. Liquefied petroleum gas (LPG)

propane and butane are the two main components of liquefied petroleum gas (LPG), which is using for cooking purposes as domestic fuel and as raw material for manufacturing of perochemicals. Its quality is based on vapor pressure and purity.

1.5.4. Distillate fuels

Diesel fuels boiling point is ranging from 400 to 700 °F. Their qualities are based on controlled flash and pour points, clean burning, no deposit formation in storage tanks, and its uniform combustion efficiency depends upon cetane number..

1.5.5. Residual fuels

residual fuels are using on large scale like marine vessels, power plants, commercial buildings and industries etc. its quality is mainly based on viscosity and less amount of sulfur contents.

1.5.6. Coke and asphalt

The pure most carbon is called coke whereas asphaltene is the residual heavy fraction of the crude oil. The main applications of coke are from electrode, bets etc where as asphaltene is mainly uses for matlling of roads which is resistive to the chemicals and weather conditions.

1.5.7. Solvents

various liquid hydrocarbons like benzene, toluene, and xylene etc are present in crude oil, which dissolved the other substances and acts as solvents.

1.5.8. Lubricants

Good quality of lubricating oil can be prepared from the crude oil distillates with the contamination of special additives to improve its viscosity, viscosity index to perform in industries, vehicles etc at high temperature.

1.6. Common refinery chemicals

1.6.1. Leaded gasoline additives

Useful gasoline additives are xylene, toluene etc, which forms due to reforming process of crude oil. In the early time TML and TEL has been commonly used but they are not environmentally friendly chemicals. These additives mainly improve octane number of the gasoline.

1.6.2. Oxygenates

Mostly ethereal compounds like tertiary amyl methyl ether (TAME), methyl tertiary butyl ether (MTBE), Ethyl tertiary butyl ether (ETBE), for the reduction of carbon dioxide (CO) emission and octane number up gradation.

1.6.3. Caustics

Caustics like NaOH and AgNO_3 are added for the desalting of crude oil to reduce the chlorides concentration and to overcome the corrosion of refinery processing plant units

Caustics are added for desalting water to neutralize acids and reduce corrosion. They are also added to desalte crude in order to reduce the amount of corrosive chlorides in the tower overheads. They are used in some refinery treating processes to remove contaminants from hydrocarbon streams.

1.6.4. Sulfuric acid and hydrofluoric acid

During alkylation in the reforming process of hydrocarbons H_2SO_4 and HF are commonly using as catalysts.

1.7. Sulfur distribution in crude oil

in crude oil sulfur contents are present in various forms, which should have to be removed before the crude induction to the refinery because it causes various impacts on environment during combustion to produce (SO_x) and damages refinery metallic plant due to corrosion. The high quantity sulfured crude oil is termed as sour crude where as the low sulfured crudes are termed as sweet crudes.

Sulfur can be found in all the distillation fractions of crude oil, but its concentration is more in heavy fraction s as compared to light fractions. Various sulfur compounds has been observed in crude oils, which are given below:

- a. Elemental sulfur
- b. Hydrogen sulfide
- c. Mercaptans
- d. Sulfides
- e. Disulfides
- f. Non-Reactive Sulfur
- g. Thiophenes

1.7.1. Elemental sulfur

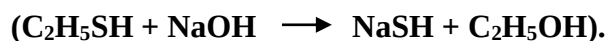
Elemental sulfur is occurring rarely in crude oil products but it form during refining and thermal cracking at high temperature. It causes corrosion to the refinery and reacts rapidly with the other compounds and creates serious problems to refiners, which can be removed with copper test easily [2].

1.7.2. Hydrogen sulfide

Hydrogen sulfide is almost present in crude oil, especially in the light fractions. It is very much corrosive in nature even if present at 1 to 2 ppm level and can be removed by treating with NaOH solution to convert H₂S to sodium sulfide. The chemical reaction is as $(\text{H}_2\text{S} + 2\text{NaOH} \longrightarrow \text{Na}_2\text{S} + 2\text{H}_2\text{O})$ [3].

1.7.3. Mercaptan

This is volatile sulfurous compound present mainly in the lower fractions of crude oil. Its general formula is R-SH and its name is thiol or thioalcohol, which has a bad odor there fore it is contaminated in Liquefied petroleum gases (LPG) and its concentration is decreasing along the boiling point of crude oil [4]. They are soluble in caustic solutions there fore can be easily remove by NaOH solution and to convert to environmentally friendly compounds as under [5-6].

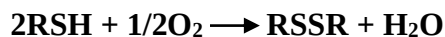


1.7.4. Sulfides

They are present in all the distillation fractions of crude oil, which are known as thioethers and their general formula is R_1-S-R_2 , which can not be removed by simple caustic treatments, therefore they have to be removed by hydrodesulphurization (HDS). Methyl ethyl sulfide ($CH_3-S-C_2H_5$) is the common example of sulfides.

1.7.5. Disulfides

These compounds are found in crude oil fractions. Disulfides are formed by the oxidation of mercaptans sulfur. Its general formula is $R_1-S-S-R_2$.



Dimethyl disulfide ($CH_3-S-S-CH_3$) is the general example disulfides.

1.7.6. Non-Reactive Sulfur

Mostly aromatic sulfurous complex compounds are examples of this category. These compounds are dominantly present in heavier fractions of crude oils. Thiophenes (C_4H_4S), Benzothiophene ($Ph-C_4H_4S$) and Dibenzothiophene ($Ph-C_4H_4S-Ph$) are the common examples of these compounds (7).

1.8. Desulfurization of crude oil

Removal of sulfur and their sulfurous analogues from the crude oil and their distillate fractions is called desulphurization. It can be categorized into two main techniques.

- a. Conventional or chemical desulfurization
- b. Biological desulphurization or biodesulfurization.

1.8.1. Conventional or chemical desulphurization

Conventional desulphurization is a particular technique, where various chemicals can be used to remove sulfurous compounds and get desulphurization. Three general methods have been used for conventional desulphurization.

- a. Catalytic hydrodesulphurization (HDS)
- b. Solvent extraction method
- c. Adsorption method.

1.8.1.1. Hydrodesulphurization (HDS)

Hydrodesulphurization involves the reaction of sulfur compounds with hydrogen in the presence of catalyst, resulting in the formation of hydrogen sulfides and hydrocarbon remnants of the original sulfur compounds. This is the most broadly applicable process for desulfurization.

(a) Hydrodesulphurization catalysts

The most commonly used desulfurization catalysts are cobalt oxide and molybdenum oxide or nickel oxide and molybdenum oxide dispersed on high surface area alumina supports [8]. Molybdenum is generally regarded as the active desulfurization component with cobalt or nickel acting as a promoter, which increase catalytic activity. The catalysts are manufactured with a metal in their oxide state. In active form they are in sulfide states, which are obtained by sulfiding the catalyst either prior to use, or with the feed during the actual use. Any catalyst, which exhibits hydrogenation, will catalyze hydrodesulfurization to some extent however the Group VIB

metals (chromium molybdenum and tungsten) are particularly active for desulfurization especially, when promoted with metals from the iron Group (iron, cobalt, nickel). **CoMo** catalysts are by far the most popular choice for desulfurization, particularly for straight run petroleum fractions. **NiMo** has often been chosen instead of **CoMo** when higher activity for polyaromatic saturation or nitrogen removal is required, or when more refractory sulfur compounds such as those in cracked stock must be desulfurized. In some applications at least, Nickel-cobalt-molybdenum (**NiCoMo**) catalyst appears to offer a useful balance of hydrotreating activity [9]. Nickel-tungsten (**NiW**) is usually chosen only when fast activity for aromatic saturation is required along with activity for nitrogen removal.

(b) Chemistry of catalytic hydrodesulfurization

As mentioned above catalytic hydrodesulfurization can accomplish more than just desulfurization. De-nitrogenation and olefin saturation can occur as well as the removal of oxygen in certain applications. Different types of sulfur compounds found in light hydrocarbon streams (boiling point below 400°F) [10]. The following reaction equations illustrate that desulfurization is accomplished by the cleavage of carbon-sulfur bonds. Carbon-hydrogen and sulfur-hydrogen bonds replace these bonds. In a similar manner nitrogen and oxygen are removed in catalytic hydrotreating by the cleavage of C-N and C-O bonds respectively.

1. $\text{H}_2\text{S} \longrightarrow \text{None- usually separated by stripping}$
2. $(\text{R-SH}) + \text{H}_2 \longrightarrow \text{R-H} + \text{H}_2\text{S}$
3. $\text{R-S-R}' + 3\text{H}_2 \longrightarrow \text{R-H} + \text{R}'\text{-H} + 2\text{H}_2\text{S}$
4. $\text{R-S-S-R}' + 3\text{H}_2 \longrightarrow \text{R-H} + \text{R}'\text{-H} + 2\text{H}_2\text{S}$
5. $\text{Cyclic sulfides (C}_5\text{H}_{10}\text{S)} + 2\text{H}_2 \longrightarrow \text{C}_5\text{H}_{12} + \text{H}_2\text{S}$

From the chemical reactions it is clear that H_2S has been the desired product in each reaction and rest of the products are purely hydrocarbons.

1.8.1.2. Solvent extraction method

Solvent extraction removes the entire sulfur-bearing molecules from hydrocarbon streams while absorption is widely employed for removing acid gases (H_2S and CO_2) from natural gas and light petroleum gases. Popular solvents for these purposes are aqueous or alcohol solutions or alkali amines and aqueous solutions of alkali carbonates. Caustic treating usually with aqueous sodium hydroxides is widely practiced to remove mercaptan and organic acids from gasoline and occasionally from light petroleum distillates. Some processes combine caustic treating with air oxidation of mercaptan to convert it to disulfides as such “sweetening” process eliminates the bad odor and corrosiveness associated with mercaptan sulfur from hydrocarbon stream. Extraction is not economically feasible for removing sulfur from heavier fraction of petroleum because of high yield losses. However during extraction of heavy gas oil with phenol to manufacture lubricating oil base stocks, sulfur compounds are concentrated in the extracts. This is observed since sulfur is pre-dominantly contained in the aromatic structures which are preferentially removed as extracts [11].

1.8.1.3. Absorption method

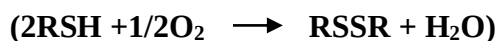
Amine treating is an absorption process that is widely used to remove H_2S , CO_2 and other acid gases from natural and light petroleum gases. The process employs an organic amine (mono-di-, triethanolamine) which will absorb H_2S at temperature up to 180°F . The process is relatively simple. The untreated gas is passed up flow through a

packed or bubbled tray tower counter to the amine solution at approximately 100°F and operating line pressure. Treated gas is removed at the top of the tower and the spent solution is regenerated by steam stripping at higher temperatures (200 to 300°F). Other processes have developed that are basically very similar to amine treating but which employ a different type of reagent. Some popular reagents are solutions of potassium, phosphate, amino acids or potassium carbonates [12], which can be alternatively used for the removal of sulfur compounds counter currently.

1.8.1.4. Caustic treating method

Caustic treating is basically an extraction process that removes organic acids, mercaptan sulfur, H_2S and phenolic sulfur compounds from petroleum fractions. The process is usually applied to light distillates, but yield losses are too great with heavier stock [13]. The major caustic treating today is the removal of mercaptan from light straight-run gasoline. Caustic treating is accomplished by contacting a 5 to 20 wt% caustic solution counter currently with untreated stream in a packed tower. The treating is carried at pressure ranging from 10 to 50 lb/in² gauges and at temperatures between 70 and 120°F. The ratio of product to caustic can be varying up to 10:1. The spent caustic can be regenerated either by steam stripping or air contacting. The regeneration condition depends on the concentration of the impurities in the caustic solution. The caustic is not 100% regenerate able. There are a number of licensed caustic treating process that have been adequately described elsewhere [14]. Many of these processes employ promoter, which are when added to the caustic either improve mercaptan removal or some other useful service. Popular solubility promoters are methanol, cresols, crystals, phenols,

phenolate, and ferrocynides, which combines caustic treating with oxidation sweetening. The caustic treating removes the H_2S and most of the mercaptan while the oxidation-sweetening step converts the mercaptan to less objectionable disulfides.



As the reaction equation indicates, oxidation sweetening does not remove the sulfur content of the stream; it only converts the sulfur to a more acceptable form. Disulfide however reduces the lead susceptibility of gasoline and for this reason processes which remove the sulfur altogether are more popular than sweetening. But still sweetening is useful process for certain applications. Some sweetening processes that have enjoyed popularity are for the doctor, copper chloride, hypochlorite and lead sulfide processes. Probably the most widely used sweetening process is inhibitor sweetening, which employs phenylenediamine type compound. This method is popular because of its low cost.

1.8.1.5. Adsorption method

Adsorption on molecular sieves is widely used to remove sulfur compound from natural gas and from light hydrocarbon streams such as propane, butane, pentane and even light gasoline. This process is effective for removing hydrogen sulfide, light mercaptan, sulfide and disulfide. It simultaneously used for drying the hydrocarbon and will also remove carbon dioxide from natural gas streams.

Molecular sieve are commonly used to selectively adsorb water and sulfur compounds from light hydrocarbon streams such as LPG, propane, butane, pentane, light olefin, and alkylation's feed. Sulfur compounds that can be removed as H_2S , sulfide,

disulfides, and mercaptan in the process [15]. The sour feed usually passes through a bed of sieves at ambient temperature. The operating pressure must be high enough to keep feed in the liquid phase. The operation is cyclic, in that the adsorption is stopped at the predetermined time before sulfur break through occurs. Sulfur and water are removing from the sieves by purging with fuel at 400 to 600°F. There are various types of molecular sieves and the choice depend on the particular application types 4A, 5A and 13X sieves are alkali metals alumino-silicates which are quite similar to some natural clays and feldspars [16]. All of these can adsorb sulfur and water, but 13X have somewhat higher capacity. In addition 13X sieves have a larger critical diameter.

Molecular sieves process has gain an importance over the years and sieves are now used for a variety of purposes in the petroleum and petrochemical industry [17-18]. Molecular sieves has an active sites, which has affinities towards sulfur containing compounds that trapped them inside petroleum products.

1.9. Biodesulfurization (BDS)

This is a particular process that removes sulfur from crude oil and their distillation fractions using a series of microorganisms. Refineries remove organic sulfur from crude oil and its fractions by hydrodesulfurization (HDS) process. HDS is a catalytic process, that convert organic sulfur to hydrogen sulfide gas by reacting crude oil fractions with hydrogen at pressures between 150-3,000 lb/in² and temperature between 290-455 °C, depending upon the feed and level of desulfurization required. Organic sulfur compounds in the lower fractions of petroleum, e.g. the gasoline range are mainly thiols, sulfides and thiophenes, which are readily removed by HDS process. However, the middle distillate fractions e.g. diesels and fuel oil range, contains significant amount of benzothiophens

(BTHs), dibenzothiophenes (DBTs) which are considerably more difficult to remove by HDS process. Among the most refractory type compounds are the DBTs with substitution adjacent to sulfur moiety compounds of this types are refer to as, sterically hindered compounds because the substitution is believed to strictly hinder, access of the sulfur atom to the catalyst surface [19]. Biodesulfurization has been studied as an alternative to HDS process for the removal of sulfur from crude oils. This is a significant way towards desulphurization of petroleum products, the high cost and inherent chemical limitations associated with HDS (chemicals desulphurization) process make alternative to this technology of interest to the petroleum industry [20-27].

1.9.1. Model systems for desulphurization

Poly aromatic sulfur hydrocarbons (PASH) are selected as suitable compounds for biodesulfurization. They have been used for many years in the development of conventional catalysts for hydrodesulphurization, because these compounds were very hard to chemical desulphurization, because structural symmetry was not fit for conventional desulphurization and bacteria can easily breakdown its C-S bond during biodesulfurization [28-29].

1.9.2. Sulfur-specific PASHs metabolism

Biodesulfurization of PASHs via (4S) metabolism pathway was introduced by Iain Campbell and Kee Rhee and it was tested on various bacterial strains by Kilbane in 1988 [30]. Sulfur moieties are insoluble in water so they are transfer in to the bacterial cell, where they convert to hydrophilic sulfurous compounds and desulfured with help of dsz genes [31].

1.9.3. Conversion to the sulfone

in this stage two step oxidation of sulfur takes place inside a cell by desulphurization gene (dszC) [32]. In this case enzyme monooxygenase flavin mononucleotide (FMN oxidoreductase) catalyzes the reaction where an electron transfers from flavin mononucleotide (FMN) to PASHs to reduce it and by self oxidized. Thus PASHs converts to PASHsO, and PASHsO₂ and FMN to FMNH and FMNH₂ in two steps [33].

1.9.4. Cleavage of the first C-S linkage

This C-S bond cleavage is, mainly performed by dszA a gene which accelerates the sulfone monooxygenase (FMNH₂: PASHsO₂ oxidoreductase activity which shifts another electron from FMNH₂ to PASHsO₂) [34].

1.9.5. Liberation of inorganic sulfur

In this step specifically catalyzed by desulfinate (dszB) genes which converts sulfurous moieties to sulfite where it reacts with hydrocarbon molecule at the last and produces oil soluble hydroxy biphenyl (HBP). Bacteria takes sulfur from an oil source and is incorporating into sulfur containing amino acid (cystene) and vitamins required for growth.

1.9.6. The supply of reducing equivalents

Reduced nicotinamide adenosine dinucleotide (NADH): FMN oxidoreductase is another enzyme which maintains the supply of reduced FMN in control equity [35].

1.9.7. Regulation of the activity in the cell

Bacteria strains are exposed to carbon rich and sulfur poor environment, which would favor cells expressing biodesulfurization genes. If the cells are grow in a sulfured environment its phenotype genes (dsz) possessing then stops presumably by repressing enzyme synthesis at the level of transcription [36].

1.9.8. Developing the technology

Development of biodesulfurization has been the desire of the refiners because sulfone like oxidized compound can be easily removed from the oil medium [37]. Monooxygenase enzyme of bacteria provide an activated oxygen on the surface of biocatalyst particularly for desulphurization, which is much specific than chemical desulphurization of crude oil. Biodesulfurization is very simple to manipulate chemical desulphurization, where one bacteria cane be replace by another for to show better performance [38].

1.9.9. Biodesulfurization processes

biodesulfurization can be applied on large industrial scale. In this technique the bacteria is introduced into a simple stirred-tank reactor where the oil may also poured into the same reactor in the presence of air and a little quantity of water. In this case the hydrophobic sulfur compounds of the oil medium may converted into hydrophilic sulfurous products and pushes it into an aqueous phase. From the reactor two streams of water water-biocatalyst-sulfur-by product stream and the oil. The oil is subjected to further refining and the oil-water-biocatalyst-sulfur-byproduct emulsion is rutun to the reactor for reused [39].

EXPERIMENTAL

2.1. Collection of samples

Sample of the crude oil was collected from Attock oil refinery Morgah Rawalpindi (Pakistan) in tin made gallons. This is situated in south east to the city of Rawalpindi. It is one of the leading refineries in Pakistan, which provides refined oil to the cities and remote areas parts of the northern parts of the country.

2.2. Distillation study of crude oil

Preliminary distillation study was performed adopting IP 24/84 standard method using Stanhope-seta limited model 11860-distillation apparatus [40]. 100 ml sample was taken in a distillation flask fitted with a thermometer (ranging up to 400°C temperature). The sample was evaporated by heating through in built heating system of the apparatus. The vaporized part of the crude was condensed in cooling tubes, and collected in a measuring graduated cylinder. When the first drop of the condensate was recovered, temperature (initial boiling point or dew point) was noted. The distillates obtained were collected in a separate bottle for further study.

Three main distillation fractions were collected, which are given below.

1. Light fraction boiling point ranges from **70°C – 200°C**.
2. Middle fraction boiling point ranges from **201°C – 300°C**.
3. Heavy fraction boiling point ranges from **301°C – 360°C**.

All the distillation fractions were safely stored separately.

2.3. Chemical treatment

The distillation fractions were scrubbed with various chemicals for the purpose of chemical desulphurization. The chemicals used are:

1. Hydrochloric acid (HCl) 1 M.
2. Sodium hydroxide (NaOH) 1%.
3. Sodium arsenate ($\text{Na}_2 \text{HAsO}_4$) 1%.
4. Ammonium vendate (NH_4VO_3) 1%.

Chemicals were put in the distillation flasks and 100 ml of each distillate was put and redistilled. The condensates from such attempt were collected separately in receiving flasks safely. The original as well as the distillates after chemical treatment were tested for different parameters and sulphur moieties.

2.4. Biological desulphurization

This is another method viewed for removal of sulfur and their respective compounds from the crude oil distillation fractions by inoculating / fermenting with two selected microbial strains i.e. *Ps.putida* and *T.Thioxidanes* .

2.4.1. Collection of the microorganism (bacteria).

Two samples were collected from the coalmines of Pakistan

1. **Much coals mine (Balochistan):** This is situated 70 Km away from Quetta city towards Sibi (Bolon pass).
2. **Tharie coal mine (NWFP).** This is 35 Km apart from Hango city towards Doaba.

Both of the samples were collected from the different spots of coalmines in polyethylene bottles. Water samples were incubated for a period of 24 hr in an incubator at 37°C. After incubation, the samples were tested with microscope for bacterial existence.

2.4.2. Confirmatory study of bacteria

Bacterial presence was confirmed by preparing more than 100 slides. Firstly, the slides were cleaned carefully, and then dried in an oven. Small spots were made from the coal mine water sample right on the mid position of the slides. The spots were fixed with the burner flame to stick the microorganism firmly. The slides were rinsed with colored ink to make the spot clear, washed and then dried again. Then, the slides were studied under microscope. Various types of microorganisms were seen under microscope and finely from their structural and physiological study, two of bacteria *Thiobacillus thiooxidans* and *pseudomonas putida* capable of desulfurizing distillates were isolated by physiological and their morphological identification. After conforming their existence, their mobility was also checked by taking a small amount of coal mine water sample in a cleaned Petri dish and added clear distilled water, it was found that they were freely moving which conformed that bacteria were in living state.

2.4.3. Microorganism, media, and culture conditions

Strains of *Thiobacillus thiooxidans* and *pseudomonas putida* were cultured according to the procedure reported else where [41-42]. Each strain was grown in 1L fermentor by using basic salts middle with glucose (2%) as the carbon source and sulfur (2%) as an energy source. Once the cultured bacteria reached to an optical density at

600 nm (OD_{600}), cells were harvested by centrifugation at $16,000 \times g$ and stored at 25°C . Culture densities were determined by diluting the suspension with distilled water and measuring the optical densities (OD) in a Beckman DU 650 spectrophotometer with a 1-cm-path-length cuvette. The cell content of the harvested paste ranged from 35-40% solids (grams dry cell weight [DCW] per gram and wet cell weight [WCW]). DCW, WCW, and percent solids were determined by placing a sample of paste on a tarred glass fiber pad, weighing the pad (WCW), and then drying it to a constant weight (DCW) in a CEM 9000 microwave oven with balance (CEM Corp.). A calibration curve was generated for correlating OD to DCW.

2.4.4. Determination of desulphurization rate

The standard procedure for desulphurization experiments was to first suspend the cell paste in 150 ml phosphate buffer, pH 5.5, with 2% glucose at a concentration of $16.7 \text{ g DCW liter}^{-1}$. Next, 750 ml of the suspension was transferred to a 2-liter stirred vessel (Applikon Inc.) equipped with temperature, agitation, pH control, and dissolved oxygen (DO) monitoring system and then allowed to mix for about 60 min at 37°C . Air flow was set to 400 ml min^{-1} to maintain DO levels above 40% of air saturation. The pH was controlled through the automatic addition of 10% NaOH in water, as needed. To initiate desulfurization, 25 ml of oil sample of known sulfur concentration was added and the contents except sulfur were mixed at 1,000 rpm [43-44].

Microbial activity was monitored by measuring sulfur concentration of the known solutions. This is measured by taking the samples with a syringe and were analyzed for sulfur compounds by adopting different IP standard techniques, and compared the data

obtained from the virgin and bacterially treated samples. Some of the sulfur contents were removed from the bacterial treated samples, which caused desulphurization of the crude oil sample.

2.5. Physicochemical properties

2.5.1. Specific gravity

Specific gravity was determined according to IP 160/87-method [45] using Stanhope-seta-limited hydrometer. A 100 mL aliquot of sample was taken in a glass cylinder. A thermometer was immersed in the sample for recording the temperature. Hydrometer was then allowed to float in the oil sample and the reading of hydrometer was noted from the graduated vertical stem. Specific gravity at 60/60 °F was then found out using ASTM / IP petroleum measurement tables (IP 1250/84). API gravity was calculated using the given formula.

$$\text{API gravity} = \frac{141.5}{\text{Sp.gr.60/60 } ^\circ\text{F}} - 131.5$$

2.5.2. Viscosity

Kinematic viscosity of crude oil was determined by Stanhope-seta kinematics viscometer adopting IP 71/ 87 method [46].

Very small amount of the sample (0.3 ml) was introduced in to the glass viscometer using a micro syringe. Temperature of bath was maintained at 100 °F. The sample was allowed to flow between the two marks encircled on the glass viscometer and the time of flow was determined with the help of timer built in the instrument. Calibration

constants were read out from the viscometer, which has a specific constant value for every viscometer. Kinematic viscosity was calculated by the formula:

$$\text{Kinematic viscosity} = C \times t.$$

Where C is the calibration constants and t is the time of meniscus flow between the two marks.

2.5.3. Aniline point

Aniline point of the crude oil sample was determined by Stanhope-seta limited aniline point apparatus adopting IP 2/78 standard method. [47].

The apparatus was thoroughly cleaned and dried in an oven. 10 mL portion of crude oil distillation fractions sample and 10 mL portion of aniline were taken in the test tube with a pipette. An agitator and a thermometer were fitted inside the tube with the help of cork. The test tube was placed in the outer jacket tube containing paraffin oil. Aniline was not miscible with sample at room temperate. The outer jacket tube was heated using a burner at a rate of 1⁰C per minute temperature rise until the sample becomes completely miscible with aniline. The temperature was thus recorded as aniline point of the sample.

2.5.4. Pour point

Pour point was determined using Stanhope-seta limited pour point apparatus adopting IP 15/67 standard method [48]. The sample of crude oil was placed in the glass jar having flat bottom up to the level mark on the tube. The jar was tightly closed with a cork fixing a thermometer with temperature measuring range of –36 to 120 ⁰F. The jar

was placed in the jacket, which was turned immersed in freezing mixture (ice +NaCl mixture). The jar was periodically brought and checked until the oil did not show any movement when the jars were horizontally tilted for 5 seconds. This temperature was recorded. To this temperature 3⁰C were added and were expressed as pour point of the sample.

2.5.5. Cloud point

For cloud point determination, the same pour point apparatus was used. The procedure adopted for cloud point determination was the same as for pour point. The temperature at which the haziness observed in the side of the jar was reported as cloud point of the sample.

2.5.6. Flash point

Flash point was determined using Pensky-Martins closed cup flash point tester adopting IP 34/87 standard method [49]. Before performing the test, all parts of the tester were thoroughly cleaned with petroleum spirit and dried. The sample cup was then filled with the sample up to the mark. Heat was supplied at the rate of 6⁰C / minute temperature rise.

The test flame was applied after each 1 ⁰C rise in the temperature until distinct flame was observed inside the cup. The temperature was recorded as flash point.

2.5.7. Ash content

Ash content of crude oil distillation fractions sample was determined adopting IP 4/81 standard method [50]. A porcelain evaporating dish of 120 ml capacity was washed, cleaned and dried at 800 ⁰C for 10 minute. Crucible was cooled to room temperature in

the desiccator and weighted. China dish was heated and cooled several time until constant weight was attained.

Crude oil sample was taken in the China dish and was re-weighted. Weighing was performed with the help of sortorius balance. The dish was heated and the sample was ignited with the flame. Temperature was maintained in such a way that sample burnt with a uniform and moderate rate leaving behind only ash and carbon residues.

The residue was heated in the muffle furnace at 800 °C until all the carbonaceous material disappeared. The dish was cooled in the desiccator to room temperature and weighted. The dish was reheated upto 800 °C for 30 minute, cooled and weighed. Heating was repeated until constant weighing was attained.

Ash was calculated as a percentage of the original sample, as follows.

$$\text{Ash percent} = w/W \times 100$$

w = weight of ash in grams.

W = weight of sample in grams.

2.5.8. Conradson carbon residue

Carbon residue was determined adopting IP 13/82 standard method [51].

A known amount of sample was taken in a silica crucible. This crucible was placed in the center of the Skidmore crucible. The Skidmore crucible was then placed in the center of iron crucible. A little sand was placed beneath the Skidmore crucible. Both the crucible was covered loosely.

The crucible was placed on the nichrome wire triangle. The sheet iron hood was then placed on the crucibles. The sample was heated strongly. When smoke appeared above the chimney, the burner was tilted strongly for vapors to catch fire. When all the

vapors burnt and caused the smoke disappeared, the burner was removed and the apparatus was allowed to cool. The Skidmore crucible was cooled in a desiccator and weighed. Carbon residue was calculated as percentage of that of the original sample.

$$\text{Carbon residue, percent} = w / W \times 100$$

w = weight of carbon residue in grams.

W = weight of sample in grams.

2.6: Determination of Various sulphur analogues

2.6.1. Sulfur (reactive to copper) ASTM IP [242/83]

Apparatus.

1. Oil bat-capable of being controlled at the required temperature $\pm 1^{\circ}\text{C}$.
2. Test tubes- 150 mm x 12.5-mm aprox.
3. Filter paper- watman No. 42.
4. Glass stirring rods.
5. Conical flasks-500 ml.
6. Distillation apparatus-consisting of a 1-liter, long neck digestion flask fitted with a nitrogen inlet, 250ml- dropping funnel and still – head.fitted to the still-head is a leibig water condenser (overall length approx. 490 mm) and a 250 ml receiver with a gas out let. Three 250-ml dreschel bottles are connected in series to the gas out let.
7. Digestion flask-1-liter round bottom long necked flask. Necked length 145 mm overall length 310 mm; B24 socket.

8. Multiple adapter-3 necks –2 parallel with B19 socket 1 angled at 45° with B 19 socket; B 24 cone.
9. Dropping funnel- 250-ml capacity with B 19 socket and cone.
10. Splash head –B19cones.
11. Liebig condenser – over all length 490 mm; B 19 socket; B 24 cone.
12. Conical flasks-250 ml; height 140 mm B 24 sockets.
13. Three dreschel bottles- 250 ml; sockets.
14. Three dreschel bottles heads –B 24 cones and sintered ends.
15. 2 Cone Screw-thread Adopters – B 19 cones to accept 6 to 7 mm glasses tubing through Screw cap.

Materials.

1. Copper-bronze powder –96-99% coppers and free from sulfur.
2. Toluene- sulfur free and conforming to the current IP specification.
3. Acetone.
4. Zinc acetate solution-approx. 0.01M.
5. Antimony trichloride solution (20 g SbCl_3 /liter) –prepared by dissolving 2g SbCl_3 in 50 ml conc. hydrochloric acid and adding 50 ml distilled water.
6. Stannous chloride-solid.
7. Hydrobromic acid-48%.

8. Ethylene Diamine Tetra-acetic Acid EDTA (Disodium salt) solution 0.01 M accurately standardized.
9. Erichrome Black T Indicator – prepared by dissolving 0.2 g of Erichrome Black Tin 15 ml of tri-ethanolamine and 5 ml ethanol.
11. Buffer solution (pH=11)-prepared by dissolving 17.5 g ammonium chloride in 142 ml 0.88 % ammonia and making up to 250 ml with distilled water.
12. Nitrogen-cylinder.

Procedure:

0.5 g of the oil and 1.0 g of the copper bronze powder was weighed in to the test tube and stirred well with a glass rod immersed the test tube in to a depth of at least 75-mm in the oil bath maintained at the correct temperature. After two hours, the test tube was removed and cooled to room temperature.

Using toluene, the copper bronze powder and oil mixture was transferred to a Wattman No.42 filter paper. The test tube and glass rod were thoroughly washed with toluene to remove all the copper bronze powder and the washings were added to the filter paper. The filter paper was washed with toluene and acetone until the washing freed from oil.

The filter paper and copper bronze powder were transferred to the digestion flask of the distillation apparatus and 125 ml distilled water was added. 50 ml of the 0.01M zinc acetate solution was put in to the first dreschel bottle and 25 ml into each of the other two. To the distillation apparatus, 75 ml Hydrobromic acid (HBr) (48%) was added. A crystal of stannous (SnCl_2) chloride was dissolved in this acid and then 2 ml of antimony trichloride (SbCl_3) was added. The nitrogen supply connection was made through side

arm to the distillation apparatus. The solution from the separating funnel was run in to the distillation flask and the nitrogen gas through an apparatus at approximately 5 ml per min was purged. The flask and contents were heated to boiling and the boiling was continued for approximately 30 min.

Dreschel bottles were disconnected and combine the contents in a 500 ml conical flask. Each dreschel bottle was washed with three 10 ml portion of distilled water and added the washing to the conical flask. A known amount of the standard 0.01M EDTA solution (in excess of 100 ml) was added followed by 2 ml of buffer solution and 2 – 3 drops of indicator solution. The solution was then titrated against the 0.01M zinc acetate solution. The end point was being indicated by the change in color from blue to wine red.

Blank titration was carried out as above using the same volume of 0.01M zinc acetate solution as charged in to the drushel bottles [52].

Calculation:

Each of the determinations of the percentage of sulfur reactive to copper was Calculate in the sample as fallow.

$$\text{Reactive sulfur, \% mass} = \frac{32 M \times (A-B)}{10 (100 + B) W}$$

Where

A = volume of 0.01 M zinc acetate solution used for the titration of the sample, ml

B = volume of 0.01 M zinc acetate solution used for the titration of blank

M = Morality of EDTA solution;

W = weight of the oil sample taken, g;

X =volume of standard EDTA solution added, ml

2.6.1. DETERMINATION OF TOTAL SULFUR BY FLASK COMBUSTION METHOD (ASTM D 1226 -IP 107)

Apparatus:

1. Combustion flask: made of borosilicate glass of 500 –1000 ml capacity and provided with a conical B24 ground glass stopper with a fused-in platinum wire. The platinum carries U-shaped platinum gauze of a platinum gauze basket.
2. Safety shield- of suitable design to protect the operator while carrying out manipulation operation during the combustion stage.
3. Teflon sleeves – to fit B24 joint.
4. Syringe: - 0.05-ml capacity with 0.01-ml divisions.
5. Lighter – any, suitable of small flame fed with sulfur free fuel. The standard wick-fed burner and flask specified in ASTM D 1226 -IP 107 and filled with alcohol, is suitable.
6. Microburette assembly –10-ml burette with 0.02 ml or finer graduation and supply bottle of borosilicate glass. A piston type burette is recommended
7. Photoelectric colorimeter -equipped with a 520 nm interference filter or an equivalent means of isolating a spectral bands at this wavelength, a stirrer and an aperture for the titration assembly. This is required only for sulfur contents of less than 0.4 % wt.
8. Daylight blue lamp- 100 watts.
9. Magnetic stirrer –optional.

Reagent and materials:

1. Purity of reagents; Analytical grade chemical shall be used in all tests unless otherwise indicated. It is intended that all should conform to established or recognized specifications for analytical reagent grade chemicals, where such specifications are available. Other may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use with the accuracy of determinations.
2. Purity of water- unless otherwise indicated, reference to water shall be understood to mean distilled water or water of equal purity.
3. Filter paper – Ash free filter paper (e.g. Watman No 40). Strips were prepared of the specific size and shape. Then the strip was folded along the dotted line to an M-shaped and stored in an open bottle in the vicinity of balance.
4. Absorbent pellets- from cellulose these wadding were prepared B.P.C standard as follows. Wet a piece of cellulose was washed thoroughly with water and squeezed out. Repeat it for four times. Then the piece of the wet wadding was taken and rolled into spherical shape of about 100-mg mass and 100 mm diameter. Dry in an oven at 120°C for two hours and stored in open bottle in the vicinity of balance. Alternatively filter paper pulp was used to prepare the pellet.
5. Oxygen- free of sulfur compounds and extraneous matter.
6. Hydrogen peroxide – 1.5 % w/v solution was dilute to 30 % w/v hydrogen peroxide with distilled water shortly before use.

7. Thorine indicator solution- 0.2 % wt aqueous solution of thorin (disodium 4-(2 arsonophenylazo)-3- hydroxynaphthalene -2, 7-disuphonate). Protect from light and labeled.
3. Sulfuric acid solution – 0.05 N and 0.01 N
4. Perchloric acid –10 % W.
5. Barium perchlorate solution –0.005 N and 0.01 N. Dissolved 0.975 and 1.95 g respectively perchlorate trihydrate in 200 ml of distilled water and make up to 1 liter with isopropanol. Adjust the apparent pH of the solution to $\frac{3}{4}$ with a few drops of 10 % perchloric acid.

Procedure:

An absorbent pellet in a filter strip was wrapped and clamped it in to the platinum basket with fused protruding. A syringe was filled with sample and weight into the nearest 0.1 mg. About 30 mg of the sample was injected into and reweigh the syringe. Care was taken to keep the fuse dries. Ignite with out delay

To the flask 4 ± 0.5 ml of the 1.5 w/v hydrogen peroxide solutions was added. Teflon sleeve in the cone of the flask was taken. Air was replaced in the flask by passing a rapid stream of oxygen through the flask for 30 second. The oxygen was introduced near the bottom of the flask but not in the absorption liquid.

The flask was holded in one hand behind the safety screen. filter paper fuse was ignited with a sulfur free flame and immediately put the stopper in to flask, holded it firmly in place. Rapidly but carefully the flask was inverted and holded it in an inclined position. It was ensured that flame does not touch the walls of the flask and that the absorption liquid

forms seals at the neck of the flask. Avoided tilting the flask at too great an angle otherwise the sample may fall out of the holder.

After the complete combustion, the flask was shaken for about 1-min. and allowed to stand for 10 min. Weted between the combustion flask and the stopper with isopropanol and open the flask allowing the liquid to be drawn in. the stopper was washed, neck of the flask, and the platinum gauze with isopropanol using a total of 16 ml isopropanol placed the stopper to one side.

Tree drops of thorine indicator solution was added and titrate with standard 0.01N barium perchlorate solution to the first permanent color changed form yellow to pink. After complete titration washed the flask thoroughly with water. For blank titration the same reagents were used but omitting the sample [53]

Calculations:

Calculate the sulfur content of the sample by means of following equation.

$$\text{Sulfur, \% wt} = \frac{(V - v) \times N \times 1603}{W}$$

Where

V = volume of barium perchlorate solution used for sample titration;

v = volume of barium perchlorate solution used for blank titration;

N = normality of barium perchlorate solution;

W = weight of the sample in milligrams

2.6.2. DETERMINATION OF TOTAL SULFUR BY BOMB COMBUSTION METHOD (IP 61/84).

Total Sulfur was found adopting IP 61/84 standard method [54]. A known weight of the sample was taken in the clean sample cup 500 mL of the aqueous solution of sodium carbonate, which was taken in the bomb. The later was rotated in such a manner that its interior got moistens by the solution, the ignition and cotton wires were set accordingly. Lid of the bomb was tightly screwed and charged with an oxygen gas of 30 bars. The sample was then fired. Ten minutes after the fired bomb was taken out, dried and the pressure was released slowly. The interior of the oil cup and the inner of the bomb cover were rinsed with a fine jet of distilled water and the washing was collected in 100 mL beaker. Based of the terminal was also washed in to the beaker. Ten mL of the bromine water was added to the contents of the beaker. The sample cup was placed in a 500 mL beaker 5 mL of the standard bromine water, 2 mL of concentrated HCl and enough distilled water were added to the cup. The contents of the beaker were heated just below the boiling point fore 3 minutes. The contents were added to the bomb washings.

The combine washing was evaporated to 20 mL on hot plate. Upon boiling 10 mL portion of BaCl_2 solution was added drop wise . The solution was stirred for 2 minutes and was then covered with a fluted watch glass. The boiling was continued till the volume was reduced to 75 mL. The beaker was removed and cooled for 1 hour. Then contents of the beaker were filtered through watman ash less filter paper. The precipitates were washed with water until it was free from any chloride. The filter paper along with the precipitate was then transfered to the pre- weighed crucible and was dried at low heat. The paper was then charred and finally ignited in bright red heat until white residue was

obtained. The crucible was cooled and re weighed. Weight of the precipitate BaSO_4 was calculated by subtracting the weight of empty crucible from the final weight.

Sulfur contents of the oil was calculated as

$$\text{S \% (by weight)} = \frac{P}{W} \times 12.73$$

P = grams of BaSO_4

W = grams of sample

2.5.3. DETERMINATION OF ELEMENTAL SULFUR SPECTROPHOTOMETRICALLY.

Apparatus

UV-160A, UV-VIS, RECORDING SPECTROPHOTOMETER (P/N204-04550)
equipped with the photo multiplier attachment, and one pair of fused quarts.

Reagents and solutions;

1. **Acetone solvent.** Fifty milliliter of water were diluted to 1 liter with technical grade acetone
2. **Sodium cyanide solution.** This solution was prepared by dissolving 0.1 g of sodium cyanide in 100 ml of acetone solvent. When first prepared, the solution was cloudy but become clear upon standing for few ours.

3. **Ferric chloride solution.** This solution was prepared by dissolving 0.4 gram of ferric chloride of hexahydrated in 100 ml of acetone solvent. Hydrolytic precipitation of part of the iron occurred, shortly after preparation. After the precipitate was settled for 24 hours the supernatant liquid was decanted into a dry bottle. The resulting solution was found to be stable for several weeks.
4. **Standard sulfur solution.** A standard solution containing 50ppm sulfur on a weight per volume basis was prepared by dissolving 50 mg of powdered roll sulfur in exactly of 1 liter of petroleum ether. The solvent had a boiling point range of 77⁰C to 110⁰C and a density of 0.73 at 25⁰C.

Procedure:

5 ml sample of the solution in a 25 ml was placed in a volumetric flask. If the sample containing more than 50 ppm elemental sulfur, a suitable dilution was made with petroleum ether to gave a sample, which contains sulfur between 5 and 50 ppm. Add 15 ml of sodium cyanide solution mixed and allowed to stand for about 2 minutes. To the mark solution was made with acetone solvent and withdraw a 5-ml aliquot. Exactly 5 ml of the ferric chloride solution was added to the aliquot and mix. Absorbance was determined at 329 nm against a blank which has been prepared by mixing 5 ml of the acetone solvent with 5 ml of the ferric chloride solution, made the absorption reading within 10 min after preparing the solution or stored the solution in darkness, if the optical measurements are to be made at a latter time. A blank correction was made in case of color sample by carrying a 5 ml aliquot through the procedure with exception that no cyanide should be added. Concentration of elemental sulfur was determine the from the calibration curve, which was prepared by treatment of a series of standard sulfur solution in petroleum ether in the same way [55].

2.6.4 DETERMINATION OF TOTAL SULFIDES BY POTENTIOMETRIC METHOD.

Reagents:

1. Potassium iodate (0.1N KIO_3).
2. Glacial acetic acid (CH_3COOH)
3. Hydrochloric acid (HCl 1N).
4. Benzene (C_6H_6)

Procedure:

The total sulfides content in the distillates were determined by titration with 0.1 N potassium iodate solution (KIO_3). The sample was dissolved in a solution of glacial acetic acid +50 ml benzene + 5ml HCl (N). Which was then potentiometrically titrated against 0.1N KIO_3 solution in 80 percent glacial acetic acid, employing a platinum electrode as indicating electrode and calomel electrode as reference electrode. It was found that 90% glacial acetic acid was recommended in the original method which does not dissolved the salt completely but by changing the concentration of the glacial acetic acid to 80 % strength of the solution, it was found that it completely dissolved the salts and there fore used in the present work. The potential drop and corresponding equivalent point of the sulfide oxidation by the reaction was observed at a potential of about 0.7v. -14) [56]

2.6.5. DETERMINATION OF ALIPHATIC SULFIDES BY SPECTROPHOTOMETRIC METHOD

Apparatus:

UV-160A, UV-VIS, RECORDING SPECTROPHOTOMETER (P/N204-04550)
equipped with the photo multiplier attachment, and one pair of fused quartz, volumetric flask, and pipette

Reagents and Chemicals:

Carbon tetrachloride,

Iodine-analytical grade,

Iodine reagent solution: Prepared by dissolving exactly 10grams of iodine in carbon tetrachloride and diluting to 1 liter.

Procedure:

Weighed sample was dissolved in carbon tetrachloride and diluted to the proper volume, dilution of this solution was prepared in order to produce absorption of the sample iodine blend falling in the range of optimum accuracy. For very small samples such as chromatographic fractions micro or semi-micro balance was used to weigh the required quantity of the samples for dilution to 25 mL in order to conserve material.

1mL iodine reagent was diluted to 10mL with the above sample solution at the proper concentration.

1 mL of carbon tetrachloride was also diluted to 10 mL with same sample solution and used this solution as the reference solution for the spectrophotometer measurements.

Immediately after preparing the sample-iodine blend and the reference solution, obtained the absorbance of the blend at 310 nm. The measurement was made as soon as the solutions have been placed in the cell compartment. As the absorbance has been noticed to increase slowly, in case of the crude oils, if the iodine-blend was allowed to stand. For blank value the iodine reagent was obtained as follow: One milliliter of the iodine reagent was diluted to 10 mL with carbon tetrachloride and the absorbance of this solution was measured at 310nm using carbon tetrachloride as reference solution. All the samples were quantitatively determined by UV-160A, UV-VIS Recording Spectrophotometer (P/N204-04550) automatically [57].

2.6.6 DETERMINATION OF MERCAPTAN SULFUR BY POTENTIOMETRIC METHOD.

Reagents and materials

1. Cadmium sulfate ($\text{CdSO}_4 \cdot 8 \text{H}_2\text{O}$)
2. Potassium iodide (KI).
3. Silver nitrate alcoholic solution (AgNO_3)
3. Distilled water (H_2O)
4. Oil Sample.

Procedure:

Sample was quantitatively tested for hydrogen sulfide (H_2S) by shaking 5 mL of the sample with 5 mL of the acid cadmium sulfate solution. The sample was analyzed

because no precipitate appeared. If the yellow precipitates were appeared then H_2S were have to be removed. Measured with a safety pipette or weighed 20-50 mL of the original or treated sample in to a 300 mL titration flask containing 100 mL of appropriate titration solvent. The beaker was Immediately placed on titration stand and adjusted its position so that the electrode was about half immersed. The burette was filled with 0.01 N alcoholic AgNO_3 solution and placed the burette in position in the titration assembly so that the tip extent approximately 25 mm below the surface of the liquid in the beaker. The speed of the stirrer was adjusted to give vigorous stirring with out separating. Recorded the initial burette and cell potential readings. A small portions of 0.01N AgNO_3 solution and was added, after waiting until a constant potential has been established, recorded the burette and meter reading. Consider the potential constant if it changes less than 6 mV/min. When the potential change was small for each increase of AgNO_3 solution, added volume as large as 0.5 ml. When the change of potential became near the end point of the titration, then 0.05 ml was added increase of 0.01 N AgNO_3 solution near the end point of the titration, 5, 10 min were elapsed before a constant potential is obtained. Although it is important to wait for equilibrium conditions, it is also important that the duration of titration be as shot as possible in order to avoid oxidation of the sulfur compounds by atmospheric oxygen. Once the titration started, it must never be interrupted and resumed later. Continue the titration until the meter reading has passed +350 mV and the cell potential per 0.1 ml of 0.01 N AgNO_3 solutions has become relatively constant. This was considered as the end point [58].

Calculations.

Mercaptan sulfur, wt = $(AN \times 3.206) / W$.

Where

A = milliners of AgNO_3 solution required to reach the end point.

N = normality of the AgNO_3 solution;

W = gram of the sample used;

= $100 \times \text{meq weight S in mercaptan.}$

2.6.7. DETERMINATION OF HYDROGEN SULFIDE BY VOLUMETRIC METHOD

Reagents

1. Cadmium sulfate ($\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$)
2. Sodium thio sulfate ($\text{Na}_2\text{SO}_3 \cdot 5\text{H}_2\text{O}$)
3. Iodine solution 0.1 N
4. Starch solution
5. Hydrochloric acid (HCl 18 %)
6. Nitrogen gas

Procedure:

A suitable quantity of the sample was added in the separating funnel containing 35 ml of the cadmium sulfate solution and shaken vigorously for 5 minutes. Content of the separating funnel was filtered. Precipitates were washed and transferred it and any

portion of filter paper to which precipitate has been adhered to a 250 ml titration flask containing 25 ml 0.1N iodine solution. 10 ml of hydrochloric acid solution (18%) was added and titrate the excess iodine with 0.1N sodium thio sulfate solution using starch as indicator. A blank test was also made by titrating a further 25 ml portion of the iodine solution in a flask containing 10 ml of hydrochloric acid solution [59].

Calculation:

$$\text{Hydrogen sulfide, \%} = N \frac{(a-b)}{W} \times 0.01$$

Where

a = volume of the sodium thio sulfate taken for the blank titration in ml;

b = volume of the sodium thio sulfate taken for the titration of the excess iodine in ml;

N = normality of the sodium thio sulfate solution;

W = weight of the sample in gram.

2.6.8. DETERMINATION OF DIBENZOTHIOPHENE

SPECTROPHOTOMETRICALLY.

10 ml of oil was filtered through a 0.22- μ m-pore-size Millex-GP filter (Millipore, Bedford, Mass.) to remove any residual cellular particles. Quality assurance samples of the known concentrated solutions of dibenzothiophene (DBT) were run before the first and after the last testing sample for calibration of the GC-MS.

For quantitative determinations of DBT an HP 5890 II plus GC with a 5972 mass spectrometer (MS) (Hewlett-Packard) operated in selective ions monitoring (SIM) mode was applied. A 1- μ l filtered oil sample was injected onto a 0.25-mm-

interior-diameter; 30-m column with a 0.5- μm film RTX-1 column (Restek) operated at 50.7-cm s⁻¹ linear velocity of helium carrier gas. The injector and detector temperatures were set to 90 and 220°C, for light fraction (200°C) then 195 and 305°C for middle fraction (300°C) whereas 295°C and 370°C for heavy fraction (360°C) respectively [60].

2.5.9. DETERMINATION OF BENZOTHIOPHENE SPECTROPHOTOMETRICALLY.

Procedure.

10 ml of the testing oil was filtered through a 0.22- μm -pore-size Millex-GP filter (Millipore, Bedford and Mass) to remove any residual cellular particles. For quantification of benzothiophene (BTH) an HP 5890 II plus GC with a 5972 mass spectrometer (MS) (Hewlett-Packard) operated in selective ion monitoring (SIM) mode was applied. A 1- μl filtered oil sample was injected onto a 0.25-mm-interior-diameter; 30-m column with a 0.5- μm film RTX-1 column (Restek) operated at 50.7-cm s⁻¹ linear velocity of helium carrier gas. The injector and detector temperatures were set to 90 and 220°C, for light fraction (200°C) then 195 and 305°C for middle fraction (300°C) whereas 295°C and 370°C for heavy fraction (360°C) respectively [60].

GC-MS was calibrated with a standard benzothiophene (BTH) solution of known concentration and noted the results obtained, and then the treated and untreated crude oil distillates were run through GC-MS, and determined benzothiophene concentration in the understudy distillates in a similar way.

RESULTS AND DISCUSSIONS

Quality of the crude oil and its distillate fractions depends upon their chemical nature, heteroatom contents and physico chemical properties. As, heteroatom contents of these products are of serious environmental concern due to their endless consequences on the environment. Hence, in this study, crude oil distillates (light, middle and heavy fractions) were studied for chemical and biological removal of sulphur moieties in order to mitigate the environmental pollution associated with their utilization as traffic fuels and to comply with the environmental codes and regulations to ensure clean and clear environment. Sulphur and its different moieties (analogues) in the original and variously chemically and microbially desulphurized samples were determined using different analytical protocols. For chemical desulphurization, hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium arsenate (Na_2HAsO_4) and ammonium vendate (NH_4VO_3) were used in order to evaluate the capability of each leachant for removal of the aforementioned sulphur moieties. Two bacterial strains i.e *Ps.Putida* and *T.Thioxidans* were cultivated with the distillates and their capability for removal of sulphur moieties as a function of inoculation time was measured. The inoculation was performed for time duration of 12 hr, 24 hr, 48 hr, one week and one month. The reduction (%) was calculated in each residual sample and reported. The physico-chemical properties of the original and the residual samples from chemical and biological desulphurization attempts were determined using standard ASTM methods with the objective to investigate any changes in such properties upon two different desulphurization approaches. The data obtained is reported in Tables-1(a) to Table-1(d) and discussed in the proceeding sections.

3.1. Physicochemical properties

3.1.1 Chemically treated crude oil and its distillate fractions

The physicochemical properties determined in case of original crude oil sample were: specific gravity; 0.873, API gravity; 24.7, kinematic viscosity; 10.35 cst, aniline point ; 143.7 oF, cloud point; 28.00 oF , pour point ; 21.8 oF , flash point; 183.3 oF, ash content; 0.056 %, Conrad son value ; 2.49 % and carbon residue ; 2.80 % as provided in Table-1(a).

The physicochemical properties of the original light fraction (b:pt 25-100 0C), middle fraction (b:pt 100-200 0C) and heavy fraction (b:pt 200-300 0C) were also determined. The values are provided in Table-1 (a). These were: specific gravity;0.713, API gravity;15.3, kinematic viscosity;8.2cst, aniline point ; 109.3 oF, cloud point;11.3oF , pour point ; 12.8 oF , flash point;140.5oF, ash content;0.0031%, Conrad son value ; 0.87 and carbon residue ;0.64% . The physicochemical properties determined in case of middle oil were: specific gravity;0.745, API gravity;18.4, kinematic viscosity;9.3cst, aniline point ;119.4oF, cloud point; 19.4oF , pour point ;18.3 oF , flash point; 159.6oF, ash content; 0.008 %, Conrad son value ;0.87% and carbon residue ; 0.98% and those determined in case of heavy fraction were: specific gravity; 0.798, API gravity; 21.3, kinematic viscosity; 10.10 cst, aniline point ; 135.0 oF, cloud point; 26.3 oF , pour point ; 20.8 oF , flash point; 173.5 oF, ash content; 0.029 %, Conrad son value ; 1.84% and carbon residue ; 1.87%.

It is observed that physicochemical properties of the light fraction are quit varied from middle and heavy fractions. This is because of the complexity of hydrocarbons constituting each fraction. It is also observed from the data that all the properties of

distillate fractions are lying below the values of original crude oil. It means that crude oil is more complex and contains long and complex molecular chain hydrocarbons, compared to the distillate fractions which contain low molecular weight and simple hydrocarbons.

The distillate fractions were chemically scrubbed with four chemical reagents separately i.e hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium arsenate (Na_2HAsO_4) and ammonium vendate (NH_4VO_3). The physico chemical characteristics of the fractions after chemical desulphurization were determined. The data has been assembled in Table-1 (b).

From the comparative study, it is concluded that the chemical treatments have caused a significant alteration in all the physicochemical properties. It can be suggested that such treatments may break some bonds of the sulfur complex compounds and hence, caused an alteration in physicochemical properties compared to the original crude oil.

3.1.2 Biologically treated crude oil and its distillate fractions

3.1.2.1 Removal with *P. putida* and *T. Thiooxidans*

Desulphurization of crude oil and its distillate fractions was performed using strains of *P. putida* and *T. Thiooxidans*, separately keeping in view their intrinsic capabilities to degrade sulfur through oxidative pathway. Physicochemical properties of the crude and its distillate fractions after biological desulphurization were studied. The data obtained in case of *P. putida* is given in Table-1(c) and in case of *T. Thiooxidans* in Table- 1(d).

The physicochemical properties determined in case of original crude oil sample were: specific gravity; 0.873, API gravity; 24.7, kinematic viscosity; 10.35 cst, aniline

point ; 143.7 °F, cloud point; 28.00 °F , pour point ; 21.8 °F , flash point; 183.3 °F, ash content; 0.056 %, Conrad son value ; 2.49 % and carbon residue ; 2.80 % as provided in Table-1(a).

The physicochemical properties of the residual light oil fraction from *P.putida* inoculation determined were: specific gravity; 0.60, API gravity; 14.2, kinematic viscosity; 7.2 cst, aniline point ; 107 oF, cloud point; 10.2 oF , pour point ; 9.8 oF , flash point; 135 oF, ash content; 0.027 %, Conrad son value ; 0.76 % and carbon residue ;0.53% . The physicochemical properties determined in case of residual middle oil fraction were: specific gravity; 0.67 , API gravity; 15.3, kinematic viscosity; 7.8 cst, aniline point ; 116 oF, cloud point; 18.3 oF , pour point ; 17.2 oF , flash point;155.2 oF, ash content;0.0006 %, Conrad son value ; 0.85 % and carbon residue ; 0.82 % and those determined in case of residual heavy fraction were: specific gravity; 0.70 , API gravity;18.2 , kinematic viscosity; 9.3 cst, aniline point ; 130.0 oF, cloud point; 23.2 oF , pour point ; 17.3 oF , flash point; 167.3 oF, ash content; 0.025 %, Conrad son value ; 1.75 % and carbon residue 17.2 %.

Similarly, the physicochemical properties of the residual light oil fraction from *T.Thioxidans* inoculation determined were: specific gravity; 0.57 , API gravity; 13.7 , kinematic viscosity; 6.8 cst, aniline point ; 105.0 oF, cloud point; 9.3 oF , pour point ; 9.5 oF , flash point; 113.2 oF, ash content; 0.0024 %, Conrad son value ; 0.73 and carbon residue ; 0.48 % . The physicochemical properties determined in case of residual middle oil fraction were: specific gravity; 0.64 , API gravity; 15.0 , kinematic viscosity; 7.3 cst, aniline point ; 113 oF, cloud point; 17.8 oF , pour point ; 16.3 oF , flash point; 152.3 oF, ash content; 0.005 %, Conrad son value ; 0.82 and carbon

residue ; 0.75 % and those determined in case of residual heavy fraction were: specific gravity; , API gravity; 0.68 , kinematic viscosity; 16.9 cst, aniline point ; 8.7 oF, cloud point; 128 oF , pour point ; 21.9 oF , flash point; 16.3 oF, ash content; 0.023 %, Conrad son value ; 1.72 and carbon residue ; 1.68 %.

It is clear from the data that no significant differences can be observed amongst the physicochemical properties determined in case of original, *P. putida* and *T.Thioxidans* inoculated samples. Both bacterial strains caused desulphurization in crude oil and its distillation fractions, without altering the physicochemical properties to a greater extent contrary to chemical desulphurization where these properties were altered to a great extent.

It is thus confirmed that bacterial treatment has nothing to do with the basic structures of crude or its fractions except removal of sulfur configurations. Hence, the biological desulphurization is suggested to be more effective compared to chemical desulphurization.

Table-1 (a). Physicochemical properties of crude oil and its distillate fractions

Parameters	Crude oil	Light fraction	Middle fraction	Heavy fraction
Specific gravity	0.873	0.713	0.745	0.798
Api gravity	24.70	15.30	18.40	21.30
Kinematics viscosity (cst) at 100 °F	10.35	8.20	9.30	10.10
Aniline point °F	143.7	109.30	119.8	135.0
Cloud point °F	28.00	11.30	19.40	26.30
Pour point °F	21.80	12.80	18.30	20.80
Flash point °F	183.30	140.50	159.60	173.5
Ash contents (% wt)	0.056	0.0031	0.008	0.029
Conradson value (% wt)	2.49	0.87	0.87	1.84
Carbon residue (% wt)	2.80	0.64	0.98	1.87

Table-1(b) Physicochemical properties of crude oil and variously chemically treated distillate fractions.

Parameters	Crude oil	Light fraction	Middle fraction	Heavy fraction
Specific gravity	0.85	0.693	0.730	0.82
Api gravity	21.05	15.00	19.80	20.98
Kinematics viscosity (cst) at 100 °F	9.90	7.95	8.85	9.97
Aniline point (°F)	137.0	107.20	116.3	133.20
Cloud point °F	24.00	10.98	18.80	25.820
Pour point °F	19.30	11.00	17.65	19.92
Flash point °F	175.20	136.30	155.00	170.00
Ash contents (% wt)	0.052	0.0027	0.0062	0.025
conradson value (% wt)	2.17	0.845	0.86	1.72
Carbon residue (% wt)	2.57	0.610	0.950	1.81

Table-1(c). Physicochemical properties of crude oil and its distillate fractions treated with *P. putida*.

Parameters	Crude oil	Light fraction	Middle fraction	Heavy fraction
Specific gravity	0.65	0.60	0.67	0.70
Api gravity	21.0	14.2	15.3	18.2
Kinematics viscosity (cst) at 100 °F	8.50	7.20	7.80	9.30
Aniline point °F	130	107	116	130.0
Cloud point °F	20.00	10.20	18.3	23.2
Pour point °F	14.30	9.80	17.20	17.3
Flash point °F	165.20	135	155.20	167.3
Ash contents (% wt)	0.043	0.027	0.006	0.025
conradson value (% wt)	1.93	0.76	0.85	1.75
Carbon residue (% wt)	2.00	0.53	0.82	1.72

Table-1(d). Physicochemical properties of crude oil and its distillate fractions treated with T. thioxidans

Parameters	Crude oil	Light fraction	Middle fraction	Heavy fraction
Specific gravity	0.730	0.57	0.64	0.68
Api gravity	22.30	13.70	15.0	16.9
Kinematics viscosity (cst) at 100 °F	9.20	6.80	7.30	8.70
Aniline point °F	136.0	105	113	128
Cloud point °F	23.00	9.30	17.80	21.9
Pour point °F	16.20	9.50	16.30	16.30
Flash point °F	172.30	131.20	152.3	165.20
Ash contents (% wt)	0.050	0.0024	0.005	0.023
Conradson value (% wt)	2.20	0.73	0.82	1.72
Carbon residue (% wt)	2.30	0.48	0.75	1.68

3.2. Desulphurization study of Crude and its distillate fractions

Sulfur concentration in crude oil has been found to be varied from 0.05 to 5.0% (by weight), but some time its concentration goes beyond even the permissible range (8%) and attain a value of (13.95%) (61). Concentration of the sulfur compounds increases along the boiling points of the various distillate fractions (61). Upon combustion, sulfur in petroleum products causes air pollution in terms of particulate matter and acidic gases, such as sulfur dioxide (SO_2), sulfur trioxide (SO_3) etc, which ultimately converts to sulfuric acid (62). To overcome all these problems sulfur contents in crude oils may be kept as low as possible during refining processing, because

Sulfur causes the poisoning of catalysts, where it deactivates the catalysts which cannot perform its functions properly. Sulfur contents in crude oil also corrode the metallic portion of the refinery plant and reduce the life of industry.

Various sulfurous compounds have been determined in original, chemically and bacterially treated distillate fractions in current investigation. These include sulfur (reactive to copper), total sulfur, elemental sulfur, aliphatic sulfides, total sulfides, mercaptane sulfur, hydrogen sulfide, benzothiophene and dibenzothiophen. Values of each corresponding sulfur analogue determined before and after particular chemical and biological treatment have been given in the respective tables and the graphical representations have also been shown for first glance comparison. For confirmation, UV analysis and GC-MS studies have also been performed in case of elemental sulfur, aliphatic sulfides, benzothiophene and dibenzothiophene.

3.2.1 Removal of sulfur (reactive to copper) in distillate fractions

Sulphur reactive to copper was determined adopting volumetric method (ASTM IP (242 /83).

Reactive sulfur is defined as the sulfur present in crude oil and its fractions in such a state that it will react with metallic copper, when they are heated together. This is an elemental form of sulfur, which is much reactive form of sulfur. In this case, sulfur liberates in the form of hydrogen sulfide (H_2S) gas.

Hydrodesulphurization (HDS) technique is mostly adopted by the refiners to remove sulfur contents from the petroleum based products. HDS is a catalytic process, which converts sulfur contents to hydrogen sulfide (H_2S) gas, if crude oil interacts with hydrogen gas at pressures between 150 and 3,000 lb/in² and temperatures between 290 and 455°C. The pressure and temperature conditions depend upon the nature of crude oil and level of desulphurization required (62).

Chemical Desulphurization

In the present study, chemical removal of sulphur reactive to copper was viewed in case of the three distillate fractions (light, middle and heavy). The sulfur contents observed in light, middle and heavy fractions before chemical treatments were found to be 0.0748, 0.1037 & 0.1028 %, respectively as shown in Fig.-1. The same distillate fractions were then scrubbed with four different chemical reagents i.e. hydrochloric acid (HCL), sodium hydroxide (NaOH), sodium arsenate (Na_2HAsO_4) and ammonium vendate (NH_4VO_3). These chemicals were used in order to evaluate their desulphurization potential in term of their efficiency for removal of sulphur moiety which is reactive to copper.

Among the chemicals used, HCl has the ability to convert sulfur to hydrogen sulfide (H_2S) gas, and reduces the sulfur contents in liquid petroleum, because H_2S is a volatile gas and emanates the petroleum surface at high temperature and pressure. From the chemically treated samples, it is clear that up to some extent, sulfur level has been reduced. In HCl scrubbed fractions, sulphur was found to be 0.0703 % in case of light fraction, 0.0901% in case of middle fraction and 0.0971% in case of heavy fraction as compared to the values determined in these fractions before scrubbing i.e 0.0748 % in case of light fraction, 0.1037 % in case of middle fraction and 0.1028 % in case of heavy fraction.

The influence of scrubbing the same samples with NaOH on sulphur depletion was also viewed. The sulphur observed in the residual fractions from this treatment was 0.0655 % in case of light fraction, 0.0838 % in case of middle fraction and 0.0853 % in case of heavy fraction. The data shows a considerable reduction in sulfur contents with this particular scrubber. This can be attributed due to the fact that NaOH converts sulfur to sodium sulfide (Na_2S) or may further oxidize to sodium sulfate (Na_2SO_4) during the chemical interaction, which ultimately causes desulphurization of the crude oil distillates.

In the next step, sodium arsenate (Na_2HAsO_4) was used in order to view its desulphurization capability. The sulfur observed in residual fractions from this treatment was 0.0645 % in case of light fraction, 0.0754 % in case of middle fraction and 0.0742% in case of heavy fraction. Upon comparing the desulphurization activity with HCl and NaOH, this scrubbing agent seems to be more efficient for the removal of sulphur reactive to copper. The reason can be thought for this significant sulphur depleting ability

is the conversion of sulphur to sodium sulfides and arsenic sulfides. Which then easily emanate from the bulk.

Ammonium vendate (NH_4VO_3) was also used to view its sulphurization capability. From the data obtained, it is quite clear that NH_4VO_3 has caused removal of a significant quantity of sulfur reactive to copper than the rest of the three scrubbing agents used. The sulphur levels observed using this reagent was 0.0628 % in case of light fraction, 0.0732% in case of middle fraction and 0.0639 % in case of heavy fraction. The reason can be thought for this significant depletion is this reagent has the ability to convert sulfur to ammonium sulfides and vanadium sulfides by reacting with the NH_4^+ and vanadium which are volatile products and quickly desorb from the surface and bulk of the distillates.

It can be concluded from the afore mentioned findings that the chemical reagents particularly ammonium vendate proved to be excellent sulfur extractor or depletor for fractions under study. The effect of ammonium vandate is quite significant in the heavy fraction.

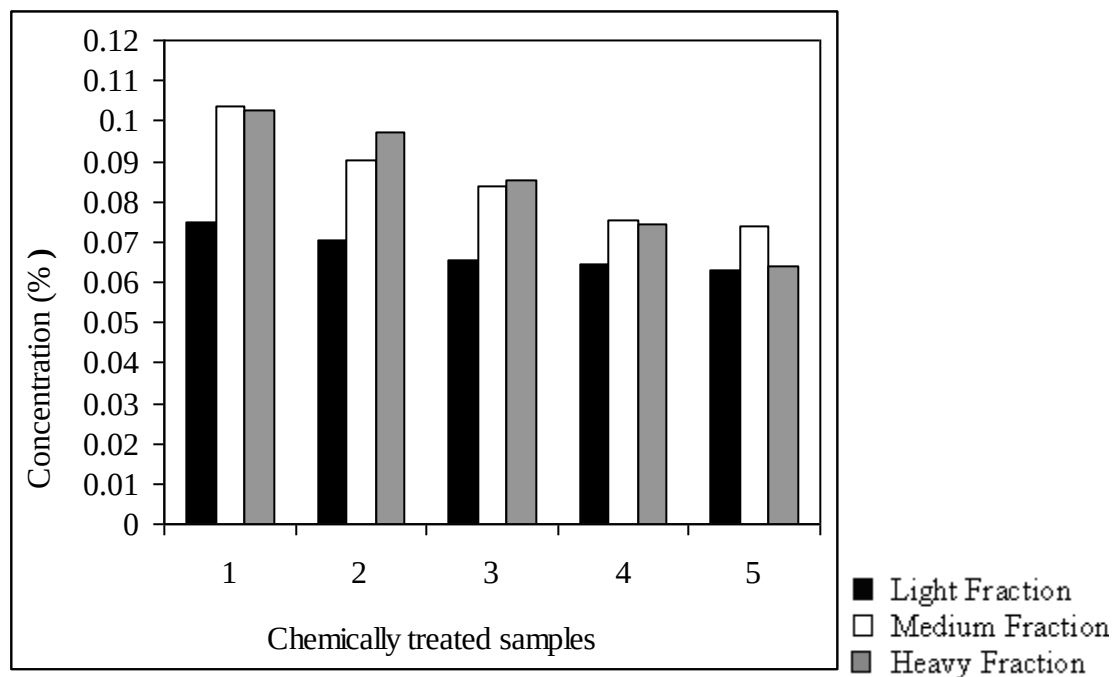
The capability of each scrubbing agent was calculated on the basis of % depletion. Fig. 2 shows the percent reduction noticed in case of these scrubbers against the value of sulphur determined in original samples. It is inferred from the results that among the reagents used, HCl has reduced 6.016 % sulphur in case of light fraction, 13.114 % in case of middle fraction and 5.544 % in case of heavy fraction. Similarly, the depletion observed using NaOH was 12.433 % in case of light fraction, 19.1530 % in case of middle fraction, and 17.0233 % in case of heavy fraction

Na_2HAsO_4 caused a significant reduction in all the three distillates studied. The depletion noticed was 13.7700, 27.4302 & 27.8210 % in case of light, middle and heavy fractions, respectively. The reduction seems to be comparatively better than HCl and NaOH.

In case of NH_4VO_3 , the reduction is quite significant, which was noted as 16.042, 28.873 & 37.8404 %, which was 10.026, 15.76 & 32 % ; 13.61, 9.72 & 20.82 % ; 2.27, 1.44 % 10.02 % times more than HCl, NaOH and Na_2HAsO_4 in case of light, middle and heavy fractions, respectively.

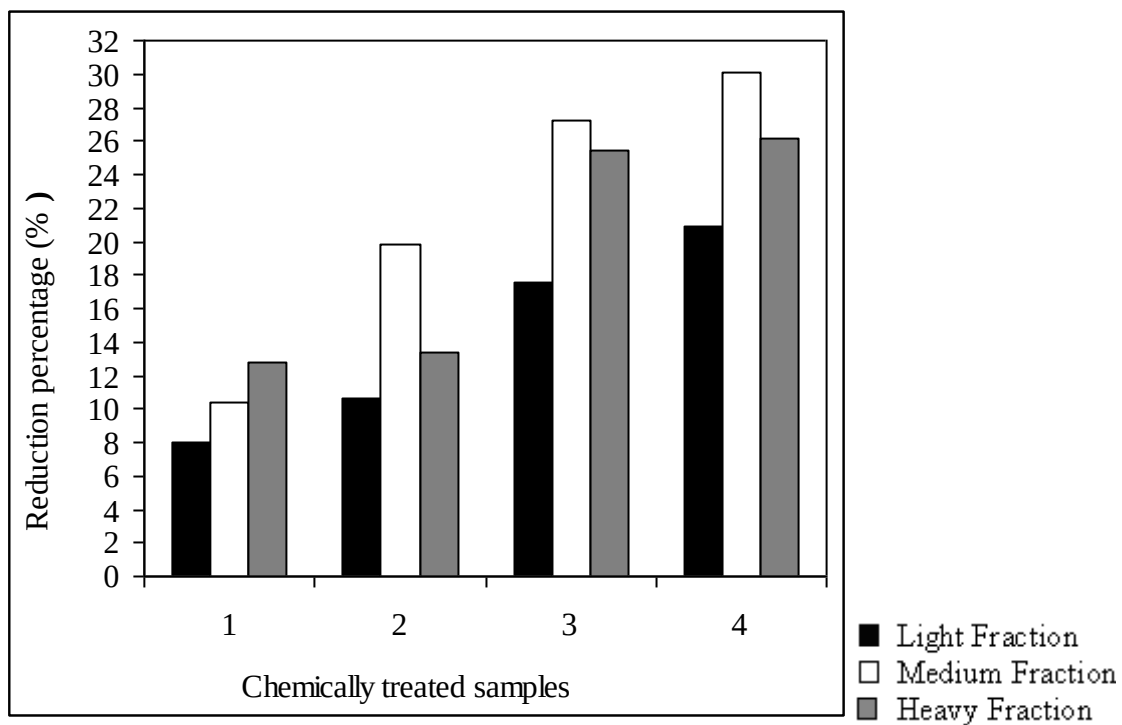
Chemical treatments have also been studied by other researches e.g. Landau et al, has studied Israeli shale oil for chemical desulphurization, where they hydrotreated the oil based products at elevated temperature and pressure for the removal of sulfur contents [63] and obtained very good results.

Figure-1: Level of copper reactive sulfur (%) determined in virgin and chemically treated distillate fractions



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.0748 (± 0.00213)	0.1037 (± 0.001955)	0.1028 (± 0.0007)
2	Hydrochloric acid	0.0703 (± 0.00335)	0.0901 (± 0.001234)	0.0971 (± 0.003214)
3	Sodium hydroxide	0.0655 (± 0.00334)	0.0838 (± 0.002193)	0.0853 (± 0.003027)
4	Sodium arsenate	0.0645 (± 0.000964)	0.0754 (± 0.002658)	0.0742 (± 0.00095)
5	Ammonium vendate	0.0628 (± 0.002631)	0.0739 (± 0.002597)	0.0639 (± 0.00109)

Figure-2: Reduction (%) of copper reactive sulfur in variously chemically treated distillate fractions.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	6.016	13.1147	5.544
2	Virgin-Sodium hydroxide	12.434	19.1530	17.0233
3	Virgin-Sodium arsenate	13.770	27.4302	27.8210
4	Virgin-Ammonium vendate	16.0428	28.8739	37.8404

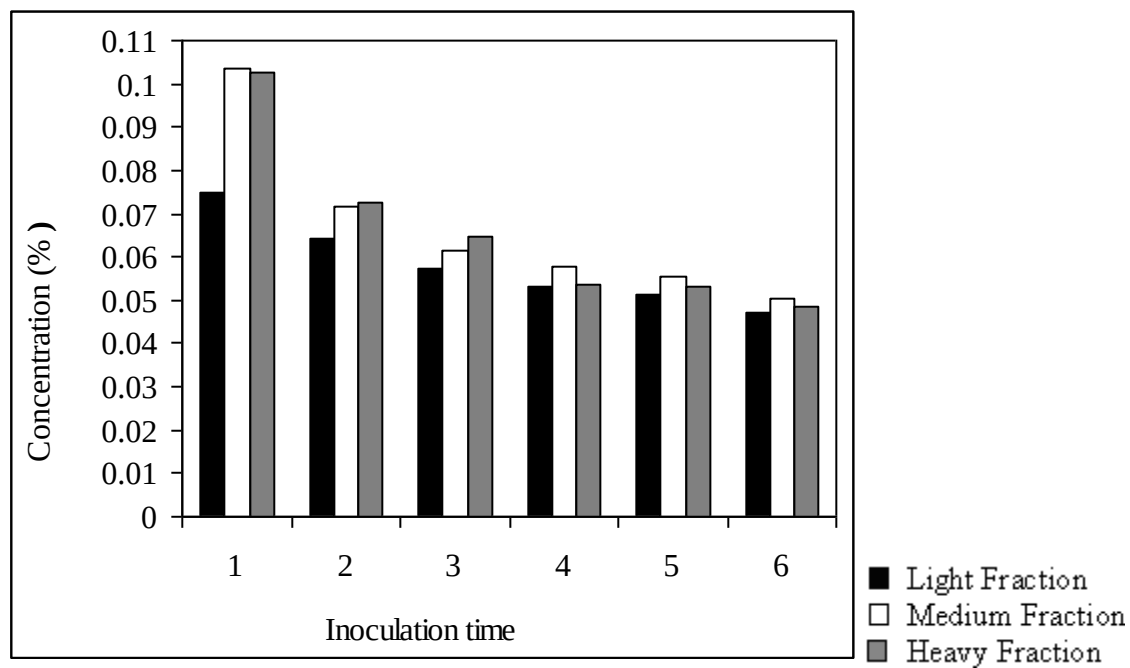
Biological Desulphurization

Desulphurization with P. Putida

In this attempt, all the crude oil distillates were inoculated with *P. putida* for a time duration selected from 12 h to one month. Considerable reduction was observed in case of 12 hr inoculation time in case of all the three distillates studied Sulphur determined in the residual fractions after 12 hr inoculation was 0.0641 % in case of light fraction, 0.0715 % in case of middle fraction and 0.0726% in case of heavy fraction. To view the effect of extension in inoculation time, the time was extended beyond 12 hr to 24 hr. As the inoculation time was extended, sulfur depletion was further found to be enroute. This is attributed due to the fact that *P. putida* has the ability to remove a wide range of sulfur compounds from the crude oil to make it available for its growth. (64). The sulphur moieties are consumed by the bacteria in the initial inoculation time that favor organisms to thrive and populate generating its blooms.

The values of sulfur determined were found to be 0.0571, 0.0614, & 0.0647 % in light, middle and heavy fractions, respectively in case of 24 hr inoculation time. The time was further increased and inoculation was performed at 48 hr. The sulphur contents determined in the residual samples were found to be 0.0533, 0.0576, & 0.0538 % in case of the three fractions studied. To view any beneficial effect of further extension in inoculation time, the time was chosen as one week. The sulphur contents determined in residual fractions from this treatment were 0.0514, 0.0553, & 0.532 % in case of light, middle and heavy fractions, where as one month inoculation period was also observed and the sulfur contents were found to be 0.0473, 0.0503 & 0.0483 respectively in light, meddle and heavy fractions as given in Fig.3.

Figure-3: Copper reactive sulfur (%) determined in *P. putida* treated distillate fractions as a function of inoculation time



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.0748 (± 0.00212)	0.1037 (± 0.00199)	0.1028 (± 0.00213)
2	12 hours	0.0641 (± 0.002641)	0.0715 (± 0.0017)	0.0726 (± 0.001769)
3	24 hours	0.0571 (± 0.002488)	0.0614 (± 0.00158)	0.0647 (± 0.002883)
4	48 hours	0.0533 (± 0.003759)	0.0576 (± 0.001823)	0.0538 (± 0.002702)
5	One week	0.0514 (± 0.00153)	0.0553 (± 0.00153)	0.0532 (± 0.000153)
6	One month	0.0473 (± 0.00020)	0.0503 (± 0.00153)	0.0483 (± 0.00208)

From the sulphur data, it is certain that upon extension in time, the required sulphur is not available for effective flourish of the microorganism and its growth and culture is suppressed.

In these inoculation time

Various sulfur specific desulphurization bacteria has been previously isolated, that are capable to desulfurize a variety of sulfurous compounds particularly found in petroleum based products through a sulfur selective oxidative pathway (65).

In order to ascertain the effect of extension in inoculation time, percent reduction was calculated in each case. Reduction between each treated and untreated sample was calculated and provided in Fig. 4. Satisfactory results have been achieved in all the inoculations e.g. 12 hr, 24 hr, 48 hr, one week and one month. During these inoculations, enough reduction has been shown by *P. putida* in light, middle and heavy fractions which was 14.304, 31.0511 & 29.377 % observed in case of 12 hr, 23.663, 40.790 & 37.062 % observed in case of 24 hr, 26.069, 44.455 & 47.665 % observed in case of 48 hr and 31.283, 46.6730 & 48.249 % observed in case of one week inoculation, respectively.

The reason for quite promising reduction observed in case of all fractions particularly in case of heavy fraction is due to the reason that bacteria have an enzymatic pathway which removes covalently bonded sulfur without disturbing carbon-carbon bonds in crude oils. The DNA sequences of these microorganisms are composed of special desulphurization genes necessary to follow the desulphurization pathway. These are particularly sulfur

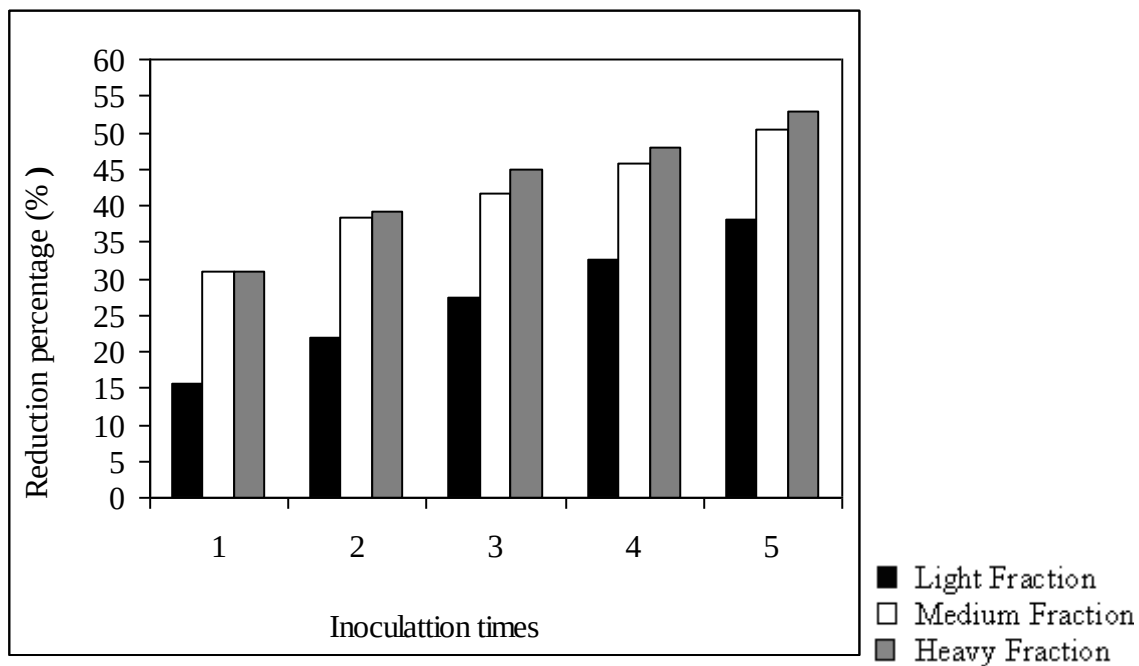
oxidatizing genes, which completes desulphurization of the crude oil middle in various stages (66).

Maximum reduction has been made by *P. putida* during its one month stay in all the oil samples, which was 58.510, 51.49 and 53.015 % in case of light, middle and heavy fractions, respectively. This indicates that as long as bacteria remain in contact with the samples, it consumes sulfur from the sample for nourishment and population.

P. putida treated samples were studied for sulfur reactive to copper as a function of inoculation time from virgin (00hr) – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, middle and heavy fractions and the values determined were found to 14.30, 31.05 & 29.37 % ; 9.36 & 7.69 % ; 2.4, 3.66 & 10.6 % ; 5.22, 2.22 & 0.58 % and 27.23, 4.82 & 4.91 %, respectively.

Comparing the reduction percentages of chemical and biological treatments, it is clear that biological method is more effective. More than fifty percent sulfur has been removed with pseudomonas treatment in one month inoculation time while with chemical treatment almost its half amount has been removed in case of ammonium vendate.

Figure-4: Reduction percentage (%) of copper reactive sulfur in *P. putida* treated distillate fraction as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	14.3048	31.0571	29.377
2	Virgin -24 hours	23.663	40.7907	37.0622
3	Virgin -48 hours	26.0693	44.4551	47.665
4	Virgin -one week	31.2834	46.6730	48.2490
5	Virgin - one month	58.5106	51.499	53.0155

Desulphurization with T. Thioxidanes

In this attempt, all the crude oil distillates were treated with *T. thioxidanes* and its desulphurization ability was evaluated. The samples were inoculated for the same contact time chosen in case of *P. putida*. *T. thioxidans* has caused sulfur removal significantly. It is because of the fact that the microorganism has the ability to erode the sulfur crystal, to reduce its size in the area immediately adjacent to the cell (67) for effective assimilation by the body.

The sulfur contents observed in residual samples from each inoculation were determined in case of 12 hr, 24 hr, 48 hr, one week and one month inoculation time. The sulphur contents observed in light, middle and heavy fractions in case of 12 hr were found to be 0.0641, 0.0718 & 0.0726 %, respectively. Similarly, the sulphur contents determined in residual light, middle and heavy fractions from the afore mentioned bacteria inoculated for 24 hr were 0.0571, 0.0614 & 0.0647 %.

The time was extended to 48 hr and the sulphur contents observed were 0.0553, 0.0576 & 0.0537 %. Inoculation time was further increased to one week. The sulphur contents determined in the residual fractions from this treatment were 0.0513, 0.0553 & 0.0532 %. The sulphur contents determined in the residual fractions from one month inoculation time were 0.0473, 0.0504 & 0.0483 %, in case of light, middle and heavy fractions, respectively. The sulphur contents determined in all cases show a significant depletion compared to original samples (0.0748, 0.1037 & 0.1028 % in light, middle and heavy fractions) as shown in Fig.-5.

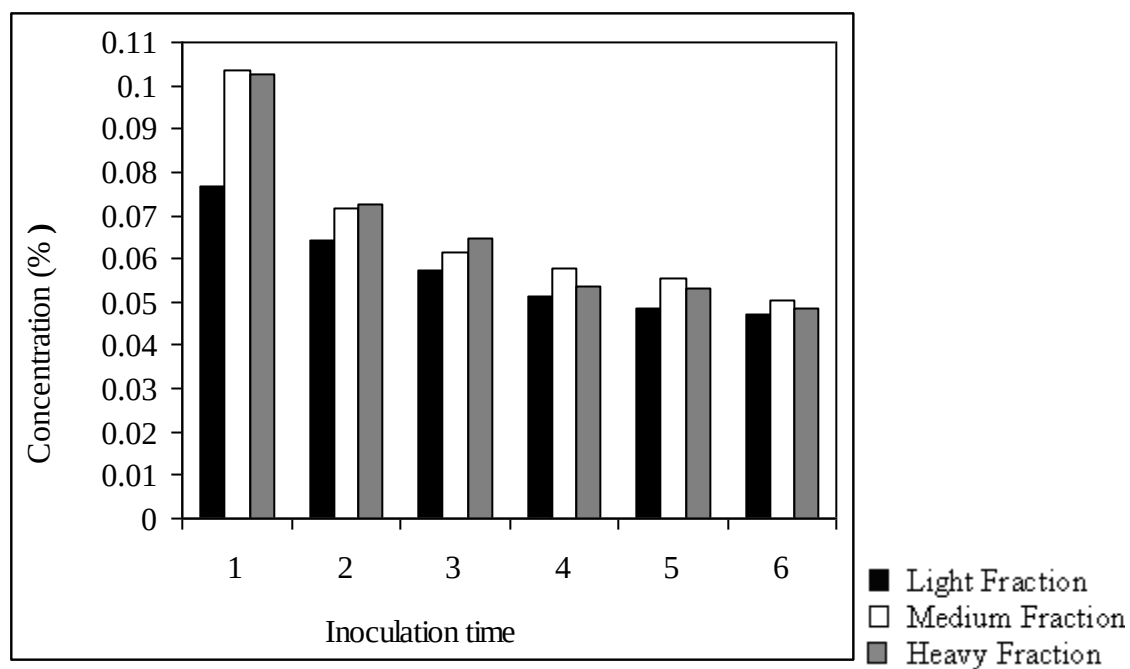
The percent reduction in each case was also calculated and represented in Fig. 6.

Where 14.305, 30.618 and 29.961 % depletion was observed in case of light, middle and heavy fractions, respectively in the first 12 hr. As the fermentation time was extended beyond 12 hr up to one month, the depletion rate also increased along with the extension in time. Same effect has been studied by other workers (68).

The percent reduction calculated in case of 24 hr, 48 hr, one week and one month was quite significant i.e. 23.663, 40.791 and 37.0622 % in case of 24 hr in light, middle and heavy fractions, respectively. Similarly, the percent reduction observed in case of 48 hr inoculation time was 26.337, 44.45 and 48.443 %, and that observed in case of one week was 31.417, 46.673 and 46.303 %. The depletion observed in light, middle, and heavy fractions in case of one month inoculation was 37.033, 51.398 and 53.015 %, respectively.

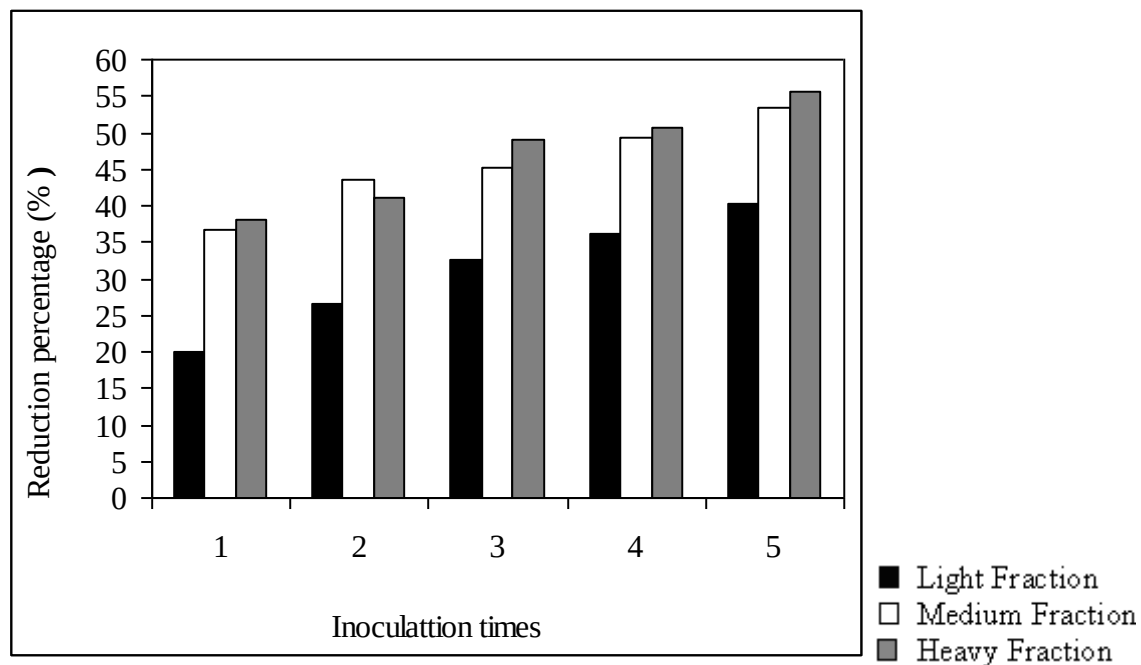
It was observed that 14.30, 30.61 & 29.96 %, 9.36, 10.18 & 7.1 % ; 2.67, 3.66 & 10.7 % ; 5.08, 2.22 & 0.48 % ; 5.62, 4.72, & 4.77 % reduction percentage has been made from one inoculation time to another in case of 12 - 24hr, 24 - 48hr, 48 - one week and one week - one month in light, middle and heavy fraction, respectively.

Figure-5: Level of copper reactive sulfur (%) determined in *T. thiooxidans* treated distillate fractions as a function of inoculation times.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.0768 (± 0.00218)	0.1037 (± 0.001955)	0.1028 (± 0.0007)
2	12 hours	0.0641 (± 0.000737)	0.0718 (± 0.001952)	0.0726 (± 0.004321)
3	24 hours	0.0571 (± 0.003156)	0.0614 (± 0.002417)	0.0647 (± 0.001656)
4	48 hours	0.0513 (± 0.001457)	0.0576 (± 0.001557)	0.0537 (± 0.001097)
5	One week	0.0487 (± 0.00210)	0.0553 (± 0.001358)	0.0532 (± 0.002631)
6	One month	0.0473 (± 0.00210)	0.0504 (± 0.001834)	0.0483 (± 0.000656)

Figure-6: Reduction (%) of copper reactive sulfur in *T. thiooxidans* treated distillate fractions as a function of inoculation time



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	14.305	30.618	29.961
2	Virgin -24 hours	23.663	40.791	37.0622
3	Virgin -48 hours	26.337	44.455	46.303
4	Virgin -one week	31.417	46.673	48.443
5	Virgin - one month	37.033	51.398	53.015

3.2.2. Removal of Total sulfur in crude oil distillate fractions

Chemical treatment

All kind of organic and inorganic sulfurous compounds present in petroleum oils are termed as total sulfur. The samples are combusted in the presence of oxygen and the combustion products are absorbed in hydrogen peroxide (H_2O_2) solution, and the amount of sulfuric acid (H_2SO_4) formed is determined by titration with standard barium perchlorate solution using thiorine as indicator.

Crude oil distillates were analyzed for total sulfur using ASTM D method (1226-IP 107).

In case of virgin samples, total sulfur concentration was found to be 0.4312, 0.523 & 0.7684% in case of light, middle and heavy fractions, respectively. All the distillation fractions were then scrubbed with the four chemicals reagents i.e. HCl, NaOH, Na_2HASO_4 and NH_4VO_3 . The depletion profile is given in Fig.-7.

In case of HCl treatment, the sulphur contents observed in the residual fractions were found to be 0.4208, 0.4910 & 0.7162 % in case of the three distillate fractions studied. Upon comparing the values with the original samples, the removal seems to be quite insignificant. Thus, this scrubbing agent is not recommended for effective removal of total sulphur. NaOH was viewed as reagent for total sulphur depletion. The data is provided in the same Fig. It is evident that NaOH has reduced some amount of total sulfur (0.4117, 0.4654 & 0.6698 %). Na_2HASO_4 and NH_4VO_3 also caused depletion of total sulphur. The total sulphur contents determined in light, middle and heavy fractions in case of residual fractions from Na_2HASO_4 treatment were 0.3747, 0.4321, & 0.6425 % and those in case of NH_4VO_3 were 0.3539, 0.4061, & 0.6231 %, respectively.

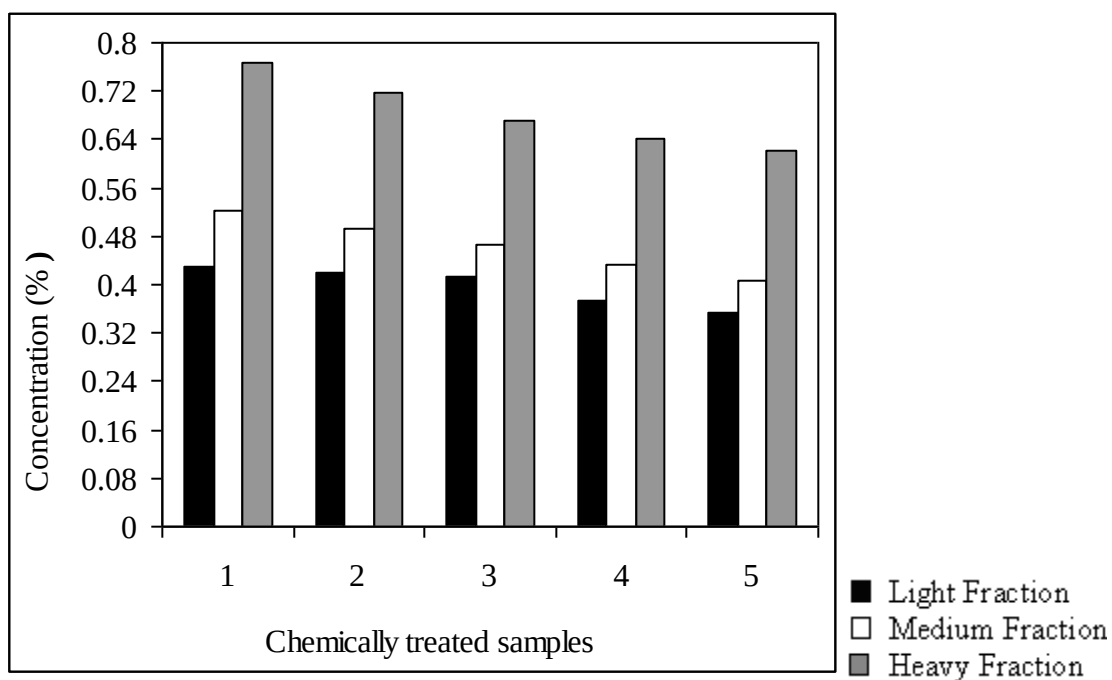
It is clear from this figure that most of the sulfur contents have been reduced in case of ammonium vendate (NH_4VO_3) as compared to other chemicals.

In chemical desulphurization the scrubbing capacities of the used leaching chemicals was found to be 2.41, 6.18 & 6.79 % ; 2.11, 4.83 & 6.13 % ; 8.6, 6.37 & 3.35 % ; and 4.82, 4.97 & 0.03 % , respectively in cases virgin – HCl, HCl- NaOH, NaOH - Na_2HAsO_4 and Na_2HAsO_4 - NH_4VO_3 in light, middle and heavy fractions.

The effect of the chemicals desulphurization has also been shown in the form of reduction percentages and the values are given in Fig.-8. Where most of the sulfur was reduced in the three distillates by ammonium vendate i.e. 17.926, 22.351 and 16.410 % followed by Na_2HAsO_4 (13.102, 17.380 and 16.384 %) followed by NaOH (4.522, 11.013 and 12.831 %) where as HCl has caused the least reduction (2.411, 6.185 and 6.793 %).

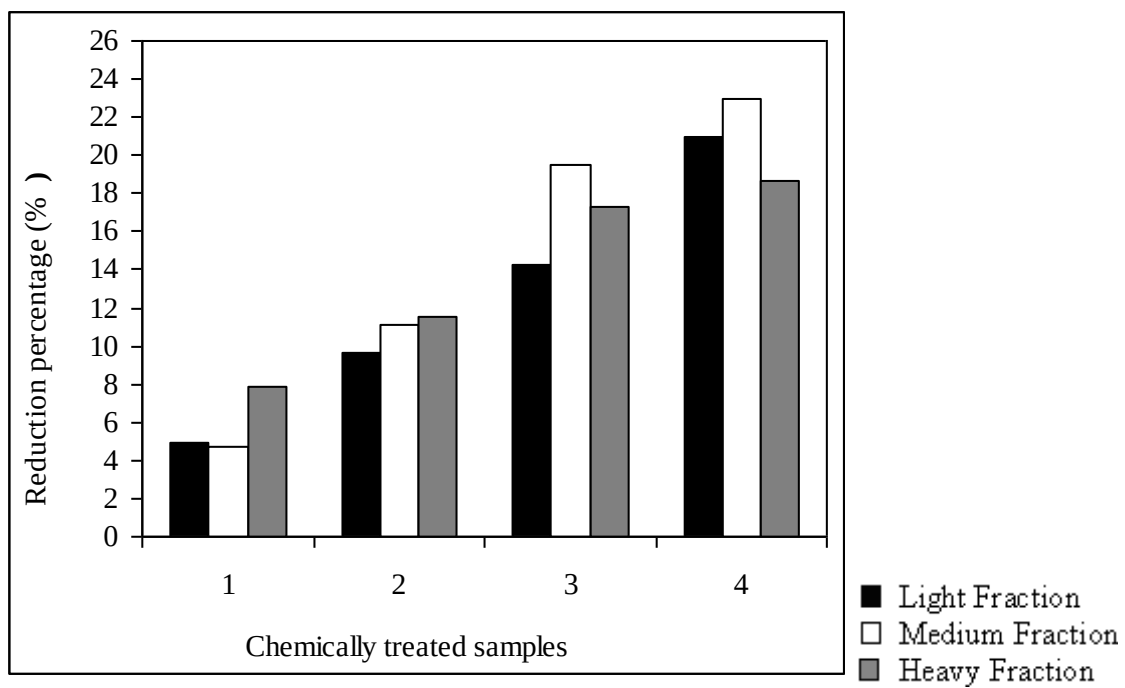
Yitzhaki has given specific methods for chemical desulphurization but only for heavy atmospheric gas oil, using catalysts (Co-Mo-Al). The effects of these catalysts were temporary sulfur adsorption upon their surfaces. They have further used oxidation regenerated catalysts $\text{Co-Mo/Al}_2\text{O}_3$ for hydrodesulphurization of heavy atmospheric gas oil (HAGO) in batch and trickle-bed reactors at high temperature, sulfur level in the hydrotreated heavy atmospheric gas oil (HAGO) was decreased by 50-350 ppm due to sulfur adsorption on the surface of catalyst for periods of 20-50 hrs [69].

Figure-7: Level of total sulfur (%) determined in virgin and variously chemically treated distillate fractions



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.4312 (± 0.00144)	0.5230 (± 0.00680)	0.7684 (± 0.00522)
2	Hydrochloric acid	0.4208 (± 0.0082)	0.4910 (± 0.00771)	0.7162 (± 0.00152)
3	Sodium hydroxide	0.4117 (± 0.00110)	0.4654 (± 0.00980)	0.6698 (± 0.00631)
4	Sodium arsenate	0.3747 (± 0.00827)	0.4321 (± 0.00101)	0.6425 (± 0.00101)
5	Ammonium vendate	0.3539 (± 0.009563)	0.4061 (± 0.0094)	0.6231 (± 0.00899)

Figure-8. Reduction (%) of total sulfur in chemically treated distillate fractions



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	2.4118	6.185	6.793
2	Virgin-Sodium hydroxide	4.522	11.013	12.831
3	Virgin-Sodium arsenate	13.102	17.380	16.384
4	Virgin-Ammonium vendate	17.926	22.351	16.410

Biological Removal

Removal of total sulfur with P. putida

Fig.9 indicates the values of sulfur depletion observed in various inoculations performed with *P. putida*. The sulfur contents observed in case of residual fractions from inoculation performed for 12 hr were 0.2801, 0.3051 and 0.5178 % in case of light, middle and heavy fractions, respectively. Upon comparing the sulphur contents determined in original fractions (0.0.4312, 0.5230 & 0.7684 %), a significant reduction in total sulphur can be noticed. The reason can be thought for this depletion is the presence of specific cells in *P. putida* which can bio catalyze the oxidation of total sulphur (70).

The influence of extension in inoculation time was viewed by extending time from 12 hr to 24 hr. The total sulphur contents determined in the residual fractions were. 0.2354, 0.2789, & 0.4971 %. Similarly, the time was further extended to 48 hr. The sulphur contents found in the residual samples from this inoculation were 0.2326, 0.2625 & 0.4675 % .The time was next extended to one week and then to one month. The sulphur contents determined in case of one week were 0.1911 , 0.2518 & 0.4460 % and in case of one month inoculation, were 0.174 , 0.2353 & 0.414 % in light, middle and heavy fractions, respectively.

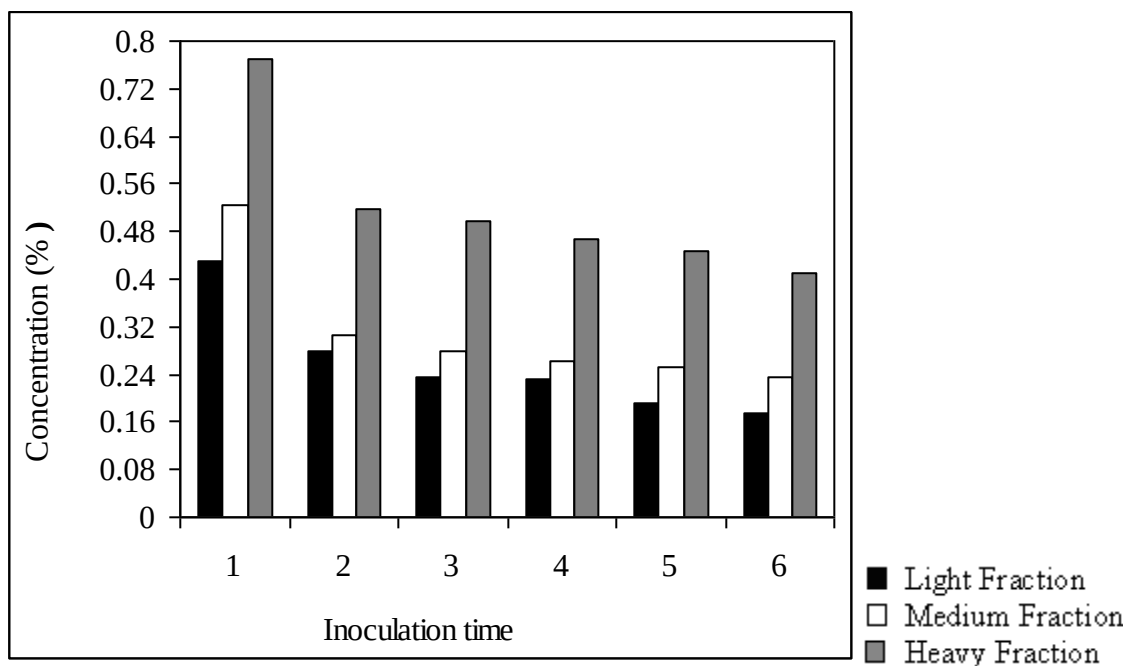
Percent reduction of all the inoculation times has been given in Fig.10. Where 35.0417, 41.663 & 32.613 % reduction can be observed in case of 12 hr A reduction of 46.0575, 46.673 & 35.307 % can be observed in case of 24h inoculation. As the inoculation time was extended beyond 24 hr to 48 hr, one week and one month its percentage reduction was further increased to (46.0788, 49.808, 39.159), (55.6818,

51.854, 41.957) and (59.647, 55.0095, 46.460) % respectively in the cases of 48 hr, one week and one month contact times as can be seen from the Fig .10.

In different inoculation times various sulphur amounts has been examined, which were 35.00, 41.66 & 32.61 % ; 11.01, 5.01, & 2.69 % ; 0.02, 3.31 & 3.85 % ; 9.61, 2.05 & 2.80 % ; and 3.96, 3.15 & 4.51 % respectively in the cases of virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month.

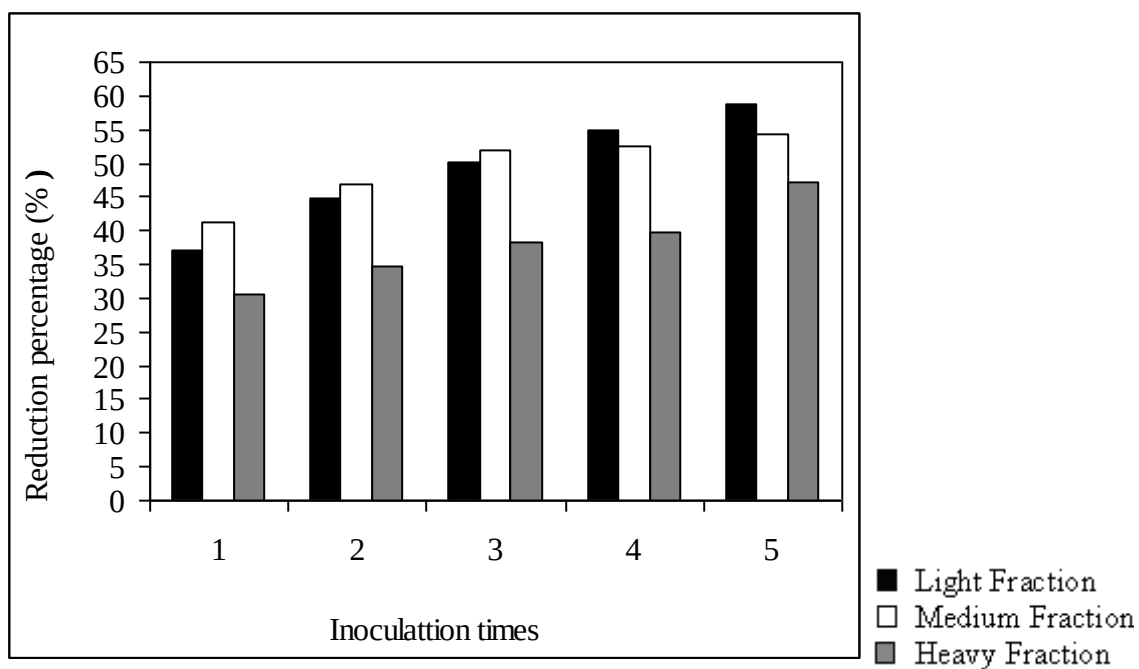
These bacteria take up the organic and inorganic sulfurous compounds from oil middle, and as a wander full desulphurization biocatalyst

Figure-9: Level of total sulfur determined (%) in *P.putida* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.4312 (± 0.00144)	0.5230 (± 0.00860)	0.7684 (± 0.00522)
2	12 hours	0.2801 (± 0.00334)	0.3051 (± 0.00927)	0.5178 (± 0.00993)
3	24 hours	0.2354 (± 0.00103)	0.2789 (± 0.00650)	0.4971 (± 0.00406)
4	48 hours	0.2326 (± 0.00123)	0.2625 (± 0.00889)	0.4675 (± 0.00545)
5	One week	0.1911 (± 0.00702)	0.2518 (± 0.00352)	0.4460 (± 0.00139)
6	One month	0.1740 (± 0.0087)	0.2353 (± 0.00126)	0.4114 (± 0.00785)

Figure-10: Reduction (%) of total sulfur in pseudomonas treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	35.0417	41.663	32.613
2	Virgin -24 hours	46.0575	46.673	35.307
3	Virgin -48 hours	46.0788	49.808	39.159
4	Virgin -one week	55.6818	51.854	41.957
5	Virgin - one month	59.647	55.0095	46.460

Removal with T.Thioxidans

The effect of inoculation time selected in case of *T.putida* was also studied by cultivating the same oil fractions with *T.Thioxidans*. The results obtained have been presented in Fig.11. Significant amount of this particular form of sulfur has been depleted by this strain, which take up the sulfur from the crude oil distillates for its growth and decreases its concentration in the oil middle. *T.Thioxidans* is strictly autotrophic bacteria, which grow on sulfur present in petroleum based products, build up keto acids, and as long as it remains inside the oil middle, it continuously decreases the sulfur concentration (71).

The study was undertaken in the same fashion and inoculations were performed for time duration ranges from 12 hr to a maximum of one month. The total sulphur determined in residual samples from all these inoculations shows a considerable depletion. From the data collected, it can easily be concluded that there is a progressive depletion occurred when the time was extended to 12 hr, then to 24 hr, 48 hr, one week and one month. Significant depletion can be observed in case of inoculation performed for one month. Sulphur contents have been dropped to 0.2554, 0.2707, 0.3740 %, 0.2361, 0.2420 & 0.3404 %, 0.2217, 0.2224 & 0.3140 %, 0.2105, 0.1933 & 0.2624, and 0.2075, 0.1716 & 0.224 % , respectively in cases of 12 hr , 24 hr, 48 hr, one week and one month in all the distillation fractions. It can also be seen from the compiled data that the depletion is quite noticeable in case of heavy fraction when cultivated with the strain under study for one month where the amount of total sulphur has reduced from 0.7685 to 0.224 %.

The effect of bio desulphurization on some petroleum fractions has been studied previously [72]. Our results are quite satisfactory particularly in case of heavy fraction.

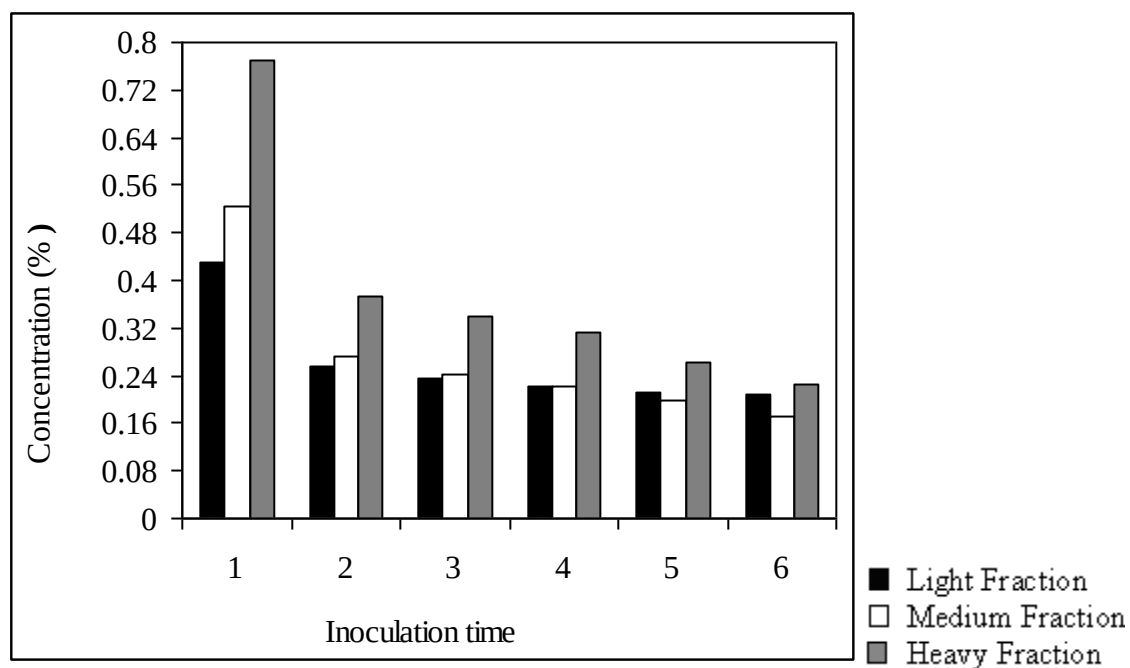
Reduction percentages were also calculated to know about the percent depletion made in each inoculation. The results are provided in Fig. 12. A depletion of 38.4508, 48.240 & 51.3337 % can be seen in case of 12hr. It has increased to 45.2458, 53.728 & 55.7059, 48.5853, 57.476, & 59 in the 24hr and 48hr inoculations, respectively.

Reduction of 51.182, 63.040 & 65.855 % and 51.878, 67.189 & 70.8522% can be noticed in case of one week and one month inoculation time.

It was studied that 38.45, 48.24 & 51.33 % ; 6.79, 5.48 & 4.37 % ; 3.34, 3.75 & 3.44 % ; 2.6, 5.57 & 6.71 % and 0.69, 4.14 & 5.00 % reduction was being observed in inoculation times from virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month in light, meddle and heavy fractions.

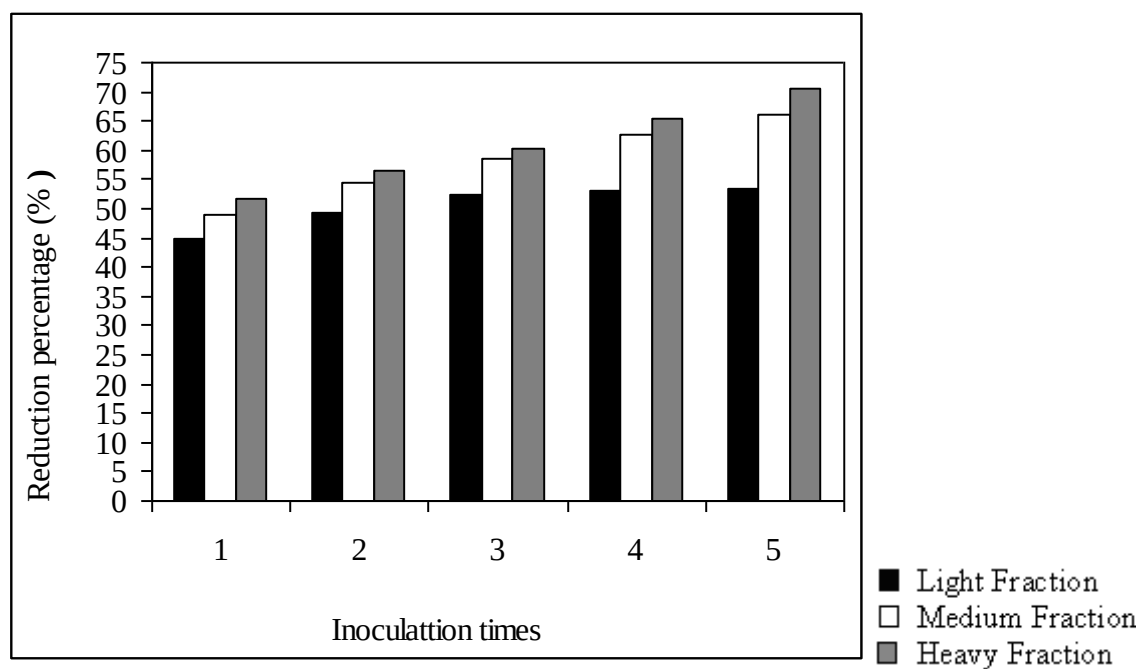
Comparing the results with the *P. Putida*, it is concluded that *T. Thiooxidans* has caused much focused desulphurization. It is therefore, suggested that *T. Thiooxidans* is the best sulfur removal strain for distillates fractions under study particularly heavy fraction.

Figure-11. Level of total sulfur determined (%) in *T. thiooxidans* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.4312 (± 0.01442)	0.5230 (± 0.0086)	0.7685 (± 0.00522)
2	12 hours	0.2554 (± 0.00105)	0.2707 (± 0.00425)	0.3740 (± 0.0104)
3	24 hours	0.2361 (± 0.00996)	0.2420 (± 0.00331)	0.3404 (± 0.00654)
4	48 hours	0.2217 (± 0.00908)	0.2224 (± 0.00589)	0.3140 (± 0.00107)
5	One week	0.2105 (± 0.00715)	0.1993 (± 0.00716)	0.2624 (± 0.0072)
6	One month	0.2075 (± 0.00475)	0.1716 (± 0.00538)	0.2240 (± 0.00116)

Figure-12: Reduction (%) of total sulfur in *T. thiooxidans* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	44.915	49.121	51.578
2	Virgin -24 hours	49.152	54.298	56.435
3	Virgin -48 hours	52.288	58.693	60.381
4	Virgin -one week	52.965	62.622	65.464
5	Virgin - one month	53.258	66.259	70.704

3.2.3. Total sulfur in crude oil distillate fractions by bomb combustion method

Total sulfur in samples under study was determined by bomb combustion method (ASTM IP (61/84)).

This method describes the procedure for the determination of total sulfur in petroleum products, which cannot be burnt completely in other combustion methods.

The sample is oxidized by combustion in a bomb containing oxygen under pressure. The sulfur, as sulfate in bomb washing is titrated with barium chloride (BaCl_2) and is determined as barium sulfate (BaSO_4) gravimetrically. This is to compare the results for the two methods for crosschecking.

Chemical removal

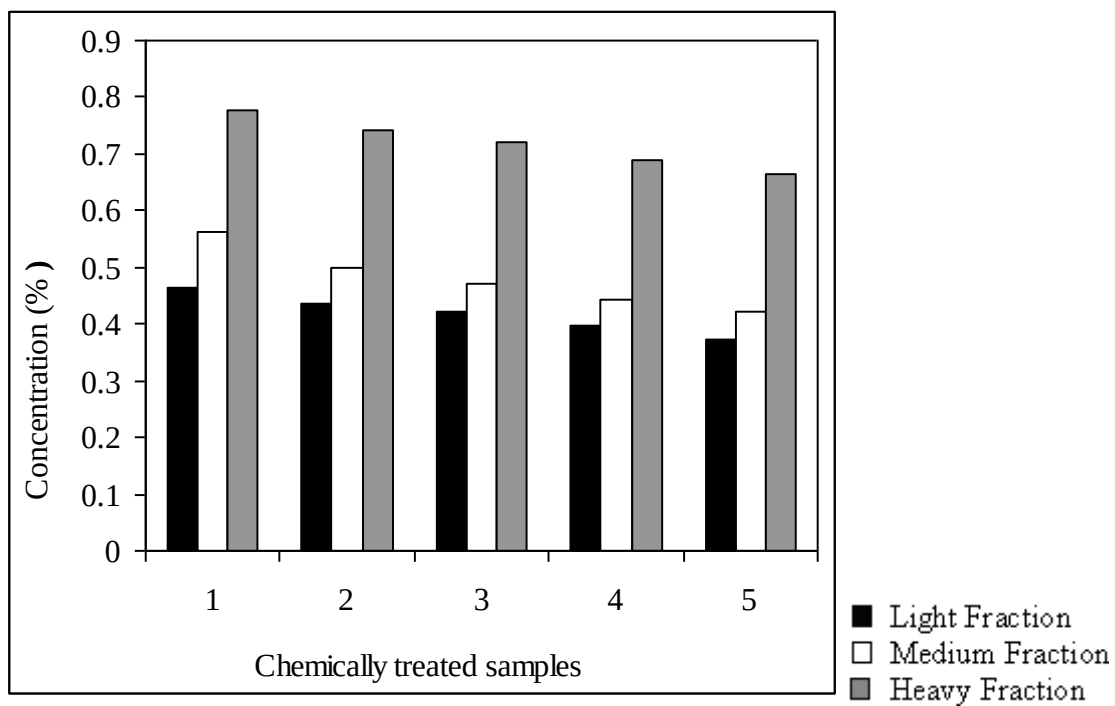
The contents of total sulfur determined in all the original distillate fractions were analyzed and the cumulative data has been shown in Fig-13. The sulphur contents of these samples were: light fraction, 0.4652 %; middle fraction, 0.5612 % and heavy fraction, 0.7786%. The fractions were then chemically desulphurized. The sulphur was determined in each chemically scrubbed sample. The values are graphically provided in Fig.13. The sulphur contents determined in HCl treated samples were: 0.4344, 0.4986, & 0.7432 %, where as those determined in NaOH scrubbed samples were: 0.4235, 0.4712, & 0.7213 %. Similarly, in case of Na_2HASO_4 scrubbed fractions, the values noted were: 0.3985, 0.4432, & 0.6905 %. The scrubbed fractions from NH_4VO_3 treatment show 0.3733, 0.4212, & 0.6655 % sulfur levels determined in case of light, middle and heavy fractions, respectively.

Variety of chemical reagents has been used in term of chemical desulphurization of fuel products with different hydrodesulphurization (HDS) techniques [73].

Comparative investigations in the form of reduction percentages are provided in Fig-14 for original as well as chemically treated crude oil distillates. Significant reduction is noticed in case ammonium vendate treated samples (19.754 , 24.946 & 14.526 %) followed by sodium arsenate (14.337 , 21.206 , & 11.315%), followed by sodium hydroxide (8.963 , 16.037, & 7.359 %) and followed by hydrochloric acid (6.620 , 11.154, & 4.546 %) in case of light, middle and heavy fractions, respectively.

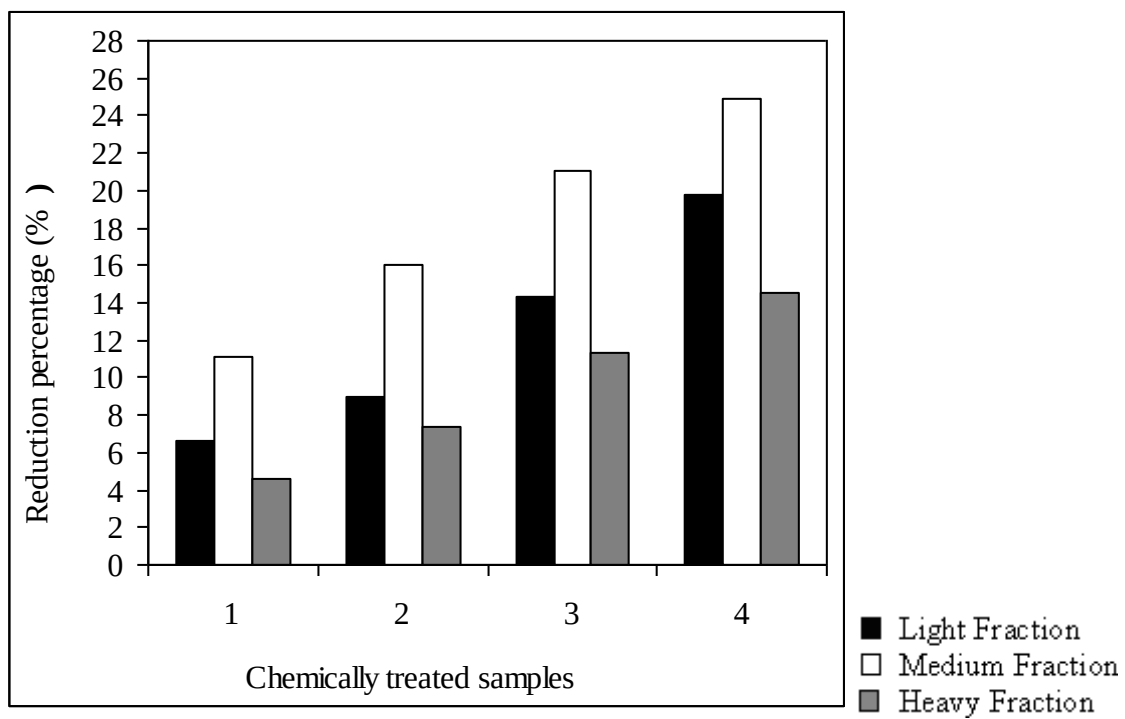
From the data it is evident it is clear that 6.62, 11.15 & 4.54 % ; 6.30, 4.88 & 2.76h , 5.37, 4.99 & 3.96 % and 5.42, 3.92 & 3.21 % has been fonud in the cases of virgin – HCL, HCL - NaOH, NaOH - Na₂HAsO₄ and Na₂HAsO₄ - NH₄VO₃ in light, middle and heavy fractions.

Figure-14: Reduction (%) of total sulfur determined in chemically treated distillate fractions.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.4652 (± 0.000153)	0.5612 (± 0.000153)	0.7786 (± 0.00010)
2	Hydrochloric acid	0.4343 (± 0.000153)	0.4986 (± 0.000153)	0.7432 (± 0.000153)
3	Sodium hydroxide	0.4235 (± 0.000153)	0.4712 (± 0.000208)	0.7213 (± 0.000153)
4	Sodium arsenate	0.3985 (± 0.000153)	0.4432 (± 0.000208)	0.6905 (± 0.000208)
5	Ammonium vendate	0.3733 (± 0.00010)	0.4212 (± 0.000153)	0.6655 (± 0.000153)

Figure-14: Reduction (%) of total sulfur determined in chemically treated distillate fractions.



S #	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	6.620	11.154	4.546
2	Virgin-Sodium hydroxide	8.963	16.037	7.359
3	Virgin-Sodium arsenate	14.337	21.026	11.315
4	Virgin-Ammonium vendate	19.754	24.946	14.526

Removal of Total sulphur with P. Putida

Total sulfur removal capability of *p. putida* was investigated. The inoculation time was kept same as in aforementioned investigations. The data obtained is graphically represented in Fig.15. It can be see that in virgin sample, sulfur has reduced when it has been kept cultivated with *pseudomonas putida* bacterium. In case of 12 hr inoculation time, the total sulphur contents determined were 0.322 , 0.334 & 0.554 % compared to their values determined in uncultivated samples (0.465 , 0.5612 and 0.778%).

The inoculation time was increased beyond 12hr to 24hr. The sulfur contents decreased to 0.287, 0.300 and 0.434%, and then further depletion was made by this bacteria with onward increase in inoculation time and finely during the longest contact time (one month), sulfur contents observed were 0.199, 0.234 and 0.345 % , respectively in light, middle and heavy fractions as shown in Fig.15.

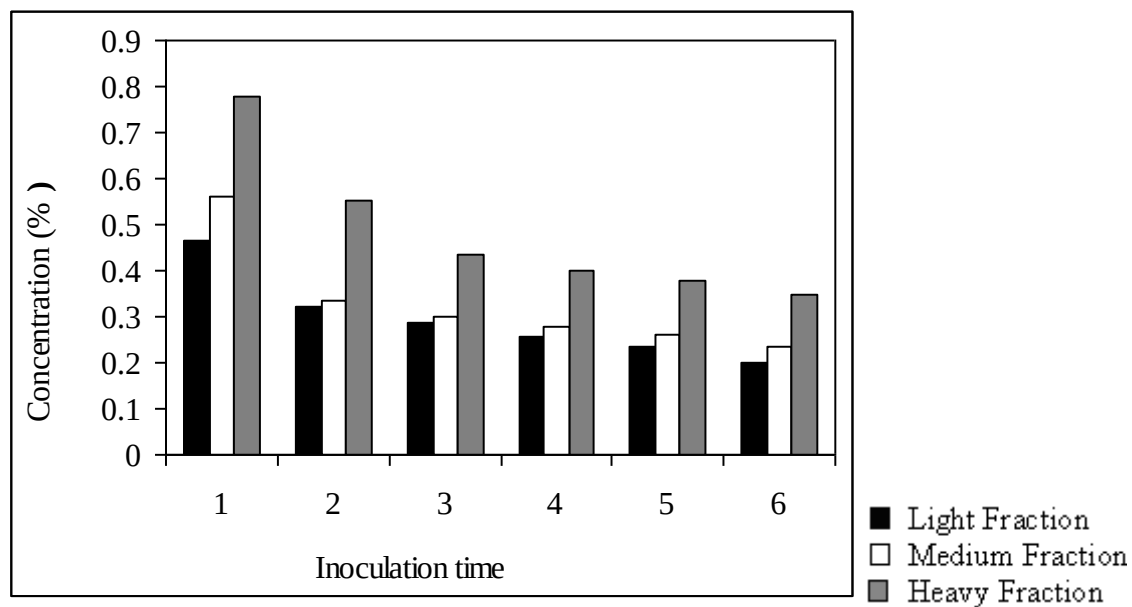
Percent reduction between the treated and untreated samples was calculated. The data is graphically represented in Fig.16. A significant depletion can be observed in all inoculation times. In the initial contact time (12hr), the reduction was 30.71, 40.396 & 28.80 % determined in light, middle and heavy distillation fractions, respectively.

P. putida is a solvent tolerant microorganism, which has the potential to remove sulfur contents from the crude oil. In the beginning, sulfur contents are available in much more concentration, there fore; *P. putida* has rapidly increased its colonies in the first cultivation time i,e 12 hr and reduced about 30.73, 40.377 % 28.53 % of the total sulfur in light, middle and heavy fractions. Total sulphur reduction has been further increased as long as the bacteria remained in the sample oil fraction, which may be used for its growth. In case of 24 hr, 48 hr, one week and one month fermentation brewing time, the

reduction attained values of 38.17 , 46.43 , & 44.19 % ; 44.62 , 50.55 , & 48.93 %; 49.57 , 53.72 , & 51.50 % and 57.09 58.26 , & 55.59 % in light, middle and heavy distillates, respectively as shown in Figure-16.

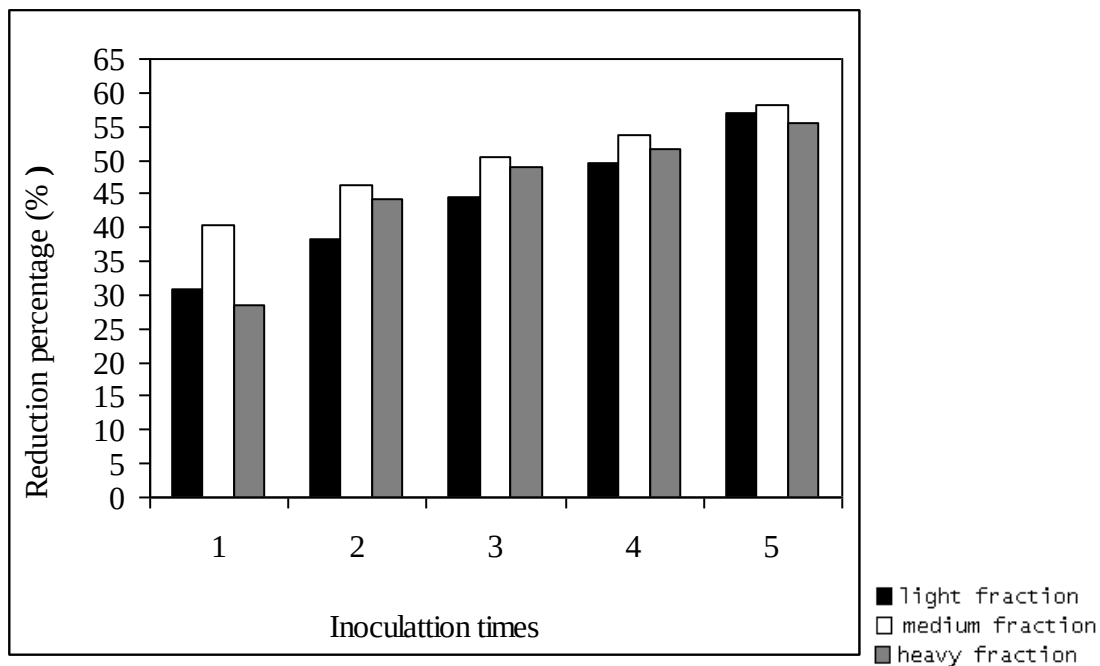
From the figure it seems that 30.73, 40.37 & 28.53 % ; 7.44, 6.06 & 15.66, 6.45, 4.12 & 4.74 % ; 4.95, 3.17 & 2.57 % and 7.52, 4.54 & 4.09 percent reduction has been observed in various inoculation times from virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month in light, meddle and heavy fractions.

Figure-15: Level of total sulfur (%) determined in *Ps.putida* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.4652 (± 0.000153)	0.5612 (± 0.000153)	0.7786 (± 0.00010)
2	12 hours	0.3222 (± 0.000153)	0.3346 (± 0.000153)	0.5543 (± 0.00010)
3	24 hours	0.2876 (± 0.000153)	0.3006 (± 0.000252)	0.4345 (± 0.000153)
4	48 hours	0.2546 (± 0.000153)	0.2775 (± 0.000153)	0.3987 (± 0.000153)
5	One week	0.2346 (± 0.000153)	0.2597 (± 0.00010)	0.3776 (± 0.000153)
6	One month	0.1996 (± 0.000153)	0.2342 (± 0.000153)	0.3457 (± 0.000153)

Figure-16: Reduction percentage (%) of total sulfur determined in *P. Putida* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	30.739	40.377	28.536
2	Virgin -24 hours	38.177	46.436	44.194
3	Virgin -48 hours	44.625	50.552	48.933
4	Virgin -one week	49.570	53.724	51.502
5	Virgin - one month	57.0393	58.267	55.599

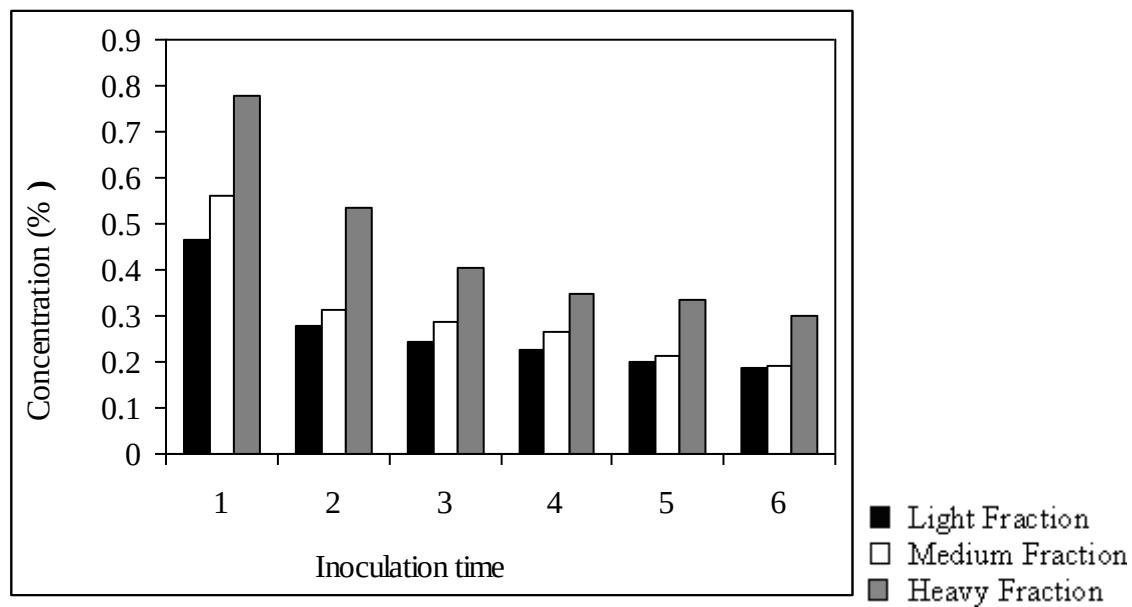
Removal of total sulphur with T thioxidans.

The effect of cultivation / inoculation of oil fractions with *T. thioxidans* was also studied and the results were compared with the virgin samples. The complete data has been presented in Fig.17. The inoculations were performed for a period of 12hr, 24hr, 48hr, one week and one month .The sulphur contents determined in case of 12 hr inoculation were 0.2786 %, 0.3114 %, & 0.5342 %. The sulphur contents observed in case of inoculation performed for time duration of 24 hr were 0.2455 , 0.2875 , & 0.4043 %. Further, the time was extended to 48 hrs, one week and one month. The sulphur contents determined in the residual samples from these inoculations were 0.2244 , 0.2665, & 0.3459 %; 0.1992, 0.2135, & 0.3353 %; 0.1876, 0.1906 ,& 0.3005 %, respectively in cases of light, middle and heavy fractions.

Percent reduction of all the inoculation times was calculated and graphically represented in Fig.18. The values were 40.111, 44.511 & 31.389 % in case of 12 hr inoculation. Similarly, the values determined in case of 24 hr inoculation were 47.226, 48.770, & 48.086 %, and those calculated in case of 48 hr inoculation were 51.762, 52.512, & 55.574 %. Reduction of 57.179, 61.956, & 56.935 % and 59.673, 66.03, & 61.405 % was observed in case of one week and one month inoculations, respectively. It is again very much clear that *T. thioxidans* is a good strain for sulfur removal from the petroleum fractions under study

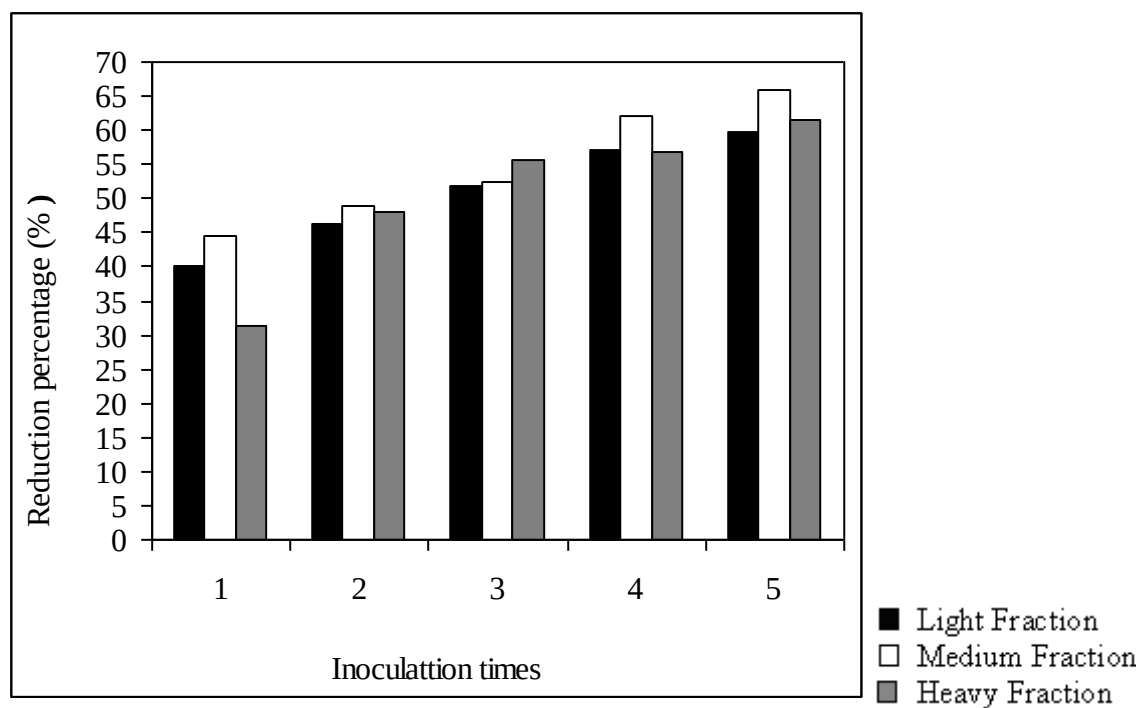
Similarly step wise inoculation times effect has also been studied as 40.11, 44.51 & 31.38 % ; 7.11, 4.26 & 16.7 % ; 4.56, 3.74, 7.49 & 7.49 % ; 5.41, 9.44 & 1.36 % and 2.50, 4.08, 4.47 % in virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month contact times respectively in light, meddle and heavy fractions.

Figure-17: Level of total sulfur (%) determined in *T. thiooxidans* treated distillate fractions as a function of inoculation time



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.4652 (± 0.000153)	0.5612 (± 0.000153)	0.7786 (± 0.00010)
2	12 hours	0.2786 (± 0.000252)	0.3114 (± 0.000252)	0.5342 (± 0.000153)
3	24 hours	0.2455 (± 0.000208)	0.2875 (± 0.000153)	0.4043 (± 0.00010)
4	48 hours	0.2244 (± 0.000208)	0.2665 (± 0.000153)	0.3459 (± 0.000153)
5	One week	0.1992 (± 0.000153)	0.2135 (± 0.000153)	0.3353 (± 0.00020)
6	One month	0.1876 (± 0.00020)	0.1906 (± 0.000153)	0.3005 (± 0.000153)

Figure-18: Reduction percentage (%) of total sulfur determined in *T. thiooxidans* distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	40.111	44.511	31.38940
2	Virgin -24 hours	46.226	48.770	48.086
3	Virgin -48 hours	51.762	52.512	55.574
4	Virgin -one week	57.179	61.956	56.935
5	Virgin - one month	59.673	66.030	61.405

3.2.5 Desulphurization of Elemental sulfur in crude oil distillates fractions.

A rapid and accurate method for the determination of elemental sulfur in hydrocarbons has been adopted in this section because it has been found that in acetone solution, sulfur react rapidly.

Chemical removal of elemental sulphur

Various oil fractions i.e. light, middle and heavy fractions were chemically scrubbed to view the influence of each chemical reagent on elemental sulphur removal. The elemental sulfur contents were determined in virgin samples (untreated) and residual fractions from each chemical treatment. The data is presented in Fig.19. The sulphur levels found in original samples were 63.422, 49.380 & 45.929 ppm. After the chemical treatment, these values were decreased to 55.715 , 44.042, & 43.228 ppm in case of scrubbing the fractions with HCl. NaOH was also used to evaluate its elemental sulphur removing capability. The elemental sulphur contents determined in residual oil samples from this treatment were 53.470, 42.079 & 38.597 ppm, Na_2HAsO_4 and NH_4VO_3 were used as sulphur scrubbing agents. The sulphur contents determined in the residual samples from these treatments were 50.762, 37.347, & 35.070 ppm, in case of Na_2HAsO_4 treatment and 46.574, 34.459, & 31.231 ppm in case of NH_4VO_3 treatment. This UV spectra of virgin and variously chemically treated, fractions as provided in profiles-1 to profiles-3 for light, middle and heavy fractions, respectively. It was found that up to some extent, desulphurization has been made by the chemicals reagents used as shown by the decrease in the intensity of corresponding absorption bands in the UV profiles.

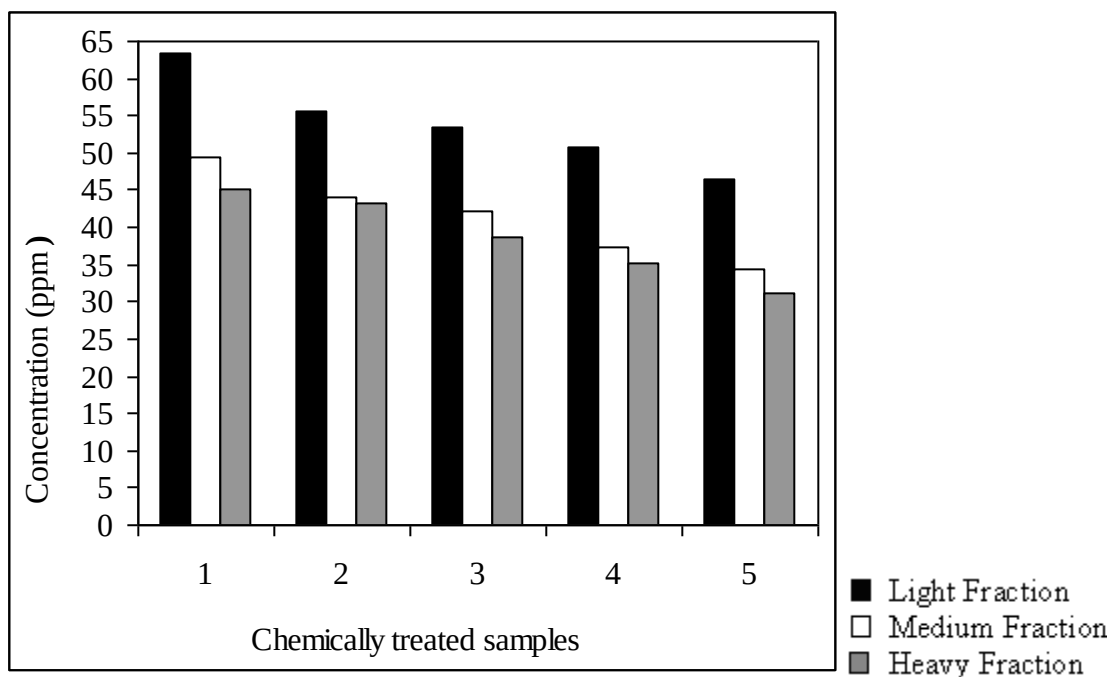
A variety of the chemical catalysts have been designed, which has the ability to remove sulfurous compounds from the petroleum based products. These chemicals

accelerate the C-S cracking activities to cause the maximum desulphurization of the crude oils [74].

Percent reduction was calculated in each case and plotted in Fig. 20. It is seen that considerable amount of sulphur was removed by ammonium vendate i.e 26.564, 30.216 & 32.000 % in case of light, middle and heavy fractions, respectively. Sodium arsenate caused 19.960, 24.360 & 23.643 % while sodium hydroxides caused 15.691 , 14.785 & 16.068 % and hydrochloric acid caused 12.151, 10.511 & 5.880 % sulfur removal in light, middle and heavy fractions, respectively.

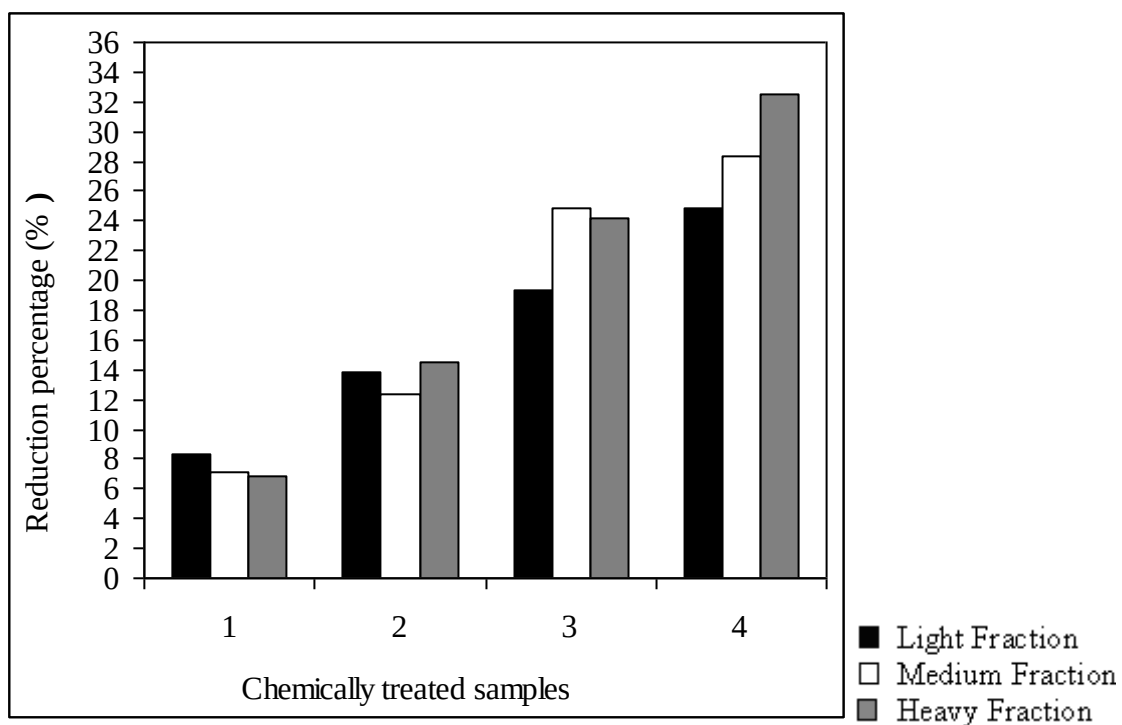
It was found that 12.15, 10.811 & 5.88 % ; 3.54, 3.97 & 10.18 % ; 4.27, 9.58 & 7.58 % and 6.66, 5.58 & 8.36 % in the cases of virgin – HCL , HCL – NaOH, NaOH- Na₂HAsO₄ and Na₂HAsO₄- NH₄VO₃ in light , middle and heavy fractions.

Figure-19. Level of elemental sulfur (%) determined in virgin and chemically treated distillate fractions



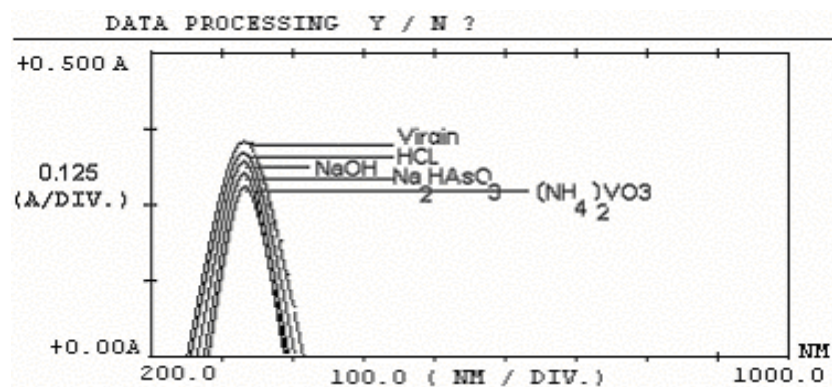
S#	Sample treated	Light fraction (ppm)	Middle fraction (ppm)	Heavy fraction (ppm)
1	Virgin	63.4220 (± 1.0547)	49.3809 (± 0.795)	45.2066 (± 0.629)
2	Hydrochloric acid	55.7157 (± 1.523)	44.0423 (± 1.488)	43.2283 (± 1.112)
3	Sodium hydroxide	53.4704 (± 0.973)	42.0797 (± 0.7271)	38.5972 (± 1.0013)
4	Sodium arsenate	50.7626 (± 1.730)	37.3475 (± 0.6799)	35.0700 (± 0.675)
5	Ammonium vendate	46.5743 (± 0.8947)	34.4598 (± 1.0613)	31.2319 (± 0.669)

Figure-20: Reduction (%) of elemental sulfur in chemically treated distillate fractions

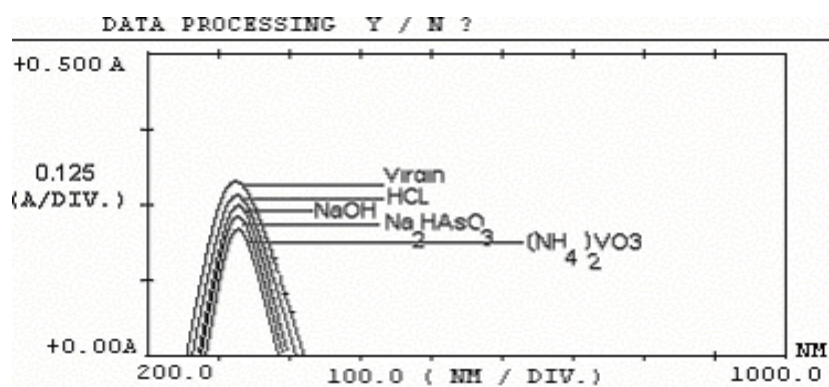


S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	12.151	10.8110	5.880
2	Virgin-Sodium hydroxide	15.691	14.785	16.068
3	Virgin-Sodium arsenate	19.960	24.360	23.643
4	Virgin-Ammonium vendate	26.564	30.216	32.000

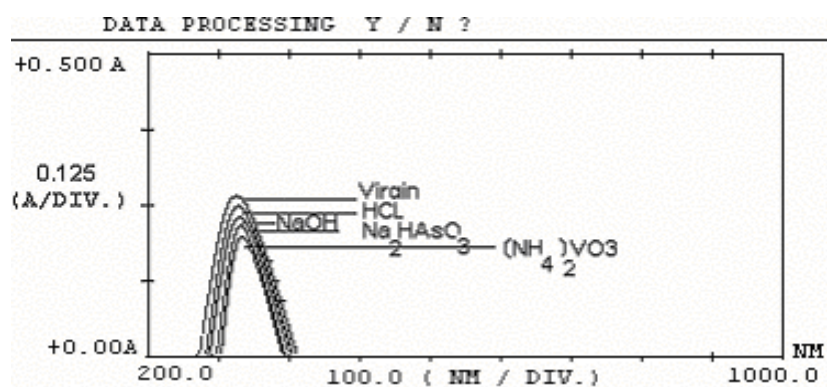
Spectrum profile-1. Elemental sulfur UV spectra of virgin and variously chemically treated light fraction



Spectrum profile-2. Elemental sulfur UV spectra of virgin and variously chemically treated middle fractions.



Spectrum profile-3. Elemental sulfur UV spectra of virgin and variously chemically treated heavy fractions.



Biological removal of elemental sulphur

Removal with P.putida

The removal efficiency of *P.putida* in term of elemental sulphur was viewed. The samples were cultivated with the strain for different inoculation times like 12 hr, 24 hr, 48 hr, one week and one month. The elemental sulphur was determined in the samples understudy before and after different inoculations. The data is represented in Fig.21. *P. putida* has desulfurized the oil distillates significantly. The sulphur contents observed in residual samples from 12 hr inoculation were 44.738, 40.947 & 34.153 ppm. In case of 24 hr inoculation, the sulphur contents determined in the residual samples were 38.842, 33.859 & 28.478 ppm and in case of inoculation performed for 48 hr, the sulphur contents determined in the residual samples were 34.459, 24.245 & 23.370 ppm, in light, middle and heavy fractions, respectively.

As the contact time was extended, sulfur level was further reduced to 28.071, 23.369 & 21.751ppm, and 23.0949, 21.352 & 19.830ppm in one week and one month inoculations. Comparing the results with the chemical desulphurization, it is clear that biodesulfurization is more helpful than chemical treatment. Almost more than half of elemental sulfur was removed in this treatment.

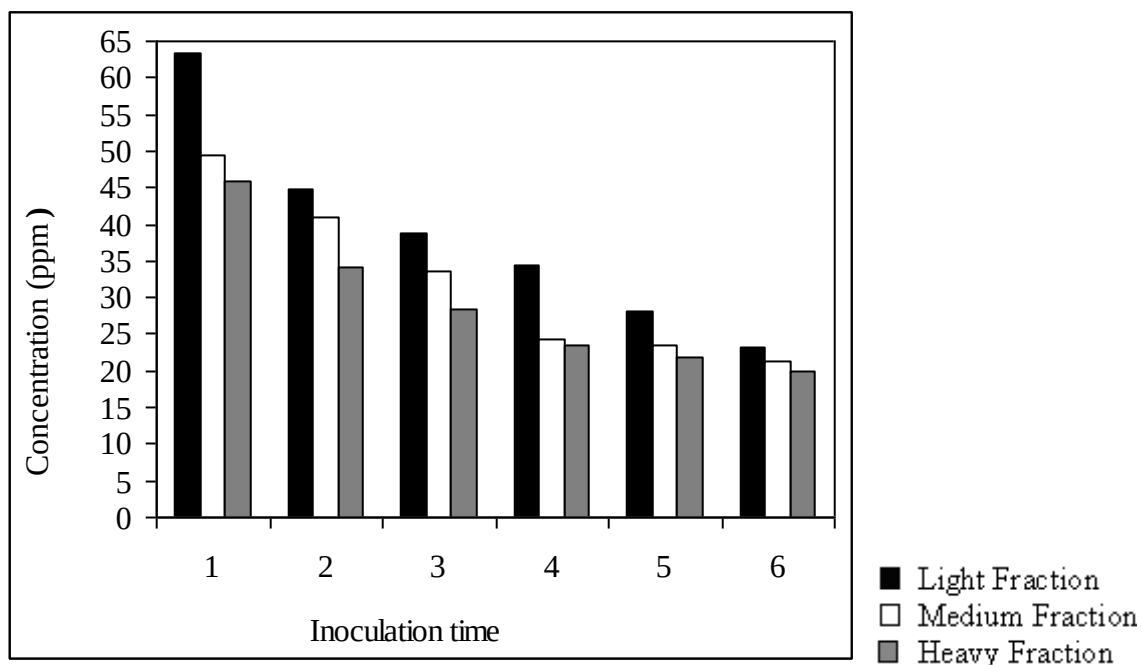
The depletion was calculated in percentages between the virgin and all the treated samples. The data has been shown in Fig. 22.. A gradual reduction has been made by *P. putida* of elemental sulfur. In inoculations performed for 12hr, 24hr, 48hr and one week period, the reduction observed was 29.45, 17.07 & 25.64 %; 39.358, 31.979 & 37.99 %; 45.66, 50.88 & 49.11 % and 63.42, 52.67 & 52.64 %, respectively for the three distillate fractions.

Different values were being observed in various inoculation times, that were 29.45, 17.07 & 25.64 %; 9.9, 14.9 & 12.35 %; 6.31, 18.91 & 11.12 %; 17.76, 1.79, 3.53

% and 0.16, 4.08 & 3.96 %. % in virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month contact times respectively in light, meddle and heavy fractions.

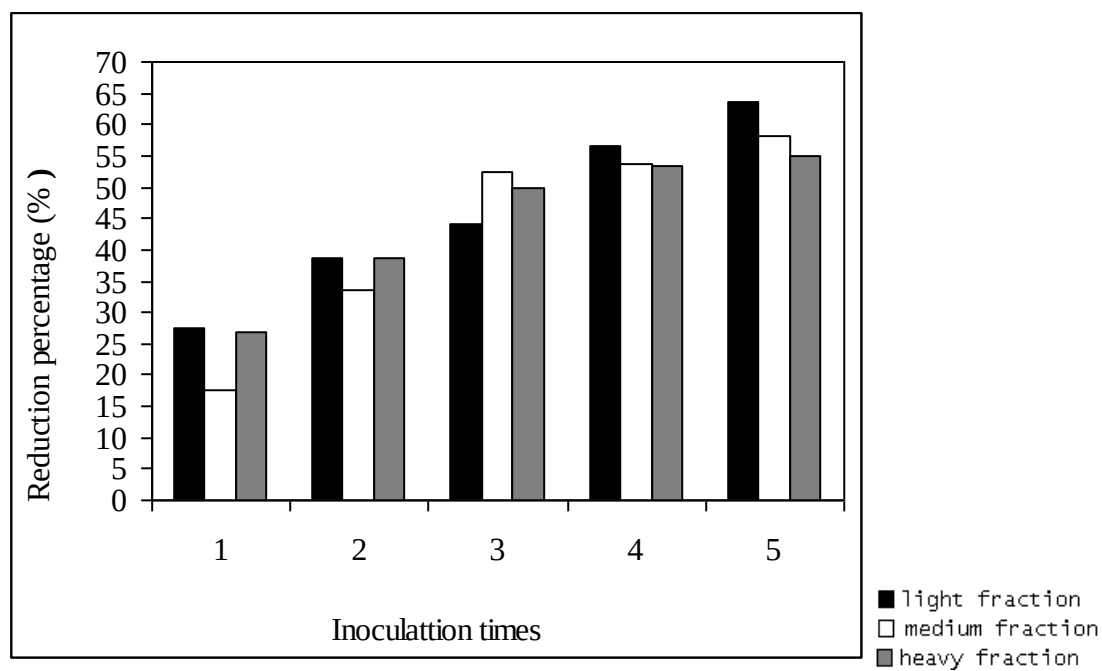
Maximum reduction has been made by one month inoculation. The depletion can be seen as in the form of decline in the intensity of the corresponding bands in VU spectrum profiles-4 to spectrum profiles-6 for all the three distillates.

Figure-21. Level of elemental sulfur (ppm) determined in *P.putida* treated distillate fractions as a function of inoculation time.



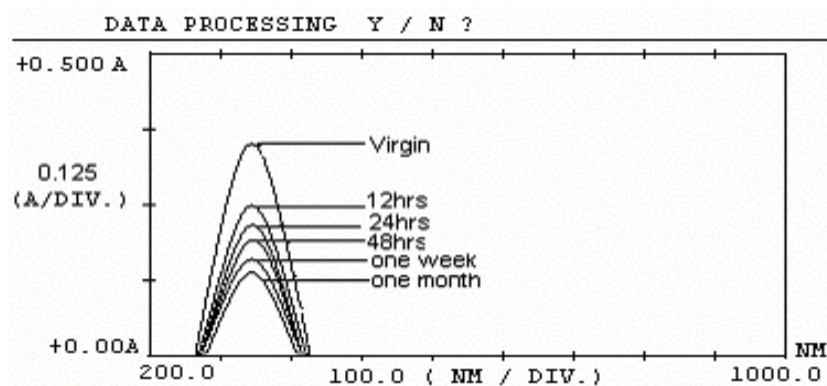
S#	Sample treated	Light fraction (ppm)	Middle fraction (ppm)	Heavy fraction (ppm)
1	Virgin	63.4220 (± 1.05477)	49.3809 (± 0.7955)	45.9294 (± 0.629)
2	12 hours	44.7384 (± 0.4140)	40.9477 (± 0.804)	34.1531 (± 0.927)
3	24 hours	38.8422 (± 0.7129)	33.5892 (± 1.5077)	28.4787 (± 0.653)
4	48 hours	34.4598 (± 1.0632)	24.2457 (± 1.113)	23.3701 (± 0.831)
5	One week	28.0710 (± 0.632)	23.3697 (± 0.8134)	21.7511 (± 0.6070)
6	One month	23.0949 (± 0.261)	21.3526 (± 1.019)	19.8301 (± 0.5732)

Figure-22: Reduction (%) of elemental sulfur determined in *P.putida* treated distillate fractions as a function of inoculation time.

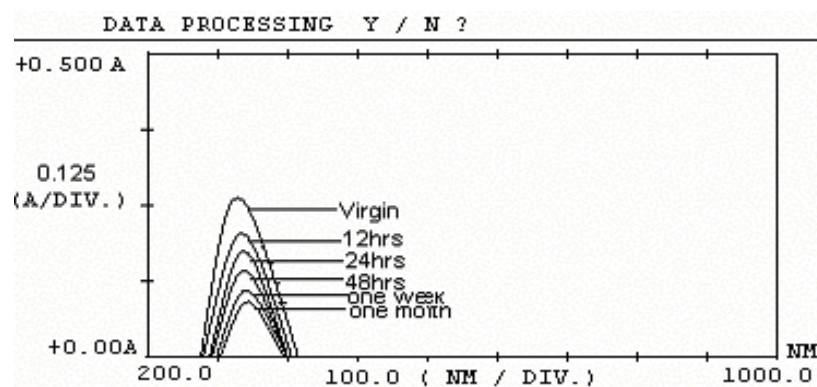


S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	29.459	17.077	25.640
2	Virgin –24 hours	39.358	31.979	37.994
3	Virgin –48 hours	45.666	50.880	49.117
4	Virgin –one week	63.422	52.674	52.642
5	Virgin - one month	63.585	56.759	56.608

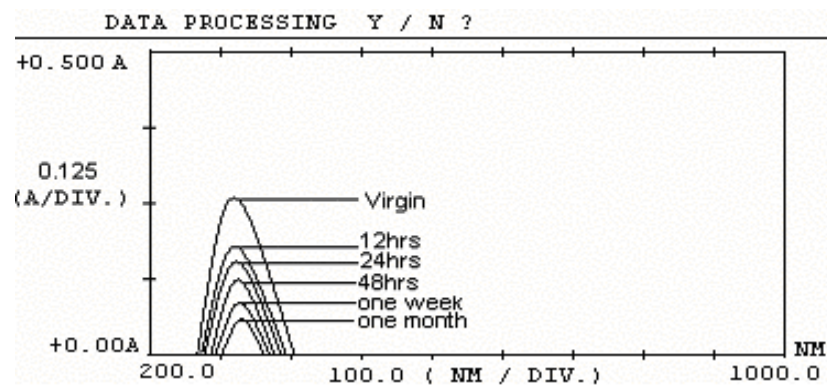
Spectrum profile-4. Elemental sulfur UV spectra of virgin and *P. Putida* treated light fractions



Spectrum profile-5. Elemental sulfur UV spectra of virgin and *Ps. putida* treated middle fractions



Spectrum profile-6. Elemental sulfur UV spectra of virgin and *P.putida* treated heavy fractions



Removal of elemental sulphur with T. thioxidans

Removal efficacy of *T.thioxidans* for elemental sulphur was performed by cultivating distillates fractions for the inoculation times selected in case of *P.putida*. The results are given in Fig. 23. The elemental sulfur contents determined were 41.09, 31.88 & 26.79 ppm in case of 12hr inoculation and 33.21, 27.01 & 23.66 ppm in case of 24 hr inoculation time when compared with original distillates.

The inoculation time was extended beyond 24 hr to one month. The elemental sulfur contents determined were 25.22, 23.27 & 20.65 ppm, 21.93, 21.19 & 18.28 ppm and 18.28, 15.31 & 11.69 ppm in residual samples from 48hr, one week and one month inoculation times, respectively in light, middle and heavy fractions. It means that elemental sulfur was significantly reduced in longer period of contact time to almost one by fourth times that of the original amount present. The depletion can be observed from the decrease in the intensity of bands correspond to elemental sulphur in the UV Spectra given in profiles 7 to 9.

Percent reduction between the treated and untreated samples has been given in Fig.24, which shows 33.93, 35.42 & 41.66 %; 47.62, 45.30 & 48.48 %; 60.23, 52.79 & 55.39 %; 65.41, 57.07 & 60.24 %; and 71.16, 66.26 & 74.54 % depletion in 12h, 24h, 48h, one week and one month inoculations, respectively.

Step wise difference of percentage reduction was also calculated from the table where as in case of virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month inoculation time were found to be 33.93, 35.42 & 41.66 % ; 13.69, 9.88 & 6.82 % ; 12.61, 7.49 & 11.76 % ; 5.18, 4.28 & 6.55 % and 5.75, 9.19 & 14.30 respectively in light, meddle and heavy fractions.

Elemental sulfur reduction percentage has been found to be increased along with increase in fermentation time, because *T.Thiooxidans* cells oxidizes elemental sulfur to sulfite, with 1 mol of O₂ consumption per mol of sulfur oxidized to sulfite (75).

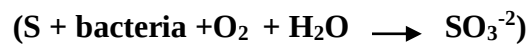
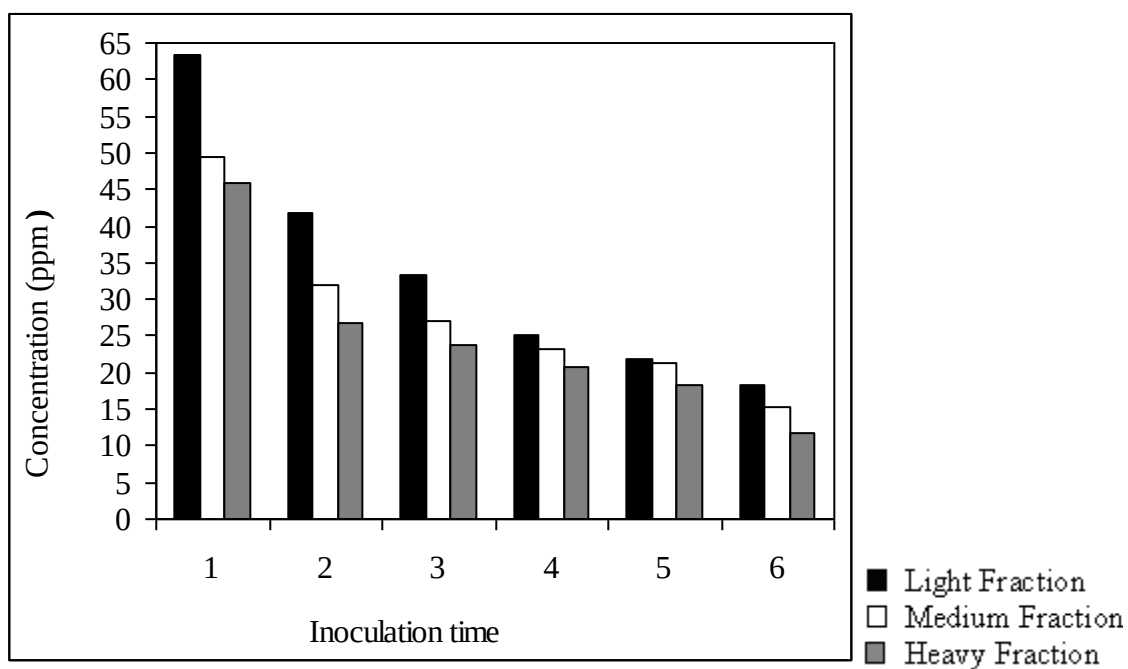
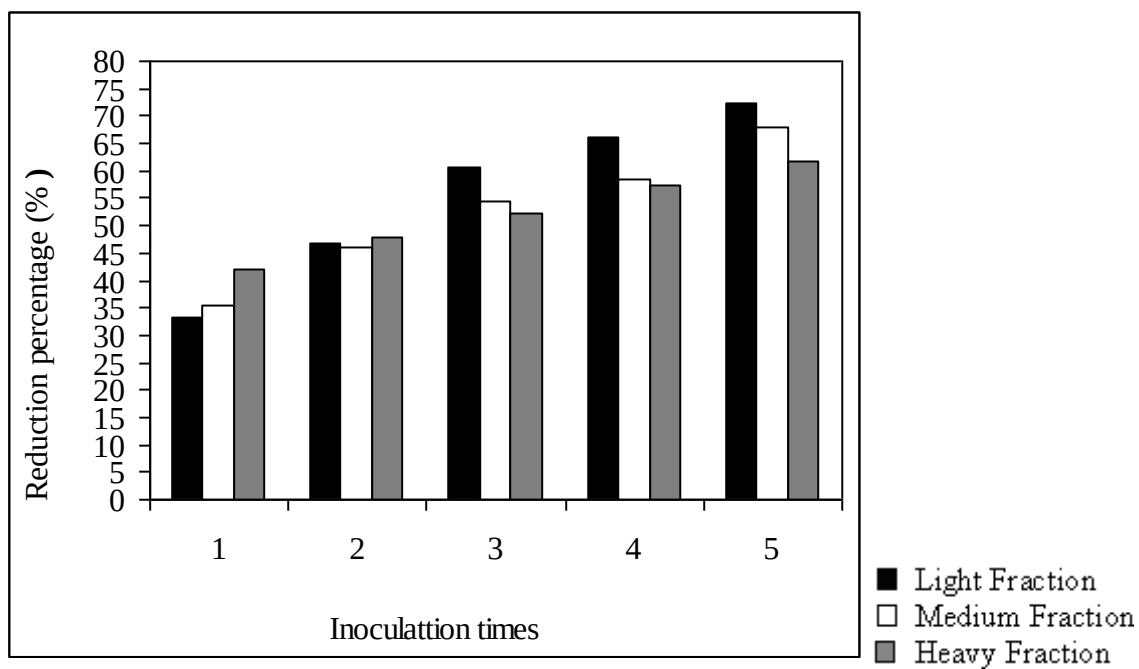


Figure-23. Level of elemental sulfur (ppm) determined in *T. thiooxidans* treated sample as a function of inoculation time.



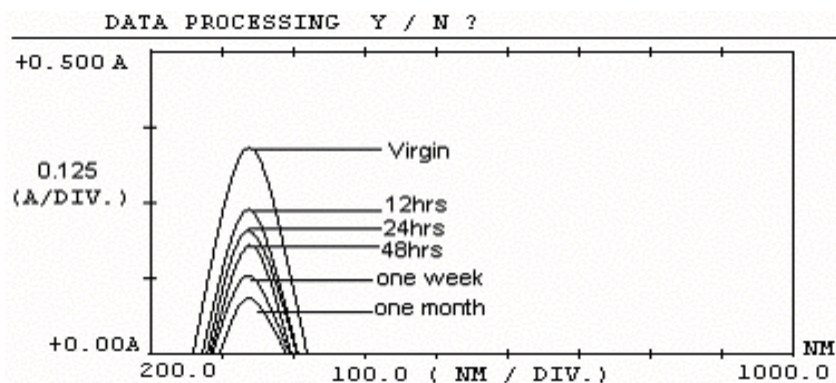
S#	Sample treated	Light fraction (ppm)	Medium fraction (ppm)	Heavy fraction (ppm)
1	Virgin	63.3334 (± 1.05477)	49.3809 (± 0.7955)	45.9294 (± 0.6293)
2	12 hours	41.9011 (± 0.4037)	31.8883 (± 0.4308)	26.7936 (± 0.6583)
3	24 hours	33.2175 (± 1.0012)	27.0110 (± 0.6811)	23.6624 (± 0.5599)
4	48 hours	25.2209 (± 0.6467)	23.273 (± 1.0887)	20.6510 (± 0.8127)
5	One week	21.9334 (± 1.1150)	21.197 (± 0.9280)	18.2878 (± 0.9330)
6	One month	18.2884 (± 0.6824)	15.311 (± 0.9562)	11.6928 (± 0.5274)

Figure-24.Reduction percentages (%) of elemental sulfur determined in thiobacillus thiooxidans treated samples as function of inoculation distillate fractions

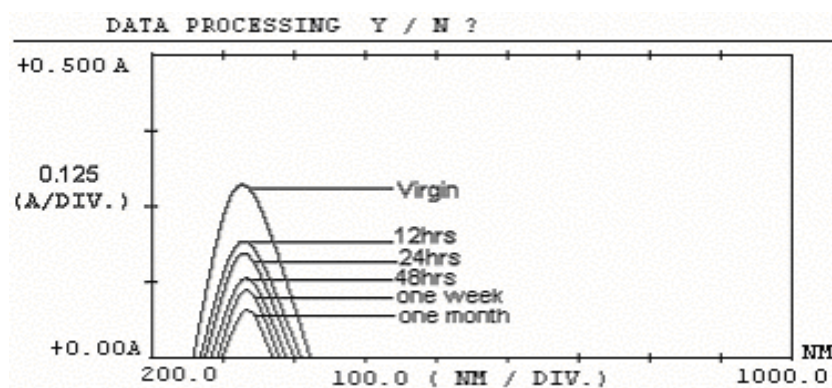


S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	33.9333	35.4230	41.6630
2	Virgin -24 hours	47.6240	45.3007	48.4809
3	Virgin -48 hours	60.2335	52.7920	55.0390
4	Virgin -one week	65.4165	57.0734	60.2480
5	Virgin - one month	71.1637	66.2604	74.5411

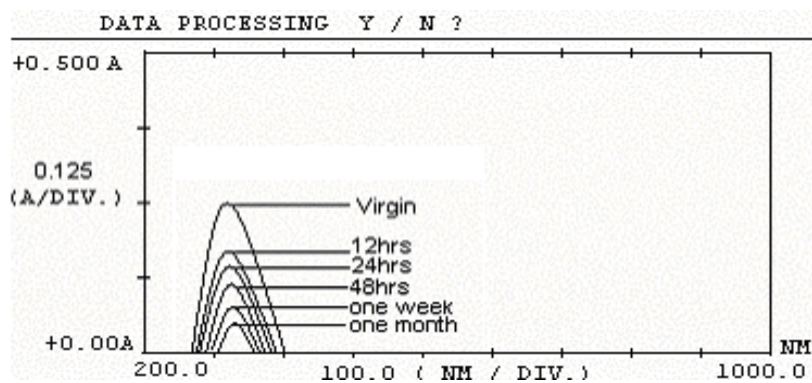
Spectrum profile-7. Elemental sulfur UV spectra of virgin and *T. thiooxidans* treated light fractions



Spectrum profile-8. Elemental sulfur UV spectra of virgin and *T. thiooxidans* treated middle fractions



Spectrum profile-9 Elemental sulfur UV spectra of virgin and *T. thiooxidans* treated heavy fractions



3.2.6. Total sulfides in crude oil distillates

Total sulfide contents in crude oil distillates were determined by titration with 0.1 N potassium iodate solution (KIO_3). The sample was dissolved in a solution of glacial acetic acid +50 ml benzene + 5ml HCl (N). Which was then potentiometrically titrated against 0.1N KIO_3 solution in 80 percent glacial acetic acid.

Chemical Treatment

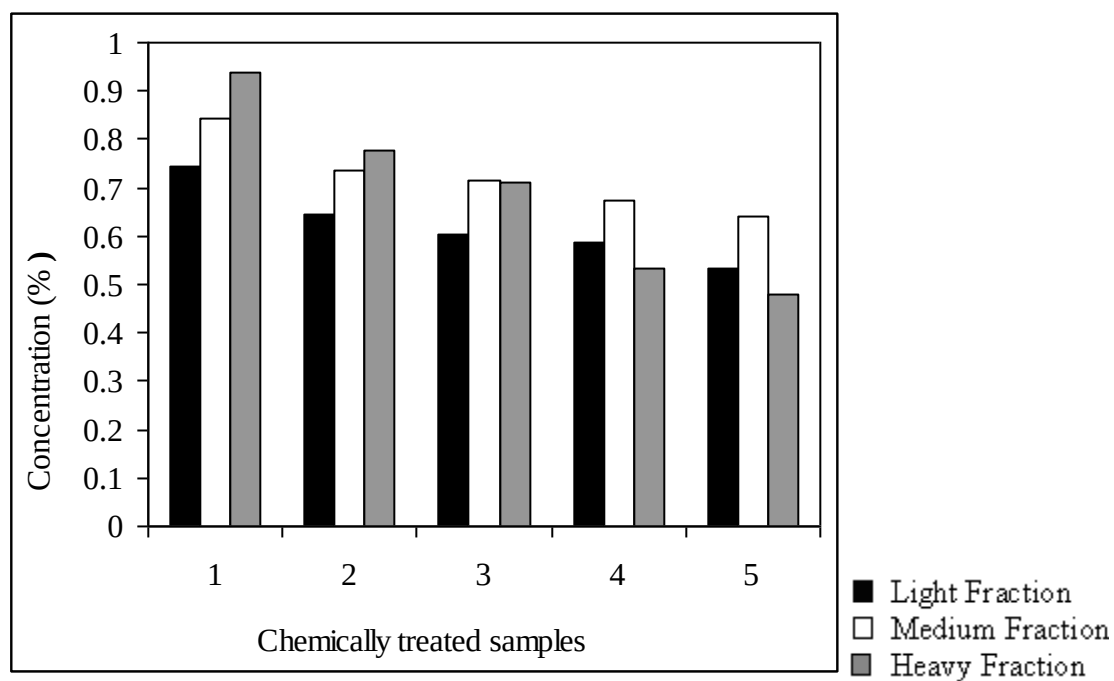
All the three distillation fractions were quantitatively tested for the total sulfides and the data has been compiled in Fig.25. In case of virgin samples, the values were 0.7425, 0.8413 & 0.9375 % in light, middle and heavy distillates, respectively. Scrubbing with some chemical reagents was viewed for removal of this sulphur moiety. The results indicate that the chemical reagents used caused removal of total sulphides. The sulphur contents determined in case of residual samples from HCl scrubbing were 0.6437, 73.51 & 81.49 %, in case of NaOH scrubbed samples were 0.6051, 71.42 & 0.7099 %, in case of Na_2HASO_4 were 0.5886, 0.6755 & 0.5336 %, and in case of NH_4VO_3 were 0.5310, 0.6392 & 0.4807 % determined in light, middle and heavy fractions, respectively.

Percent reduction between original and treated samples was calculated. The data is graphically represented in Fig.26, where a significant depletion of the total sulfides can be seen in case of NH_4VO_3 i.e. 28.48, 24.02 & 48.72 % while depletion caused by Na_2HASO_4 noted as 20.72, 19.70 & 43.08 %. Depletion observed in case of NaOH was 18.50, 15.10 & 24.27 %. HCl caused a poor depletion of 14.65, 12.62 & 13.07 % in case of light, middle and heavy fractions, respectively.

The used chemical reagents has shown different reductions, which were 14.65, 12.62 & 13.07 % ; 3.85, 2.48, & 11.2 % ; 2.22, 4.60 & 18.81 % ; 7.76, 4.32 & 5.64 %

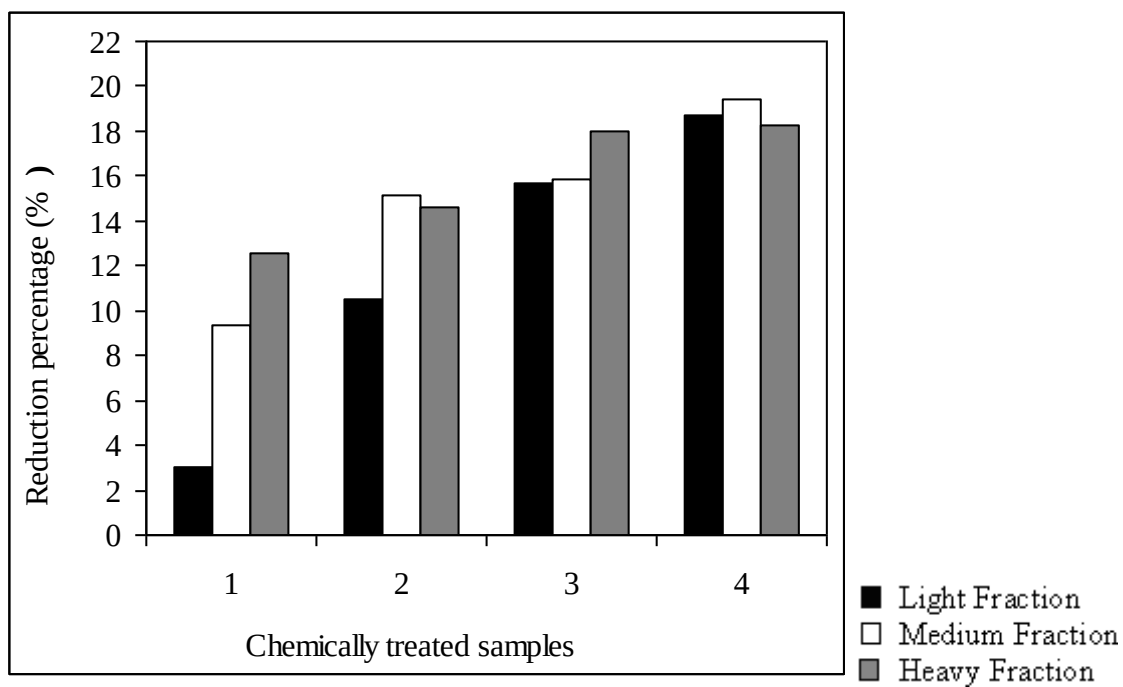
cases of virgin – HCL , HCL – NaOH, NaOH- Na₂HAsO₄ and Na₂HAsO₄- NH₄VO₃
in light , middle and heavy fractions.

Figure-25: Level of total sulfides (%) determined in chemically treated distillate fractions



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin	0.7426 (± 0.00959)	0.8413 (± 0.00767)	0.9375 (± 0.00575)
2	Hydrochloric acid	0.6437 (± 0.0821)	0.7351 (± 0.216)	0.7754 (± 0.0123)
3	Sodium hydroxide	0.6051 (± 0.00743)	0.7142 (± 0.0115)	0.7099 (± 0.0101)
4	Sodium arsenate	0.5886 (± 0.0078)	0.6755 (± 0.005745)	0.5336 (± 0.00807)
5	Ammonium vendate	0.5310 (± 0.004008)	0.6392 (± 0.003035)	0.4807 (± 0.01522)

Figure-26: Reduction (%) of total sulfides determined in chemically treated distillation fractions



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	14.6530	12.6230	13.0773
2	Virgin-Sodium hydroxide	18.5050	15.1070	24.2770
3	Virgin-Sodium arsenate	20.7272	19.7075	43.0820
4	Virgin-Ammonium vendate	28.4840	24.0223	48.7250

Biological Removal of Total Sulphides

Removal of total sulfides was also investigated by cultivating the fractions under trial with two different strains.

Removal with *P.putida*

The crude oil distillates were inoculated with the strain for previously selected times. The reduction in total sulphide noticed in each case is provided in Fig.27. The sulphur contents determined in residual fractions from 12 hr inoculation time were 0.5078, 0.6135 & 0.4185 %. In case of 24hr inoculation time its contents were reduced to 0.4497, 0.5291 & 0.3584 %. Microorganism grow on oxidized sulfur (sulfate, sulfite) as sulfur source and gets energy for its growth. Its growth rate increases in the presences of more organic aromatic sulfurous compounds. The whole surface of the sulfurous compounds are colonized by thick mycelium desulfurizing bacteria, there fore they remove sulfur contents during incubation times in the oil middle. The inoculation time was extended to 48 hr, one week and one month, where the total sulphides determined were found to be 0.3988, 0.4359 & 0.3212 %; 0.3250, 0.3555 and 0.2836 % and 0.2647, 0.2684 & 0.2264 %, respectively in light, middle and heavy fractions.

The deference between the original and microbial treated samples is clear from Fig.28, where a considerable depletion can be seen in case of 12hr contact time, which was 31.60, 27.07 & 55.36 %. With the extension of inoculation time beyond 12hr, reduction further increased, and a maximum reduction can be seen in case of one month contact time i.e 64.35, 68.09 and 75.85 %, in light, middle and heavy fraction, respectively.

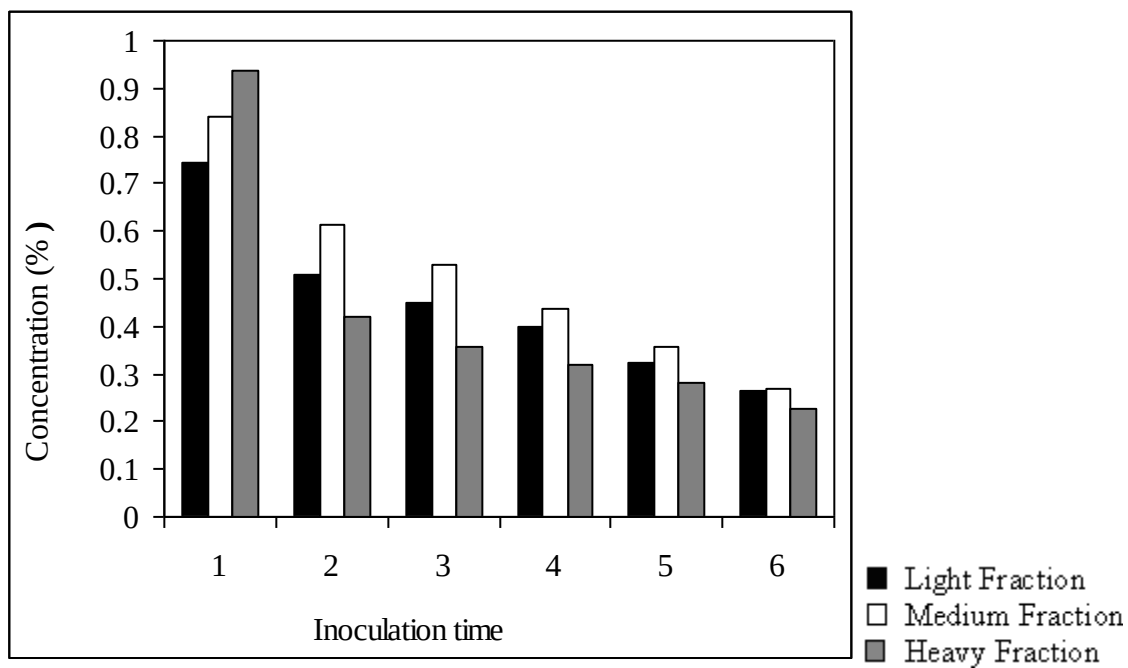
Total sulfides has been checked from among the various inoculation time which were found to be 31.60, 27.07 & 55.36 % ; 7.83, 10.03 & 6.41 % ; 6.85, 11.08 & 3.96 %, 9.94, 9.54 & 4.01 % and 8.13, 10.37 & 6.11 % in the selected times from virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

The reason can be thought for this significance removal in case of the bacterial strains is sulfides are consumed by microbial enzymes such as cytochrome oxidase, . Bacteria producing these enzymes can assist this oxidation depicted as under:



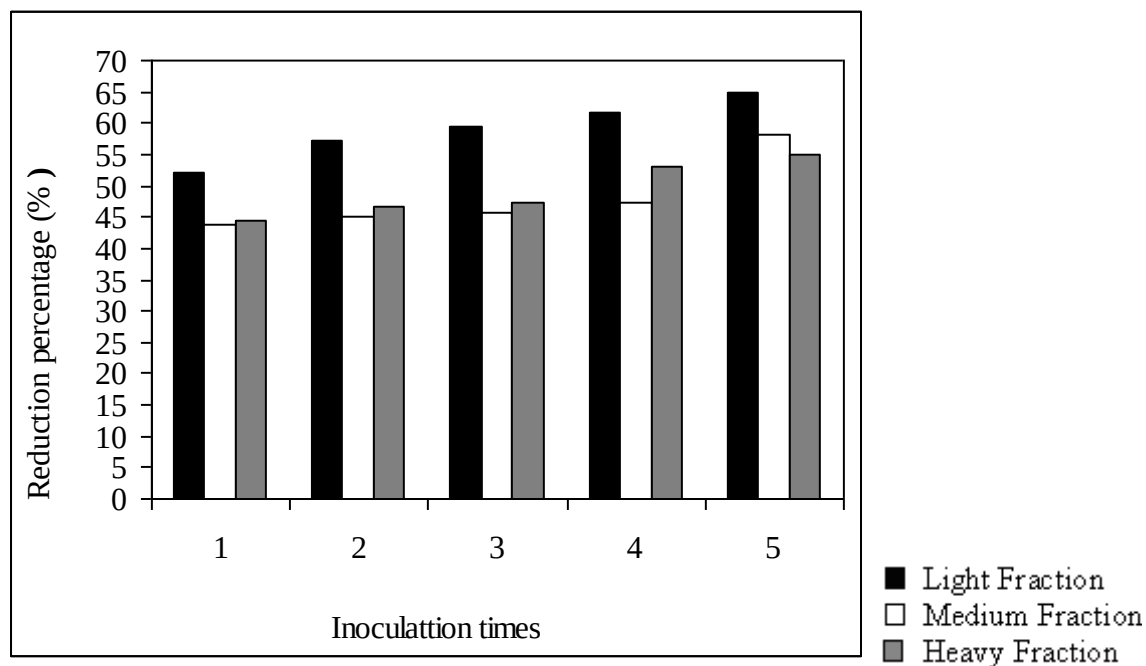
Hence, the sulphides i,e in the form of H_2S or HS are oxidized and converted to elemental sulphur which in turn is utilized by the organisms understudy for their rapid growth and population. Bacteria mostly consume inorganic sulfur in the form of elemental sulfur (S), hydrogen sulfides (H_2S) for its growth and nourishment (76)

.Figure-27: Level of total sulfides (%) determined in *P.putida* treated distillate fractions as function of inoculation time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin	0.7426 (± 0.00959)	0.8413 (± 0.00767)	0.9375 (± 0.0095)
2	12 hours	0.5078 (± 0.0056)	0.6135 (± 0.01053)	0.4185 (± 0.0044)
3	24 hours	0.4497 (± 0.003761)	0.5291 (± 0.0129)	0.3584 (± 0.0129)
4	48 hours	0.3988 (± 0.00355)	0.4359 (± 0.0095)	0.3212 (± 0.00227)
5	One week	0.3250 (± 0.0100)	0.3557 (± 0.00892)	0.2836 (± 0.00175)
6	One month	0.2647 (± 0.00667)	0.2684 (± 0.00147)	0.2264 (± 0.00696)

Figure-28: Reduction (%) of total sulfides determined in *P.putida* treated distillate fractions as a function of inoculation time



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	31.6090	27.0770	55.360
2	Virgin -24 hours	39.4343	37.109	61.770
3	Virgin -48 hours	46.289	48.1870	65.738
4	Virgin -one week	56.228	57.720	69.749
5	Virgin - one month	64.350	68.096	75.850

Removal with T.Thioxidanes

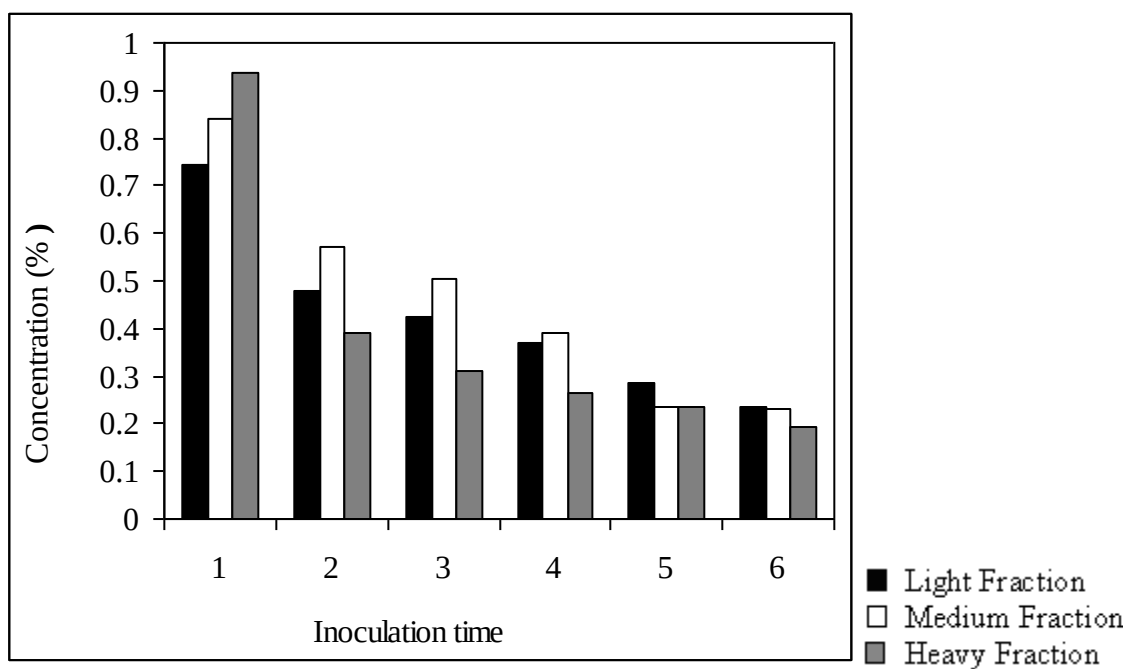
T. Thioxidans was also studied for desulphurization of this sulfur moiety by cultivating the strain with the oil fractions under study for different inoculation times. The results have been summarized in Fig.29. The inoculation was likewise performed for the inoculation time selected from 00 hr- one month. It is evident from the data compiled that total sulphides could be removed by cultivating the oil fractions with strain under study. The contents of this sulphur moiety were determined in the residual samples from each inoculation. In case of inoculation performed for 12 hr, the contents found were 0.4810, 0.5750 & 0.3904. Similarly, depletion was noticed when the same fractions were inoculated for 24 hr. The contents determined in the residual samples were 0.4236, 0.5082 & 0.3106. The time was extended to 48 hr. the results obtained were 0.3704, 0.3926 & 0.2349. The inoculation time was further increased and the cultivation was performed for time duration of one week and then one month. The sulphur contents determined were...0.2862, 0.2336 & 0.2349 and 0.2348, 0.2331 & 0.1933.

Fig.30 represents the reduction percentages obtained in case of different inoculation times versus the original fractions. From the data collected, it has been concluded that *T Thioxidans* has shown better desulphurization results in case of light fraction.

In the initial inoculation time of 12hr and 24hr, the percentage reduction been made by *T. thioxidans* was 35.21, 31.65 & 58.35 %, and 42.64, 39.59 & 66.86 %. Reduction was further enhanced up to 68.37, 72.29 & 79.38 % in case of one month inoculation time.

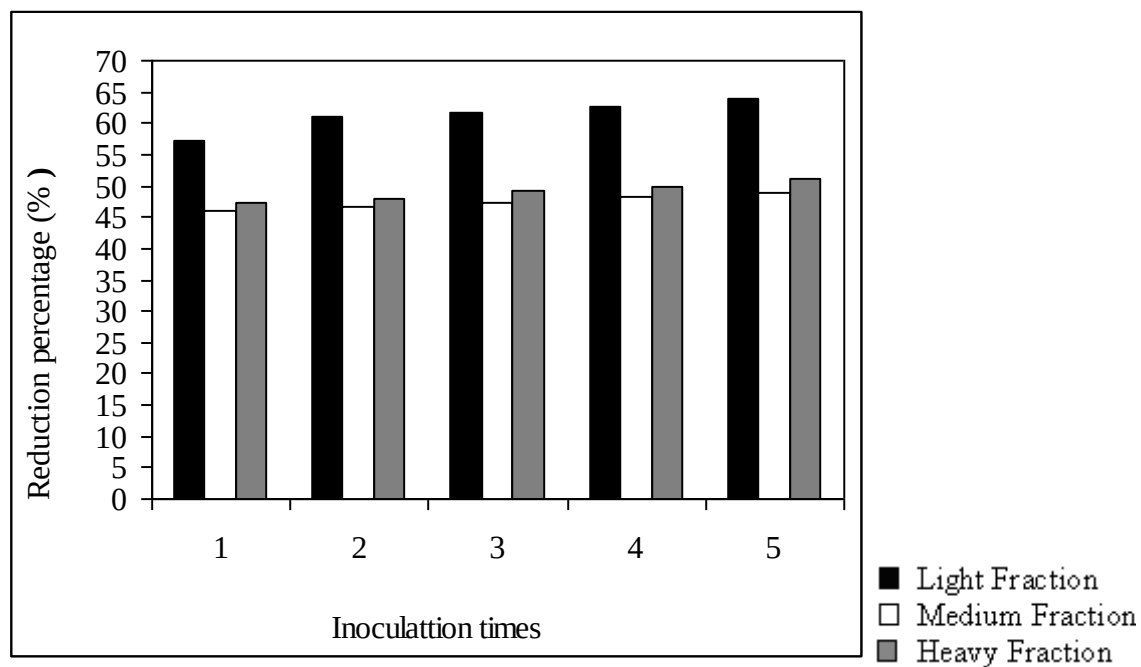
Similarly the difference of the total sulfides among the variously inoculated times was determined, and the data observed was found to be 35.21, 31.65 & 58.35 % ; 7.73, 7.94 & 8.51, % ; 7.17, 13.74 & 4.48 % ; 11.13, 18.9 & 3.24 % and 7.13, 0.06 & 4.44% in cases of virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

Figure-29: Level of total sulfides (%) determined in *T.thioxidans* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin	0.7425 (±0.00959)	0.8413 (±0.00767)	0.9375 (±0.00575)
2	12 hours	0.4810 (±0.01319)	0.5705 (±0.00679)	0.3904 (±0.00909)
3	24 hours	0.4236 (±0.0051)	0.5026 (±0.00386)	0.3106 (±0.00678)
4	48 hours	0.3704 (±0.0070)	0.3926 (±0.00629)	0.2653 (±0.00517)
5	One week	0.2862 (±0.0120)	0.2336 (±0.00536)	0.2347 (±0.00105)
6	One month	0.2348 (±0.0111)	0.2331 (±0.009097)	0.1933 (±0.00412)

Figure-30: Reduction (%) of total sulfides determined in *T. thiooxidans* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	35.218	31.653	58.3570
2	Virgin -24 hours	42.949	39.393	66.869
3	Virgin -48 hours	50.114	53.334	71.7013
4	Virgin -one week	61.248	72.233	74.944
5	Virgin - one month	68.377	72.292	79.381

3.2.7 Removal of Aliphatic sulfides

Aliphatic sulphides were analyzed in the original as well as variously desulphurized samples spectrophotometrically. This method is recommended for the separation of aliphatic sulfides from the petroleum products containing other organic sulfur compounds using Ultra violet spectrophotometer. Each sample was dissolved in carbon tetrachloride solvent. Iodine solution was mixed with the sample solution to get aliphatic sulfide iodine complex.

Chemical Removal

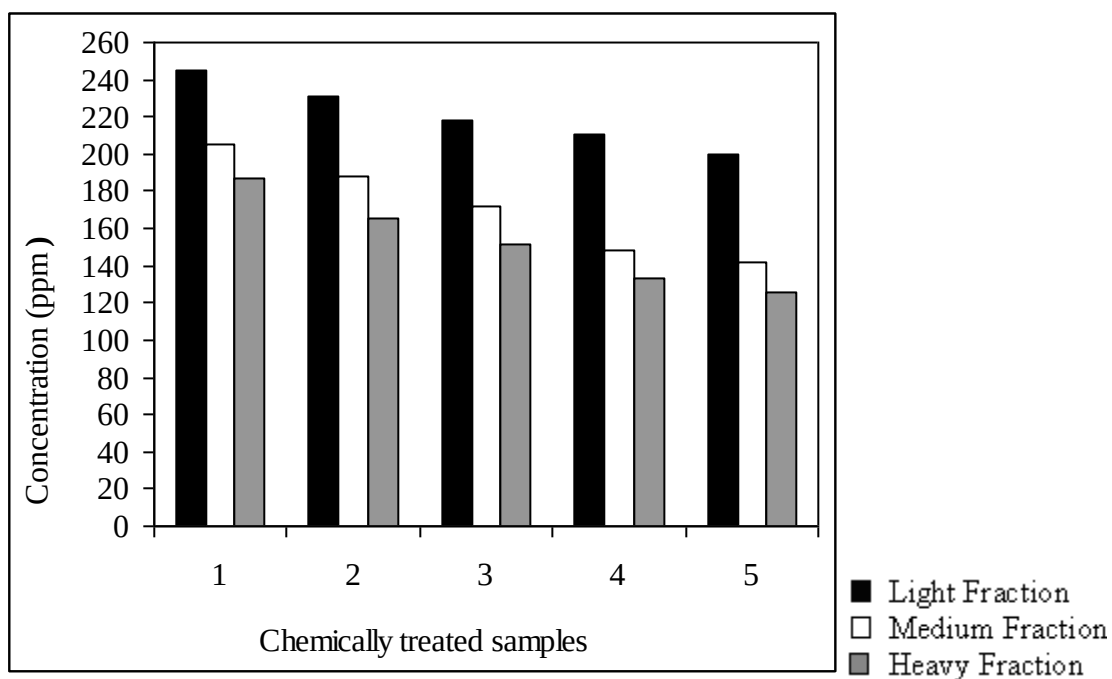
Quantitative investigation of the aliphatic sulfides determined in original samples is represented in Fig.31. The contents of aliphatic sulphide determined in original light, middle and heavy fractions were 245.3743, 20.2753 & 186.4110 ppm, respectively. All crude oil distillates were then scrubbed with four chemical reagents. The aliphatic sulphide determined in case light, middle and heavy fraction after being scrubbed with hydrochloric acid, sodium hydroxide, sodium arsenate and ammonium vendate were 231.3860, 188.3218 & 165.1925ppm; 218.3920, 171.4315 & 151.3401ppm; 210.5214, 148.331 & 133.4916 ppm and 200.3708, 141.3674 & 125.4749 ppm respectively. It is clear that ammonium vendate caused removal of the maximum amount of this sulfur moiety. The removal efficiency of sodium arsenate is found to be inferior compared to ammonium vendate. The removal efficiency of NaOH and HCl was found to be minimal compared to sodium arsenate. The depletion can be observed from the decrease in the intensity of the bands in UV spectra given as pofile-10 to 12.

Percent reduction data of the chemically treated sample versus the untreated sample is given in Fig.32. It is clear that in case of HCl, the reduction was 5.70, 8.25 & 11.38 % and in case of NaOH treated samples; the reduction noted was 10.99, 16.48 &

18.81% determined in light, middle and heavy fractions, respectively. In case of sodium arsenate treated fractions, reduction noted was 14.20, 27.74 & 28.38 %, and in case of ammonium vanadate (NH_4VO_3), the reduction noted was 18.34, 31.13 & 32.68 % in light, middle and heavy fraction, respectively.

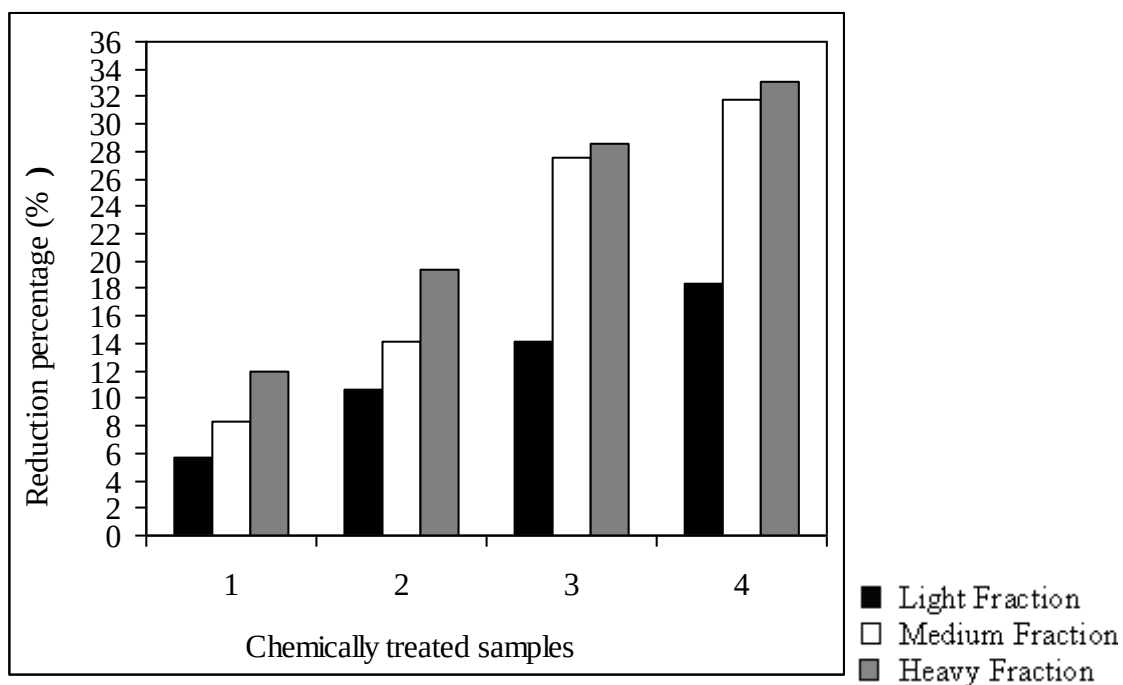
Aliphatic sulfide has been also tested for with the used chemical reagents and the data observed was 5.70, 8.25 & 11.38 % ; 5.29, 8.25 & 7.43 % ; 3.21, 11.26 & 9.57 % and 4.14, 3.39 & 4.3 % cases virgin – HCL , HCL – NaOH, NaOH- Na_2HAsO_4 and Na_2HAsO_4 - NH_4VO_3 in light , middle and heavy fractions.

Figure-31: Level of aliphatic sulfide (ppm) determined in chemically treated distillate fractions.



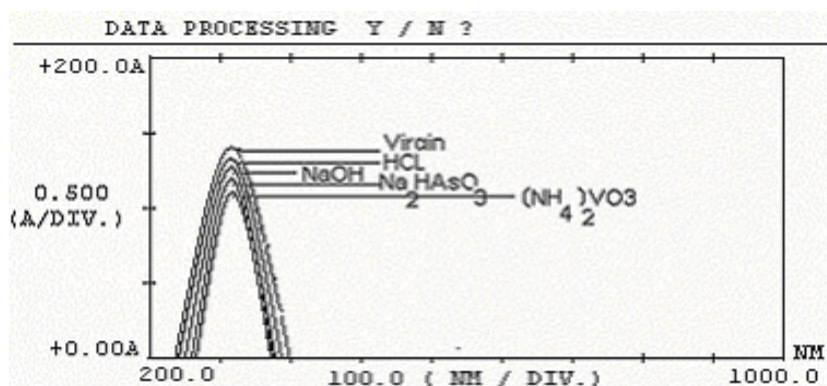
S#	Sample treated	Light fraction (ppm)	Medium fraction (ppm)	Heavy fraction (ppm)
1	Virgin	245.3743 (± 1.1116)	205.2753 (± 1.09231)	186.4110 (± 0.00951)
2	Hydrochloric acid	231.3860 (± 0.86023)	188.3218 (± 0.8025)	165.1925 (± 1.0561)
3	Sodium hydroxide	218.3920 (± 1.1217)	171.4315 (± 1.04226)	151.3401 (± 1.484)
4	Sodium arsenate	210.5214 (± 0.7667)	148.3311 (± 1.1464)	133.4916 (± 1.0965)
5	Ammonium vendate	200.3708 (± 0.8937)	141.3674 (± 0.5657)	125.4749 (± 0.9517)

Figure-32.Reduction (%) of aliphatic sulfide determined in chemically treated distillate fractions.

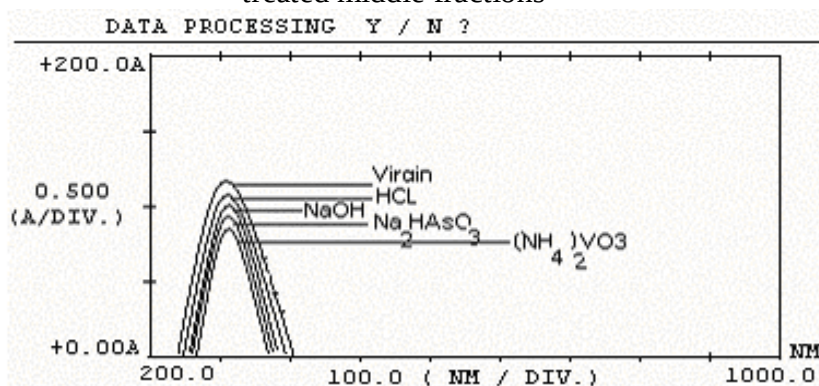


S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	5.7000	8.2589	11.3826
2	Virgin-Sodium hydroxide	10.9963	16.4870	18.8137
3	Virgin-Sodium arsenate	14.2039	27.7404	28.388
4	Virgin-Ammonium vendate	18.3410	31.1327	32.6891

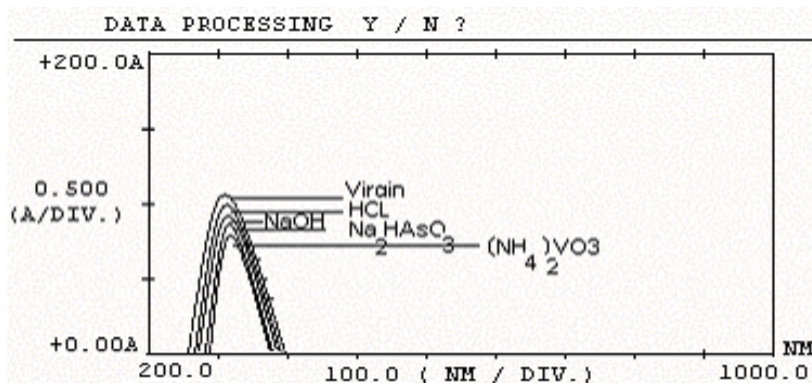
Spectrum profile-10. Aliphatic sulfide UV spectra of virgin and variously chemically treated light fractions



Spectrum profile-11. Aliphatic sulfide UV spectra of virgin and variously chemically treated middle fractions



Spectrum profile-12. Aliphatic sulfide UV spectra of virgin and variously chemically treated heavy fractions.



Microbial removal of aliphatic Sulphides

Removal with P.putida

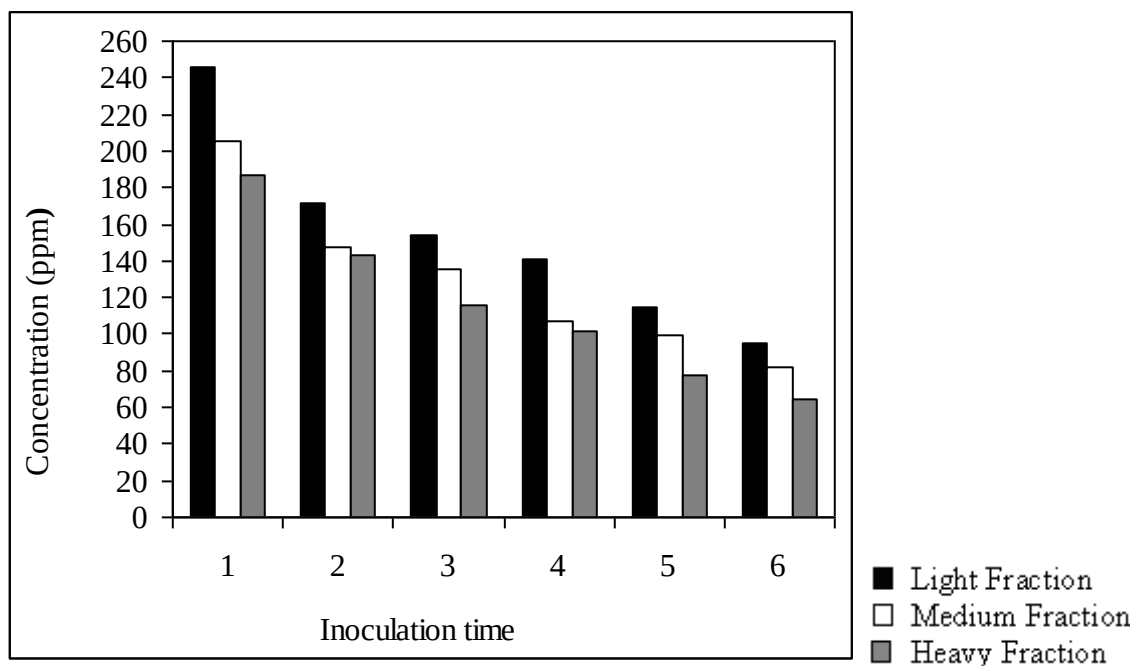
The samples were inoculated for the time duration selected in the aforementioned attempts. The strain selected was *P.putida*. The results obtained are provided in Figs.33.

It appears from the data that a significant amount of sulfur (aliphatic sulfides) has been disappeared in the bacterially cultivated samples. Aliphatic sulfide contents were determined in the residual samples from bacterial inoculations performed for time duration of 12 hr, 24 hr, 48 hr, one week and one month, separately. The contents of this sulphur moiety were 171.2667, 147.6732 & 143.3714 ppm and 154.3842, 136.2767 & 115.6020 ppm in the first two cases of 12 hr and 24 hr, respectively. Inoculation time was again extended to 48 hr, one week and even up to one month, and the sulfur contents determined were found to be 140.9232, 107.5309 and 101.1414 ppm; 114.4612, 99.0088 & 77.4478 ppm and 95.3098, 82.4571 & 64.3758 ppm, in residual light, middle and heavy fractions, respectively. Maximum depletion can be seen in case of one month. Qualitative investigation has also been given in UV spectrum profiles (13-15). A decrease in intensity of absorption bands can be seen when the time was extended from 12 hr to one month.

Percent reduction of all inoculation times (from 12 hr to one month) has been given in Fig.34. The values determined were 30.20, 28.06 & 23.08 ; 37.08, 33.61 & 37.98 ; 42.56, 47.61 & 45.74; 53.35, 51.76 & 58.45 and 61.15, 59.83 & 65.46 in case of 12 hr, 24 hr, 48 hr, one week and one month inoculation times, respectively which is also clear from their UV spectra profiles 31-33.

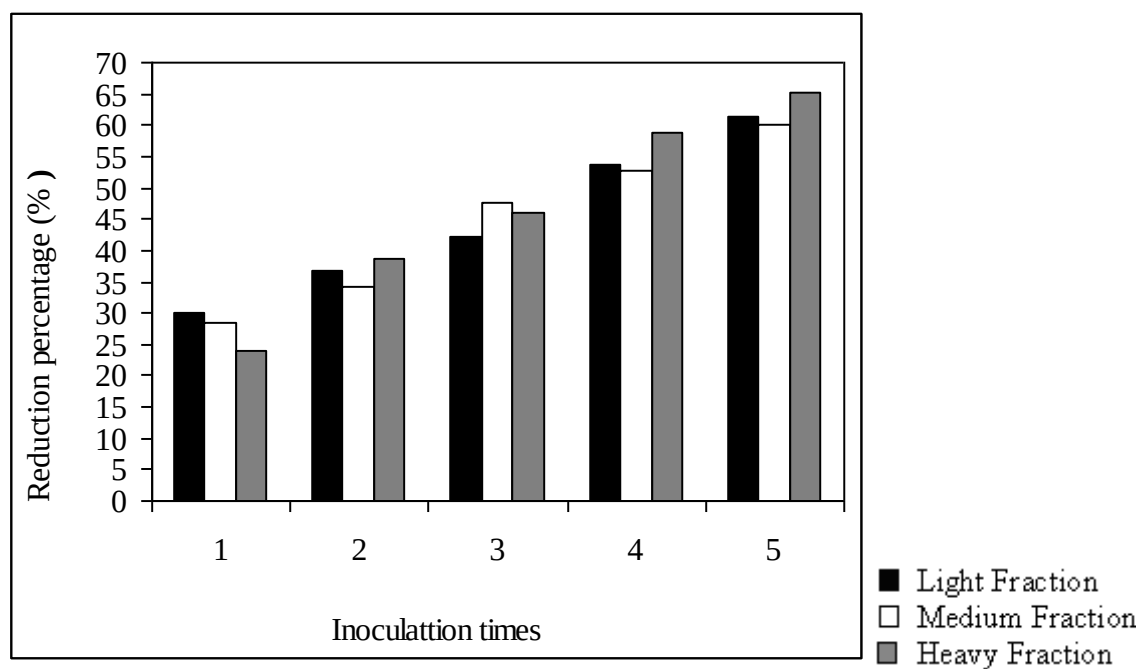
In pseudomonas treated samples similarly the difference of the aliphatic sulfides among the variously inoculated times has been tested, and the findings were 30.20, 28.06 & 23.08 % ; 6.88, 5.55 & 14.9 % ; 5.48, 14.00 & 7.76 % ; 10.76, 4.15 & 12.71 % and 7.83 ,8.07 & 7.01 % cases of virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

Figure-33: Level of aliphatic sulfide (ppm) determined in *P.putida* distillate fractions as a function of inoculation time.



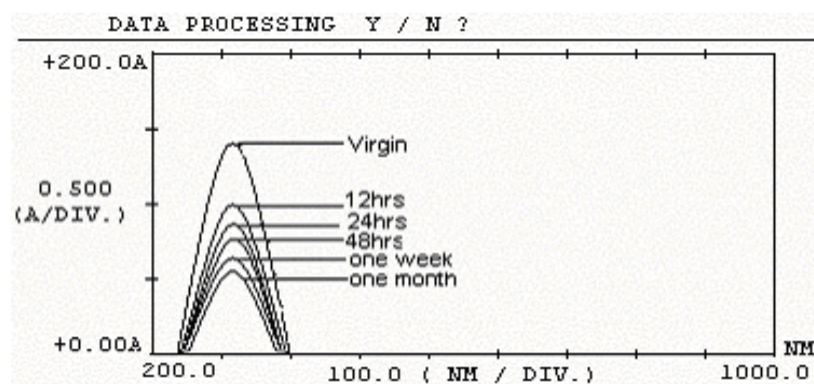
S#	Sample treated	Light fraction (ppm)	Medium fraction (ppm)	Heavy fraction (ppm)
1	Virgin	245.3743 (± 1.1116)	205.2753 (± 1.0923)	186.4110 (± 0.9513)
2	12 hours	171.2663 (± 1.0523)	147.6732 (± 1.2666)	143.3714 (± 0.8287)
3	24 hours	154.3842 (± 1.02595)	135.7065 (± 0.5913)	115.6020 (± 1.4344)
4	48 hours	140.9232 (± 0.7107)	107.5309 (± 0.8277)	101.1414 (± 0.8721)
5	one week	114.4612 (± 1.02931)	99.0088 (± 1.2391)	77.4478 (± 1.2971)
6	one month	95.3098 (± 1.02092)	82.4571 (± 1.01455)	64.3758 (± 0.4762)

Figure-34: Reduction (%) of aliphatic sulfide determined in *P.putida* distillate fractions as function of inoculation time.

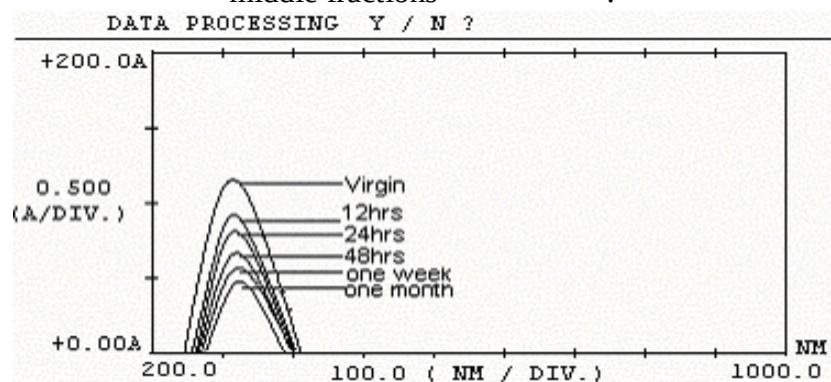


S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	30.2018	28.0609	23.0885
2	Virgin -24 hours	37.0821	33.6127	37.9854
3	Virgin -48 hours	42.5680	47.6162	45.7427
4	Virgin -one week	53.3522	51.7677	58.4532
5	Virgin - one month	61.1573	59.8309	65.4656

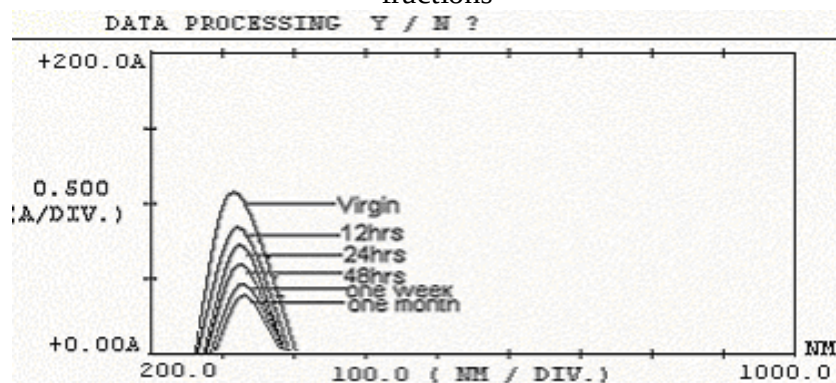
Spectrum profile-13. Aliphatic sulfide UV spectra of virgin and *P. putida* treated light fractions



Spectrum profile-14. Aliphatic sulfide UV spectra of virgin and *P. putida* treated middle fractions



Spectrum profile-15. Aliphatic sulfide UV spectra of virgin and *P.putida* treated heavy fractions



Removal of aliphatic sulphides with T. Thioxidans

Fig. 35 shows the effect of *T. Thioxidans* on the removal of aliphatic sulphides from the three distillate fractions under study. The strain was cultured and kept in contact with each fraction for time duration selected from 12 hr to one month. The reduction in this sulphur moiety can be noticed in each case. The sulphur contents observed in case of light, middle and heavy fractions were 166.6477, 136.5061 & 121.5535ppm ; 145.1804, 121.2605 & 103.4208 ppm ; 133.625, 100.7067 & 88.3729 ppm ; 102.34448, 82.1105 & 64.1688 ; 81.6817, 60.4858 & 50.2762 ppm in residual samples from 12 hr, 24 hr, 48 hr, one week and one month inoculations, respectively.

The UV spectra profiles16-18 demonstrates the data collected from the UV visible spectrophotometer in case of *T.thioxidans* inoculation in light, middle and heavy fractions, respectively. Reduction in band intensity could easily be observed confirming the depletion in this particular sulphur moiety.

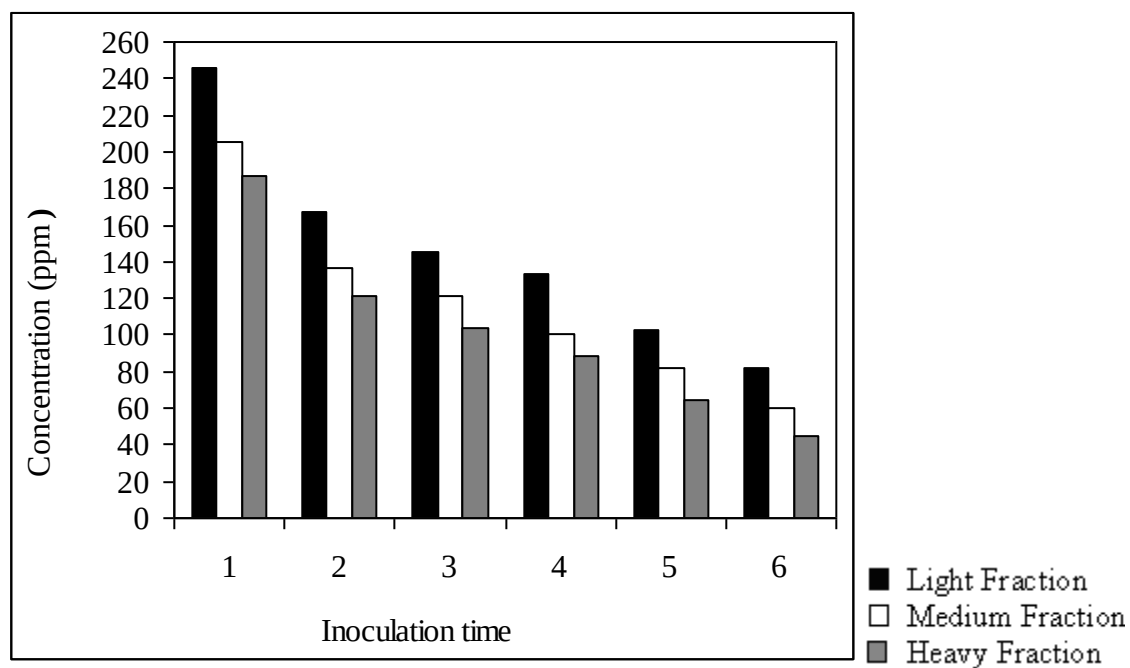
Percent reduction between the untreated virgin and treated samples with *T.thioxidans* has been given in Fig.36 for different inoculation times. The reduction observed in light, middle and heavy oil fractions in case of 12 hr and 24 hr was 32.08, 33.50 & 34.79 % and 40.83, 40.92 & 44.52 %, respectively.

In extended inoculations i.e. those performed for 48 hr, one week and one month, the reduction observed was 45.54, 50.98 & 52.59 % ; 58.28, 60.00 & 65.57 %, 66.711, 70.53 & 75.71 in case of light, middle and heavy fractions. This clarifies that treatment with *T. thioxidans* is more efficient than chemical and even *P.putida* treatment.

Aliphatic sulfides has been tested in the same way for various inoculation times were 32.08, 33.50 & 34.79 % ; 8.00, 7.42 & 9.73 % ; 4.71, 10.06 & 8.05 % ; 12.74, 90.2

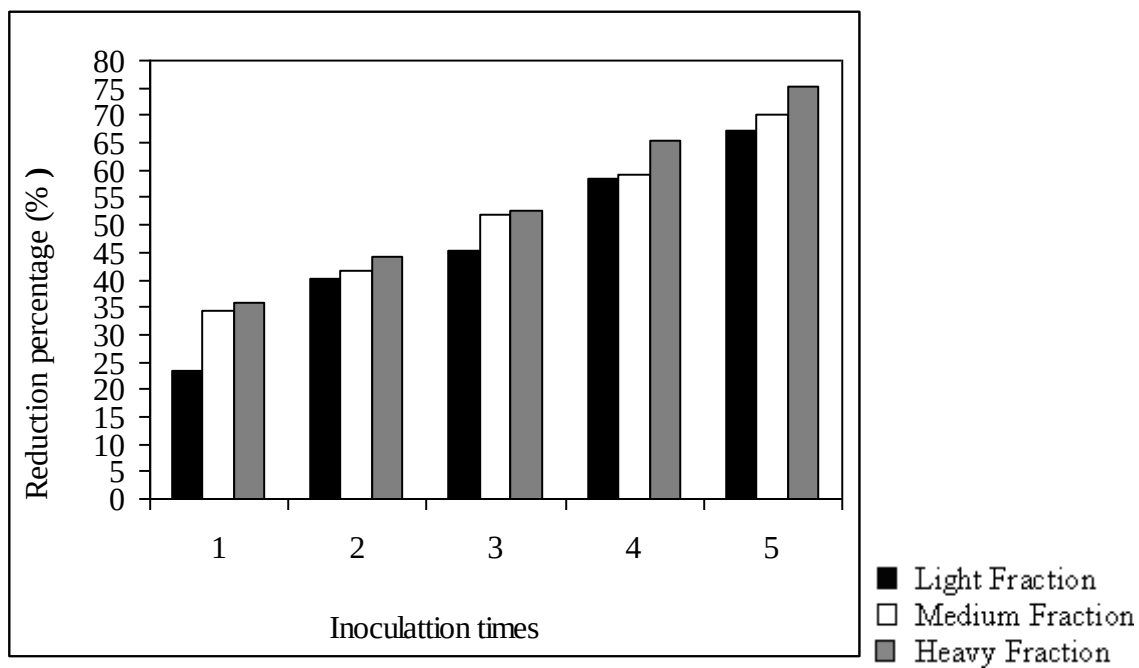
& 12.98 % and 10.14 , 10.53 & 10.14 % in cases of virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

Figure-35: Level of aliphatic sulfide (ppm) determined in *T. thiooxidans* treated sample as a function of inoculation time.



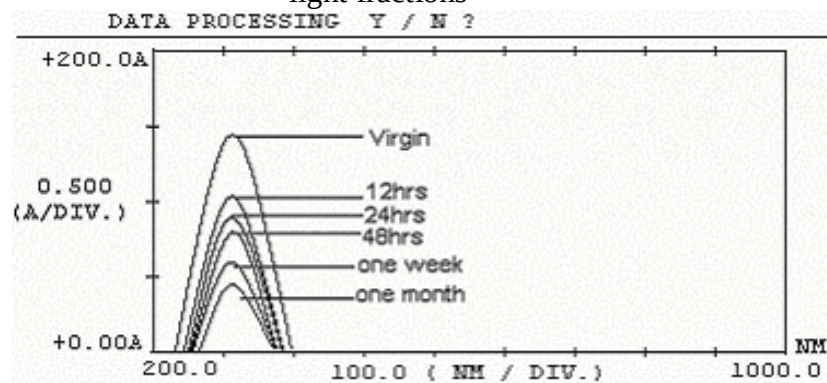
S#	Sample treated	Light fraction (ppm)	Medium fraction (ppm)	Heavy fraction (ppm)
1	Virgin	245.3743 (± 1.1168)	205.2753 (± 1.092)	186.411 (± 0.9513)
2	12 hours	166.6477 (± 1.1476)	136.5061 (± 0.9502)	121.553 (± 1.5425)
3	24 hours	145.1804 (± 0.8978)	121.2605 (± 1.0262)	103.420 (± 1.1699)
4	48 hours	133.6250 (± 0.9310)	100.7067 (± 1.4794)	88.3729 (± 0.7783)
5	One week	102.3448 (± 1.0301)	82.1105 (± 1.6224)	64.1688 (± 0.8834)
6	One month	81.6817 (± 1.4102)	60.4858 (± 0.9819)	45.2762 (± 0.9194)

Figure-36: Reduction (%) of aliphatic sulfide determined in *T. thiooxidans* treated distillate fractions as function of inoculation time.

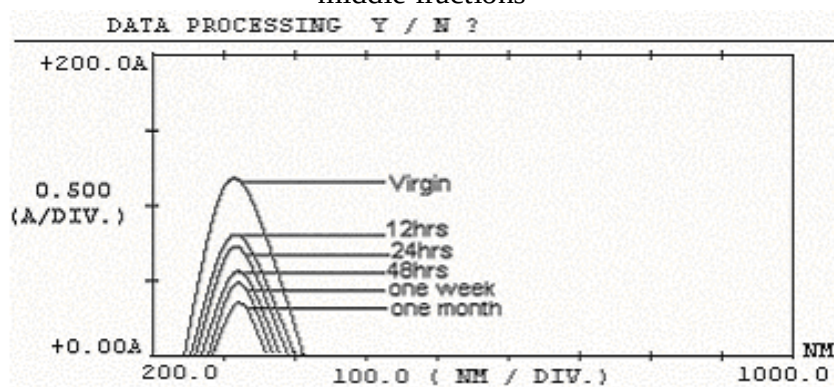


S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	32.0842	33.5009	34.7927
2	Virgin -24 hours	40.83330	40.9278	44.5200
3	Virgin -48 hours	45.5423	50.9888	52.5924
4	Virgin -one week	58.2816	60.0000	65.5776
5	Virgin - one month	66.7113	70.5343	75.7116

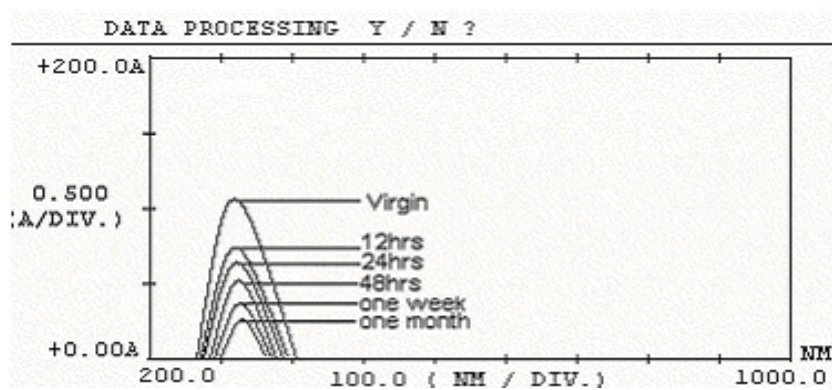
Spectrum profile-16. Aliphatic sulfide UV spectra of virgin and *T. thiooxidans* treated light fractions



Spectrum profile-17. Aliphatic sulfide UV spectra of virgin and *T. thiooxidans* treated middle fractions



Spectrum profile-18 Aliphatic sulfide UV spectra of virgin and *T. thiooxidans* treated heavy fractions



3.2.8. Removal of Mercaptan sulfur

Mercaptan sulfur was determined using ASTM Method 2, 342/79. This method covers the mercaptan sulfur in crude oil fractions. Organic sulfur compounds such as sulfides, disulfides and thiopenes don't interfere; elemental sulfur in very less amount does not interfere also. Hydrogen sulfide will interfere if not removed before treatment. The alternative procedure (color indicator method) is less sensitive and less precise. The hydrogen sulfide free sample is dissolved in an alcoholic sodium acetate titration solvent and titrated potentiometrically with silver nitrate solution. Under this condition, the mercaptan sulfur is precipitated as silver mercaptide.

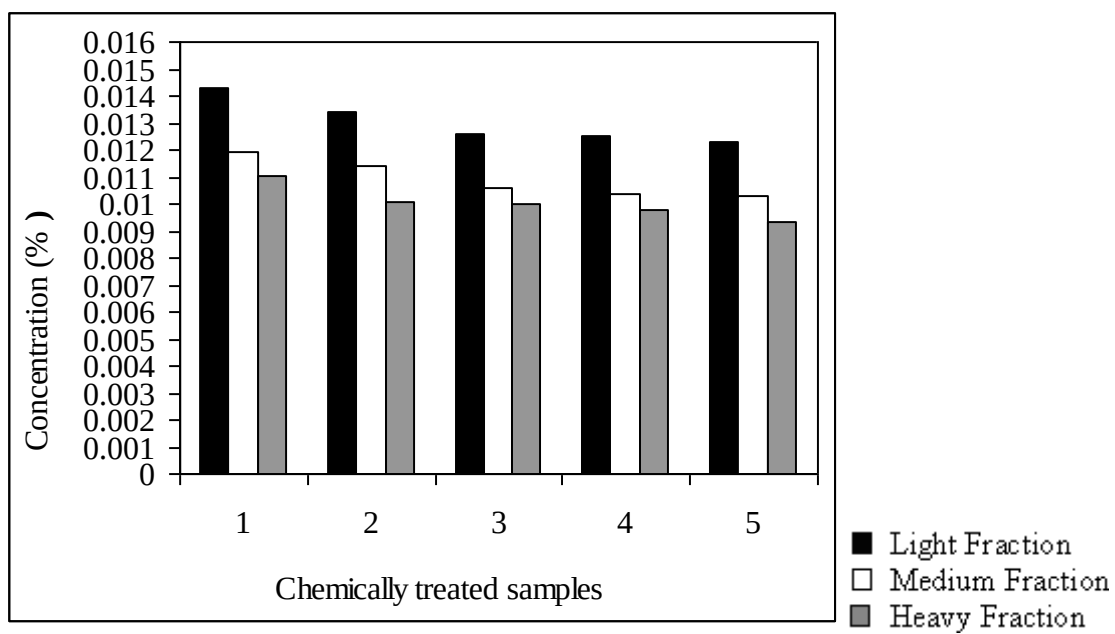
Chemical Removal

The amount of the mercaptan sulphur determined has been shown in Fig. 37 for the original distillates i.e. light, middle and heavy fractions. The contents were 0.0143, 0.019 & 0.01104 %, respectively. Then, all the three distillate fractions were reacted with the already mentioned chemicals. The depletion in mercaptan sulphur was noted in case of each chemical scrubbing. The data is provided in the Fig. where a drop in sulfur level can be seen. The sulphur contents noted were 0.0134, 0.0114, & 0.01008 %; 0.0125, 0.0106, & 0.01003 %; 0.0125, 0.0107, & 0.0098 % and 0.0123, 0.0103, & 0.0093 % in case of hydrochloric acid, sodium hydroxide, sodium arsenate and ammonium molybdate scrubbed samples, respectively. From the data collected, it is understood that these chemicals have dropped the mercaptane sulfur level up to some extent.

Percent reduction of the comparative investigations is given in Fig. 38 for virgin versus various chemically treated samples. The reduction calculated in case of virgin-HCl was 8.29, 4.20, & 8.69 %, virgin-NaOH 11.88, 10.92 & 9.14 %, virgin- Na_2HAsO_4 and

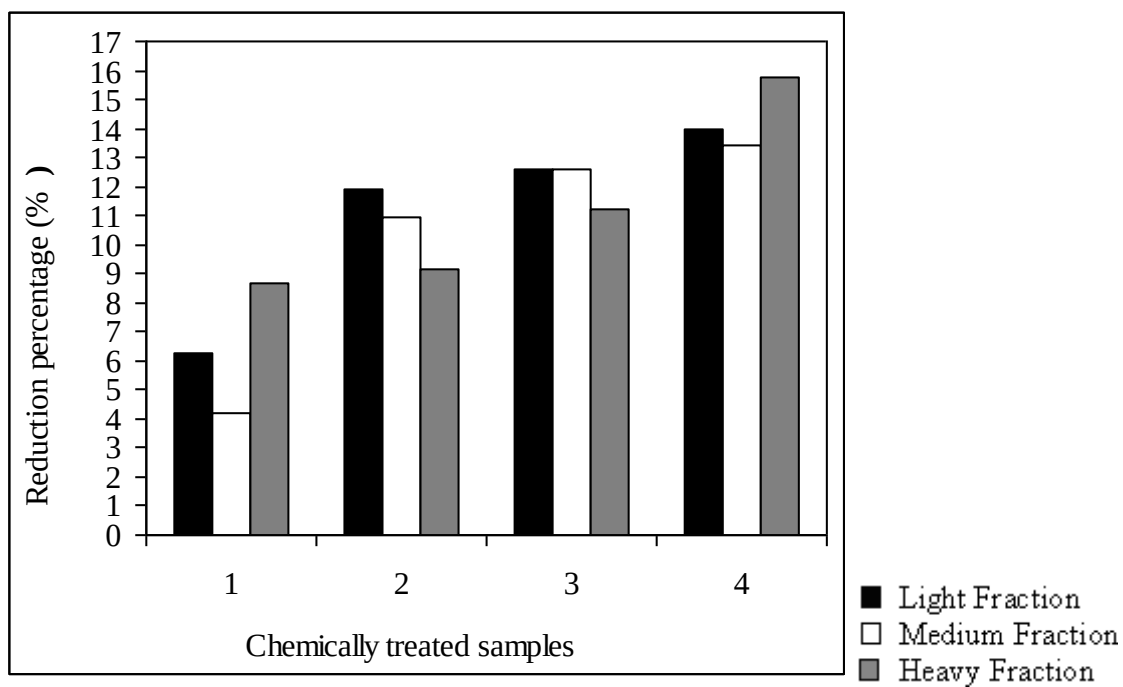
virgin-NH₄VO₃ were 12.58, 12.60 & 11.23 % and 13.98, 13.44 & 15.76 % respectively in the three distillate fractions.

Figure-37: Level of mercaptan sulfur (%) determined in chemically treated distillate fractions.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin	0.0143 (± 0.000306)	0.0119 (± 0.000321)	0.01104 (± 0.000252)
2	Hydrochloric acid	0.0134 (± 0.00025)	0.0114 (± 0.000569)	0.01008 (± 0.000053)
3	Sodium hydroxide	0.0126 (± 0.000529)	0.0106 (± 0.000450)	0.01003 (± 0.000537)
4	Sodium arsenate	0.0125 (± 0.000351)	0.0104 (± 0.000208)	0.0098 (± 0.000377)
5	Ammonium vendate	0.0123 (± 0.000458)	0.0103 (± 0.00092)	0.0093 (± 0.0003707)

Figure-38: Reduction (%) of mercaptan sulfur determined in chemically treated distillate fractions



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	6.293	4.202	8.695
2	Virgin-Sodium hydroxide	11.888	10.924	9.148
3	Virgin-Sodium arsenate	12.587	12.605	11.231
4	Virgin-Ammonium vendate	13.986	13.445	15.760

Biological removal of mercaptan sulphur

Removal with *P.putida*

The samples was interacted with *P.putida* in order to view the desulphurization ability of this strain for removal of the sulphur moiety i, e.mercaptane sulphur. The data is represented graphically in Fig.39, where enough amount of the mercaptan sulfur was observed to be consumed by *Ps.putida* during its various inoculations, where sulfur content can be used as source of energy for its growth and mobility.

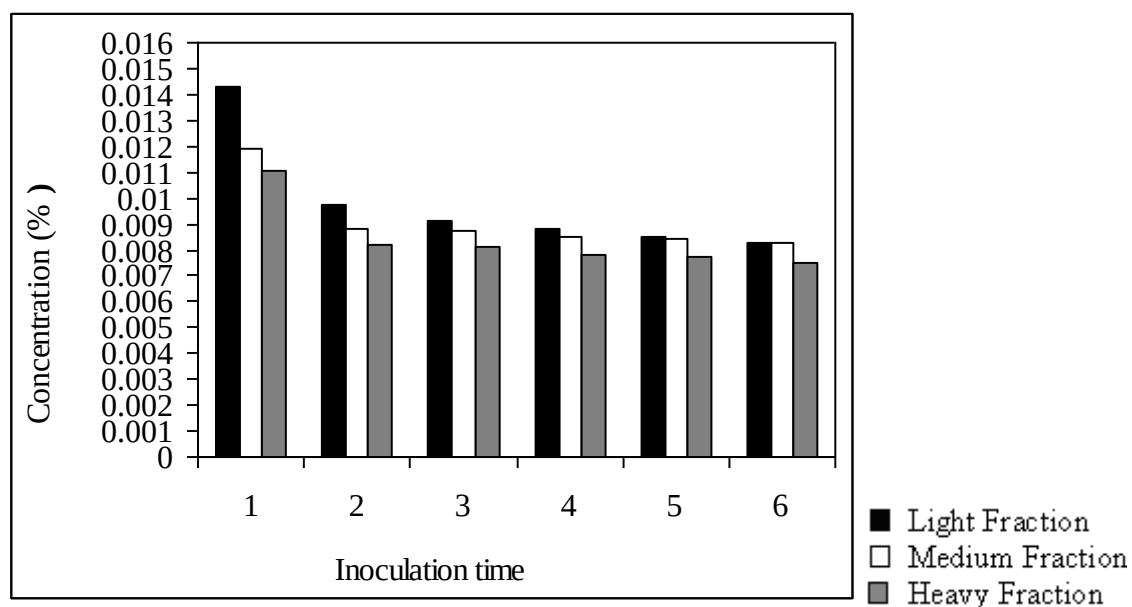
The inoculations were performed for time duration of 12 hr, 24 hr, 48 hr, one week and one month. Sulphur contents determined in residual samples from inoculation performed for time duration of 12 hr were 0.0097, 0.0088 & 0.0082 %, in case of light, middle and heavy fractions, respectively. The sulphur contents determined in residual samples from inoculations performed for time duration of 24 hr, 48 hr, and one week were 0.0091, 0.0087 & 0.0081 % ; 0.0088, 0.0085 & 0.0078 % ; 0.0085, 0.0084 & 0.0077 % . Similarly, the time was increased up to one month, where the contents of mercaptan sulfur determined were 0.0083, 0.0083 & 0.0075 % in light, middle and heavy fractions.

The percent reduction in each inoculation was calculated and given in Fig.40 where significant reduction has been observed in all the contact times, which was 31.74, 25.54 & 25.72 % in case of 12 hr .The reduction was found to be further increased in longer fermentation times and reduction of 36.15, 26.89, & 26.639 % was observed in case of 24 hr inoculation. A reduction of 38.46, 28.57, & 29.34 % was observed in case of 48 hr and reduction of 40.55, 29.41, & 30.25 % in inoculation performed for one week.

The reduction has gone up to 41.95, 30.25 & 32.06 % in inoculation performed for one month inoculation time in light, middle and heavy fractions, respectively.

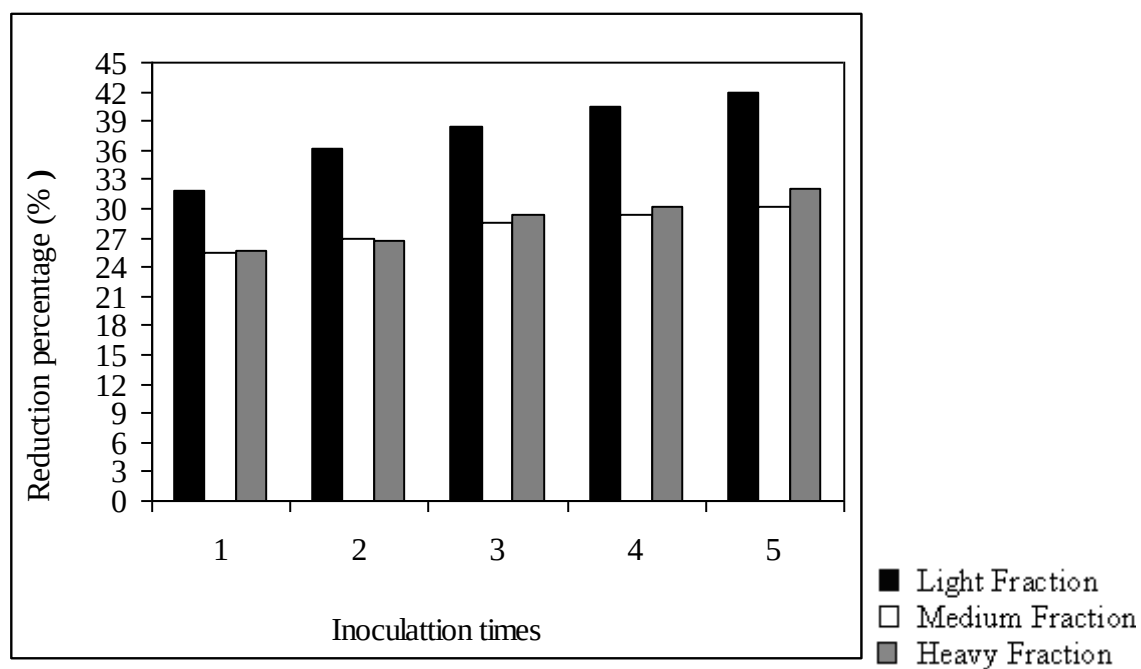
Thiol or mercaptane sulfur has been traced in various fermentation times as 31.74, 25.54 & 25.72 % ; 4.41, 1.35 & 0.91 % ; 2.31, 1.68 & 2.71 % ; 2.09, 0.84 & 0.91 % and 1.4, 0.84 & 1.81 % in the form of virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

Figure-39: Level of mercaptan (%) sulfur determined in pseudomonas treated distillate fractions as a function of inoculation times.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin	0.01430 (± 0.000306)	0.0119 (± 0.000321)	0.01104 (± 0.000252)
2	12 hours	0.00976 (± 0.000338)	0.0088 (± 0.000162)	0.0082 (± 0.000586)
3	24 hours	0.00913 (± 0.000117)	0.0087 (± 0.000252)	0.0081 (± 0.000721)
4	48 hours	0.0088 (± 0.000232)	0.0085 (± 0.000303)	0.0078 (± 0.000111)
5	One week	0.0085 (± 0.000273)	0.0084 (± 0.000357)	0.0077 (± 0.000195)
6	One month	0.0083 (± 0.000331)	0.0083 (± 0.000416)	0.0075 (± 0.000158)

Figure-40: Reduction percentage (%) of mercaptan sulfur determined in *P.putida* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	31.748	25.546	25.724
2	Virgin –24 hours	36.153	26.890	26.630
3	Virgin –48 hours	38.461	28.571	29.348
4	Virgin –one week	40.559	29.411	30.254
5	Virgin - one month	41.958	30.252	32.065

Removal with T.Thioxidanes

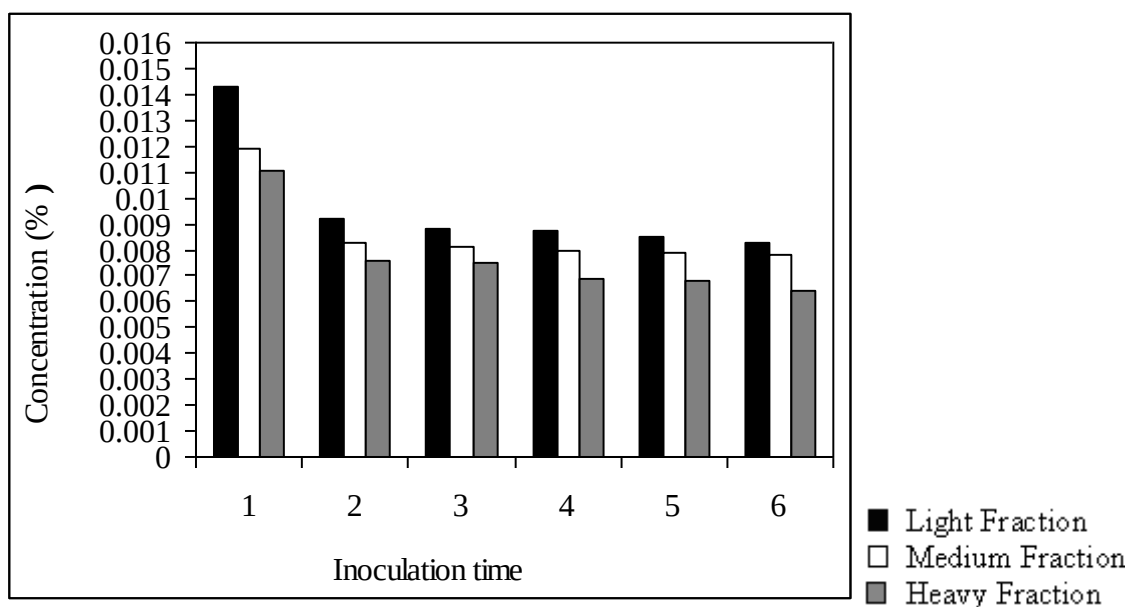
Bacterial strain *T. Thioxidans* has also been tested for mercaptan sulfur removal, where it has successfully removed enough sulfur contents, and reduced the level of mercaptane sulfur. Sulphur contents determined in residual samples from inoculation performed with the strain under study for time duration of 12 and 24 hr contact time were 0.0092, 0.0083, & 0.0076 % and 0.0088, 0.0081, & 0.0075 %. The sulphur contents determined in case of 48 hr inoculation were 0.0087, 0.0081, & 0.0069 % Mercaptane sulfur was further decreased by keeping the strain in contact for one week and one month, and the corresponding values of the mercaptan sulfur were 0.0085, 0.0079, & 0.0068 % and 0.0083, 0.0078 & 0.0064 % in light, middle and heavy fractions, respectively (Fig.41).

Percent reduction by *T. thiobacillus* is clearly shown in Fig.42, where during the first 12 hr and 24 hr, reduction of 35.66, 30.08, & 30.52 %, and 38.53, 31.42, 31.70 % was noticed. Reduction of 38.95, 32.68, & 36.86 %, 40.27, 33.52, & 37.40 % and 41.81, 34.53, & 40.02 % was observed in the inoculations performed for 48hr, one week and one month.

Similarly in *T.thiobacillus* treated samples mercaptane sulfur has been determined different contact which were found to be 35.66, 30.08 & 30.52 % ; 2.81, 1.34, & 1.18 % ; 0.18, 1.26 & 5.34 % ; 1.32, 1.01 & 5.16 % and 1.54, 1.01 & 2.62 % in virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

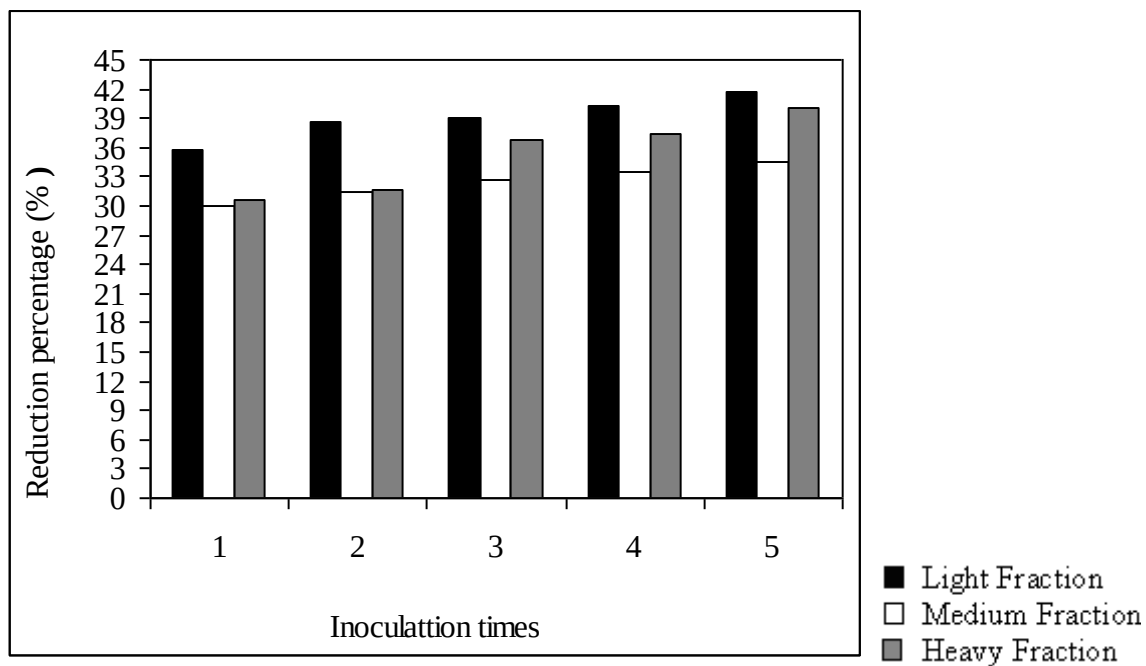
From the data given it is quite clear that *T.thioxidans* has significantly reduced mercaptane sulfur than *P.putida* as well as chemical treatments used in this study.

Figure-41: Level of mercaptan (%) sulfur determined in *T. thiooxidans* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin	0.01430 (± 0.000306)	0.0119 (± 0.000321)	0.01104 (± 0.000252)
2	12 hours	0.0092 (± 0.000153)	0.0083 (± 0.00010)	0.0076 (± 0.000312)
3	24 hours	0.0088 (± 0.000100)	0.0081 (± 0.000153)	0.0075 (± 0.000100)
4	48 hours	0.0087 (± 0.000100)	0.0080 (± 0.000115)	0.0069 (± 0.000100)
5	One week	0.0085 (± 0.000100)	0.0079 (± 0.000100)	0.0068 (± 0.000110)
6	One month	0.0083 (± 0.000153)	0.0078 (± 0.000100)	0.0064 (± 0.000153)

Figure-42. Reduction (%) of mercaptan sulfur determined in *T. thiooxidans* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	35.66	30.08	30.52
2	Virgin -24 hours	38.53	31.42	31.70
3	Virgin -48 hours	38.95	32.68	36.86
4	Virgin -one week	40.27	33.52	37.40
5	Virgin - one month	41.81	34.53	40.02

3.2.9. Removal of hydrogen sulfide

Hydrogen sulfide was determined in the original and variously inoculated samples by volumetric method (ASTM 1, 103/ 88). This method is intended for the determination of hydrogen sulfide in crude oil and their distillation fractions. The hydrogen sulfide present in the sample is converted to the cadmium sulfide by reaction with cadmium sulfate solution. The sulfide precipitate is filtered off and is acidified in the presence of measured volume of standard iodine solution, the excess of which is then determined by titrating with standard sodium thiosulfate solution. In this way, virgin fractions and the fractions, which were reacted with the four chemicals were treated and the collected data of the analyzed hydrogen sulfide is presented in Fig.43 for virgin and chemically treated samples. The amount of hydrogen sulfide (H_2S) found in virgin distillates were 0.0170, 0.0120 & 0.0090 % in light, middle and heavy fractions, respectively.

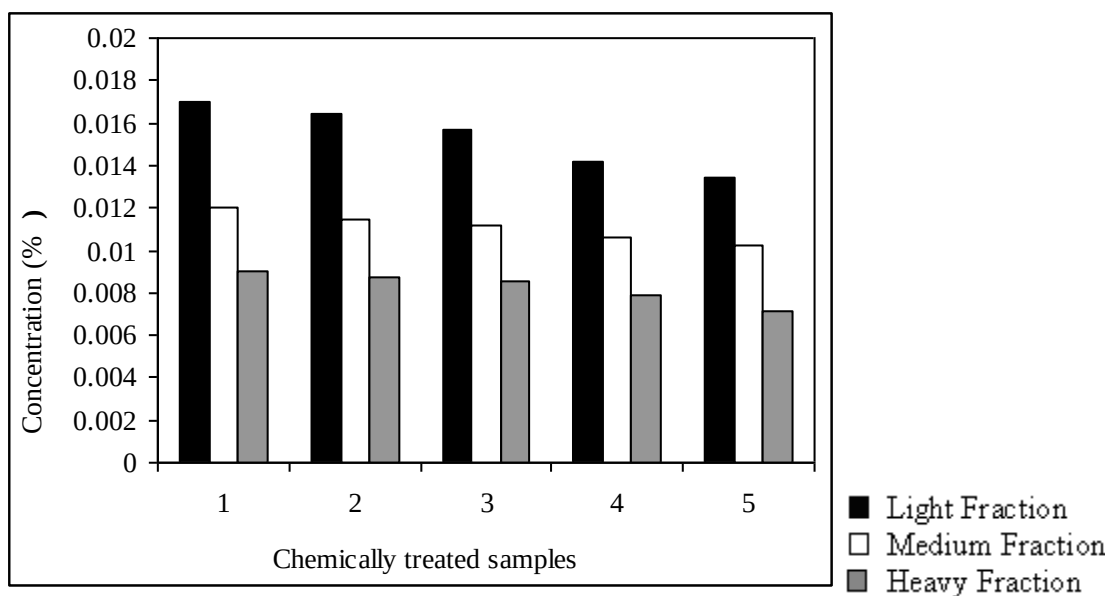
Chemical Removal

For chemical desulphurization, the fractions under study was scrubbed with the same set of chemicals used in the aforementioned sections. The values of hydrogen sulphide determined in the residual samples from these treatments were 0.0164, 0.0155 & 0.0087 % ; 0.0157, 0.0112 & 0.0085 % ; 0.0142, 0.0106 & 0.0079 % and 0.0134, 0.0102 & 0.0071 % in case of HCl, NaOH, Na_2HAsO_4 and NH_4VO_3 treatments, respectively (Fig.43). It can be seen from this data that H_2S is mainly present in the light distillate and less amount is present in heavy distillates.

The comparative investigation has been determined in the form of reduction percentage for the said applied chemicals and the data collected has been shown in Fig. 44. A reduction of .352, 4.16 & 3.33 % ; 7.64, 6.66 & 5.55 % ; 16.47, 11.66 & 12.22 % ; 21.17, 15.00 & 21.11 % was observed in case of HCl, NaOH, Na₂HAsO₄ and NH₄VO₃ treatments, respectively. It is clear that almost maximum reduction has been taken place with ammonium vendate than the rest ones.

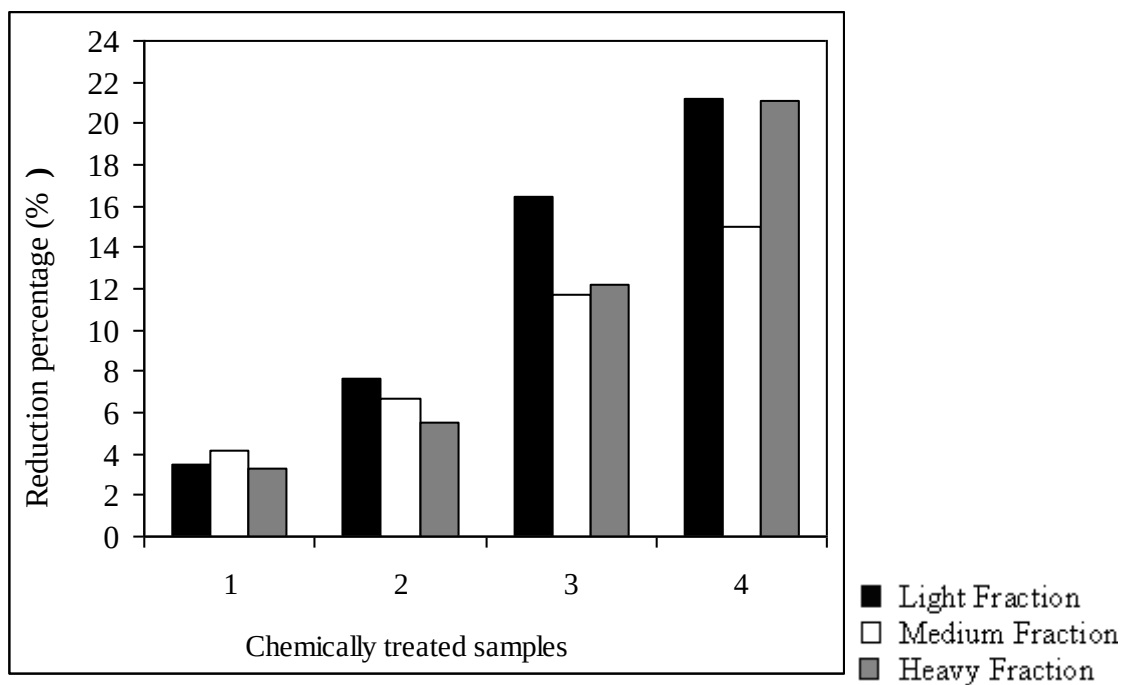
Hydrogen sulfide was determined among the scrubbing chemicals leachents which was 3.52, 4.16 & 3.33 % ; 4.12, 2.5 & 2.22 % ; 8.83, 5.00 & 6.0 % ; 4.7, 3.34 & 8.89 % respectively in the samples of virgin – HCL , HCL – NaOH, NaOH- Na₂HAsO₄ and Na₂HAsO₄- NH₄VO₃ in light , middle and heavy fractions.

Figure-43: Level of hydrogen sulfide (%) determined in chemically treated distillate fractions.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin	0.0170 (± 0.000351)	0.012 (± 0.000252)	0.0090 (± 0.000153)
2	Hydrochloric acid	0.0164 (± 0.000153)	0.0115 (± 0.000153)	0.0087 (± 0.000100)
3	Sodium hydroxide	0.0157 (± 0.00020)	0.0112 (± 0.000100)	0.0085 (± 0.000153)
4	Sodium arsenate	0.0142 (± 0.000153)	0.0106 (± 0.000153)	0.0079 (± 0.000153)
5	Ammonium vendate	0.0134 (± 0.00020)	0.0102 (± 0.000153)	0.0071 (± 0.000153)

Figure-44: Reduction (%) of hydrogen sulfide determined in chemically treated distillate fractions.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	3.529	4.167	3.333
2	Virgin-Sodium hydroxide	7.647	6.667	5.555
3	Virgin-Sodium arsenate	16.471	11.667	12.222
4	Virgin-Ammonium vendate	21.176	15.00	21.111

Biological Removal

Removal with *P.putida*

Hydrogen sulfide has been removed biologically by treating the samples under study with *Ps. putida* for various inoculation times (12hr- one month).

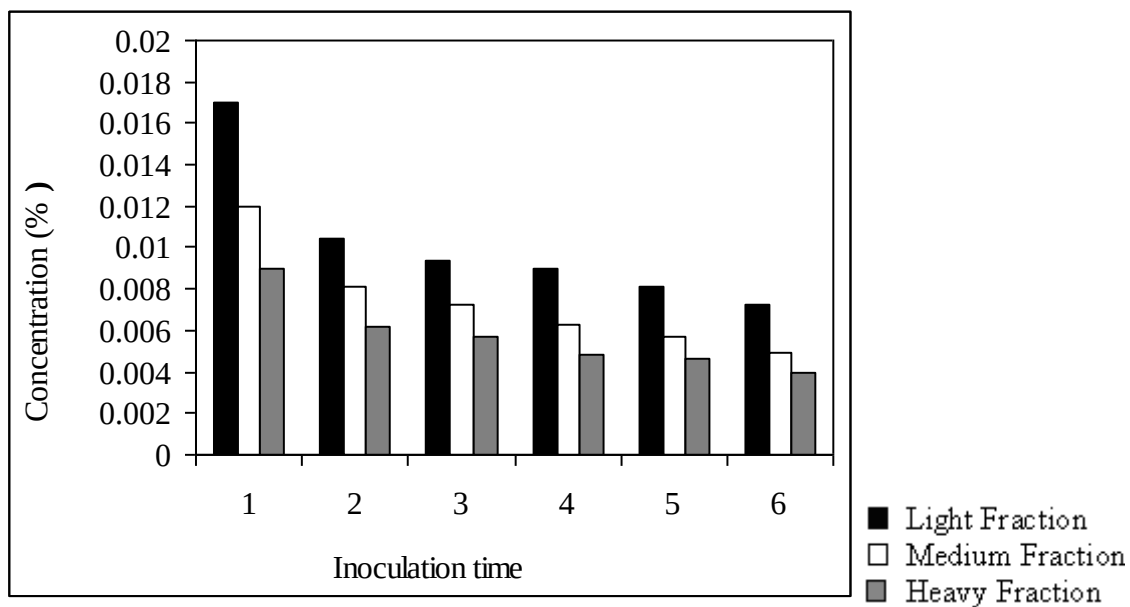
Hydrogen sulfide contents were observed to be depleted in the light, middle and heavy distillates of crude oil when these were inoculated with bacterial strain used. Original sample was analyzed for the determination of hydrogen sulfide and its values were found to be 0.0170, 0.012, & 0.0090 % in light, middle and heavy fractions respectively. The original samples were then inoculated for time duration of 12 hr, the H₂S contents observed in the residual samples were 0.0104, 0.0081 & 0.0062 %. For further removal of hydrogen sulfide, the inoculation time was extended beyond 12 hr ranges from 24 hr up to one month (720 hr). Hydrogen sulphide contents determined in the residual samples from these inoculations were found to be 0.0094, 0.0072 & 0.0057 % ; 0.0090, 0.0063 & 0.0048 % ; 0.0081, 0.0057 & 0.0046 %, 0.0072, 0.0049, & 0.0046 % in case of 24 hr, 48 hr, one week and a one month, respectively as shown in Fig.45. This indicates that a marginal depletion has been occurred due to the interaction of the applied microorganism with the oil samples under study in contact time beyond 12 hr. The reason is that the strain gets immobilized after 12 hr.

Percent reduction made by *P.putida* during variously inoculated samples has also been investigated, and all the findings are assembled in Fig.46. In case of 12 hr, 24 hr, and 48 hr, these values are 38.82, 32.50 & 31.11 % ; 44.70, 40.00 & 36.66 % ; 47.05, 47.50 & 46.66 % respectively in light, middle and heavy fractions.

The time of fermentation was extended for one week and one month, which increased the reduction percentage to 52.35, 52.50 & 61.66 and 57.64, 59.16 & 66.66 % in light, middle and heavy fractions, respectively which shows a comparatively high amount of H₂S removal from all the distillate fractions compared to chemically treated samples.

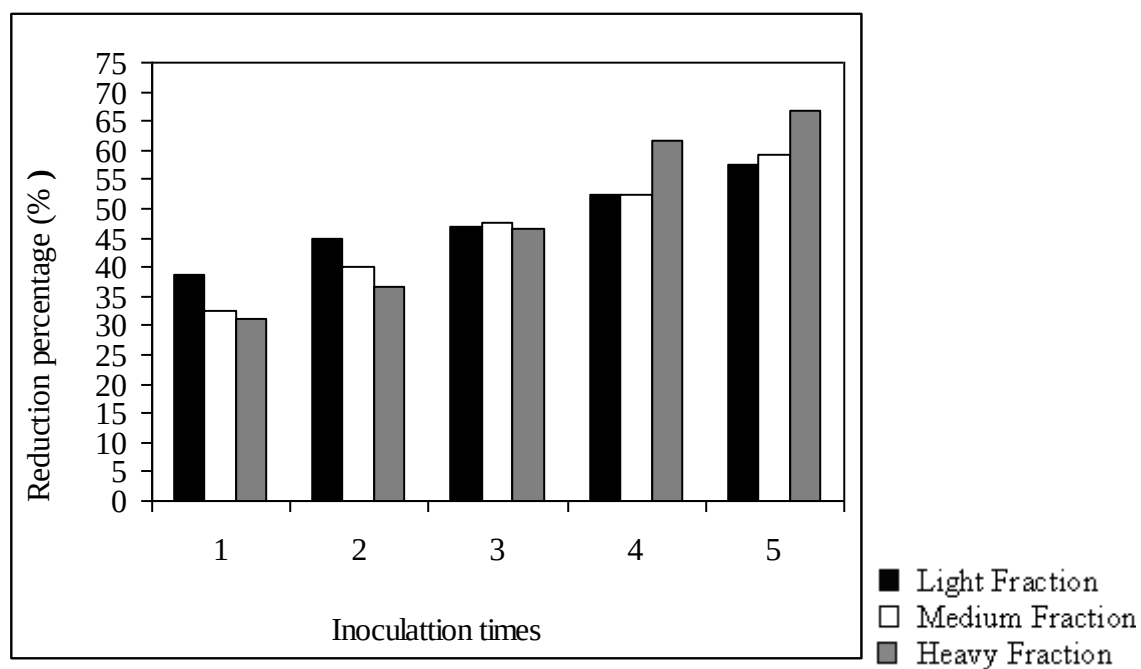
Among the mentioned inoculation times again hydrogen sulfide has been determined which was found to be 038.82, 32.50 & 31.11 % ; 5.88, 7.50 & 5.55 % ; 2.35, 7.56 & 10.00 % ; 5.3, 5.00 & 15.00 % and 5.29, 6.60 & 5.00 % in pseudomonas treated samples in different cases like virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

Figure-45: Level of hydrogen sulfide (%) determined in *P.putida* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin	0.0170 (± 0.000351)	0.0120 (± 0.000252)	0.0090 (± 0.000153)
2	12 hours	0.0104 (± 0.00014)	0.0081 (± 0.000208)	0.0062 (± 0.000153)
3	24 hours	0.0094 (± 0.000208)	0.0072 (± 0.000153)	0.0057 (± 0.00020)
4	48 hours	0.0090 (± 0.000153)	0.0063 (± 0.000252)	0.0048 (± 0.000153)
5	One week	0.0081 (± 0.000153)	0.0057 (± 0.000153)	0.0046 (± 0.000208)
6	One month	0.0072 (± 0.000208)	0.0049 (± 0.000208)	0.0040 (± 0.000153)

Figure-46: Reduction (%) of hydrogen sulfide determined in *P.putida* treated distillate fractions as function of inoculation time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	38.823	32.500	31.111
2	Virgin –24 hours	44.706	40.000	36.667
3	Virgin –48 hours	47.059	47.500	46.667
4	Virgin –one week	52.353	52.500	61.667
5	Virgin - one month	57.647	59.167	66.667

Removal with *T.Thioxidanes*

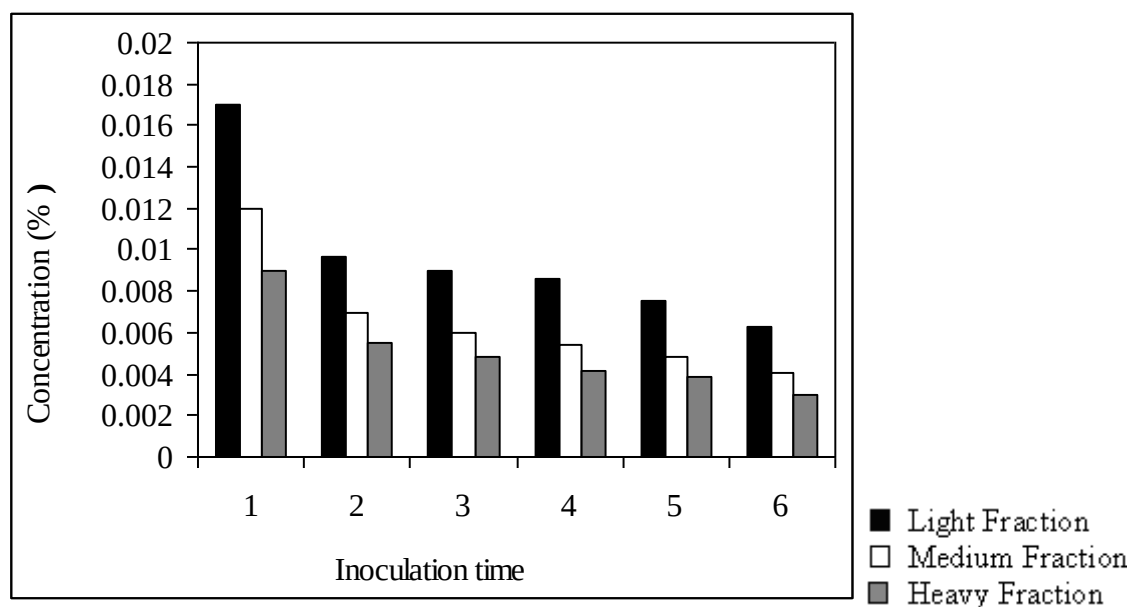
Samples were inoculated with the second strain to view its capability for removal of hydrogen sulphide. Inoculation was performed for the same inoculation times as selected in case of *P.putida*. From the Fig. 47, it is observed that in case of residual samples from 12hr, 24hr and 48hr inoculations, H₂S contents were 0.0097, 0.0070, & 0.0055 %; 0.0090, 0.0060, & 0.0048 % and 0.0086, 0.0054, & 0.0042%, in light, middle and heavy fractions, respectively. Hydrogen sulphide is a dangerous gas causes cellular respiration, which paralyses pulmonary system and ultimately the result is death. If the concentration is (15-50ppm), causes the irritation of mucous membrane, headache, dizziness and nausea. Concentration (200-300ppm) causes respiratory arrest leading to coma and if the concentration is 700 ppm and a person exposes for 30 minutes the results is death. Bacteria took H₂S from the sample and converted to elemental sulphur, which further oxidized to sulphates, with the help of CO₂ and O₂ according to the mechanism described elsewhere (77). The values of H₂S determined in the residual samples from one week and one month inoculations were 0.0075, 0.0048, & 0.0039 % and 0.0063, 0.0041, & 0.0030 % in light, middle and heavy factions, respectively which shows a good enhancement in H₂S removal compared to that of *pseudomonas putida*.

Reduction made by *T. thiooxidans* in light, middle and heavy fractions is quite clear from the Fig. 48. A reduction of 42.94, 41.66, & 38.88 %; 47.05, 50.0, & 46.60 %; 49.41, 55.00, & 53.33 % in case of 12 hr, 24 hr and 48 hr inoculation times, respectively. Reduction percentage was further increased along with increasing the inoculation time and was found to be 5.88, 60.00, & 56.60 %, and 62.94, 65.83, & 66.660 % in one week and one month inoculation times. This shows that as long as *T. thiooxidans* remains inside the samples, it oxidizes the hydrogen sulfides to elemental sulfur and

onward oxidizes to sulfate, which are hydrophilic compounds, which leaves the oil middle.

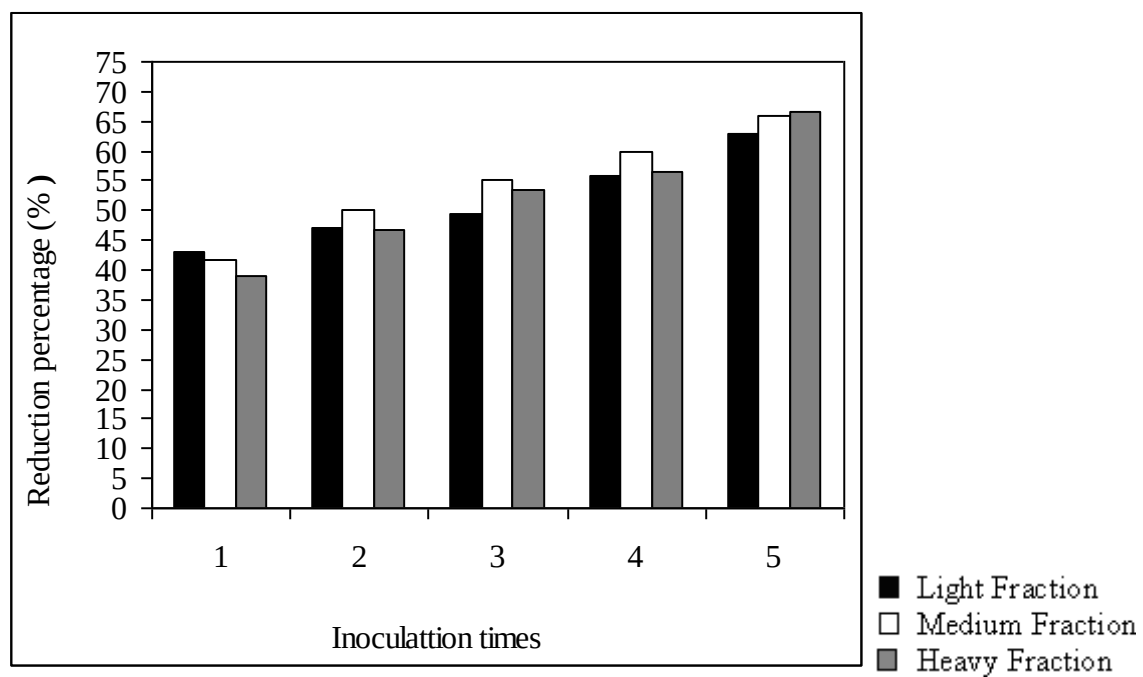
Similarly hydrogen sulfide was tested in *T.thiobacillus* inoculated samples and the determinations were compiled as 42.94, 41.66 & 38.88; 4.11, 8.34 & 7.78 % ; 2.3, 5.00 & 6.67 ; 6.47, 5.00 & 3.33 % ;and 7.06, 5.83 & 10.00% in virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

Figure-47. Level of hydrogen sulfide (%) determined in *T. thiooxidans* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin	0.0170 (± 0.000351)	0.0120 (± 0.000252)	0.0090 (± 0.000351)
2	12 hours	0.0097 (± 0.000351)	0.0070 (± 0.000351)	0.0055 (± 0.000351)
3	24 hours	0.0090 (± 0.00010)	0.0060 (± 0.000252)	0.0048 (± 0.000252)
4	48 hours	0.0086 (± 0.000200)	0.0054 (± 0.000252)	0.0042 (± 0.00010)
5	One week	0.0075 (± 0.00010)	0.0048 (± 0.000300)	0.0039 (± 0.000351)
6	One month	0.0063 (± 0.000252)	0.0041 (± 0.000210)	0.0030 (± 0.000351)

Figure-48: Reduction (%) of hydrogen sulfide determined in *T. thiooxidans* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Middle fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	42.941	41.667	38.889
2	Virgin -24 hours	47.058	50.000	46.667
3	Virgin -48 hours	49.412	55.000	53.333
4	Virgin -one week	55.882	60.000	56.667
5	Virgin - one month	62.941	65.833	66.667

3.2.10. Removal of dibenzothiophene

Determination of dibenzothiophene was done Spectrophotometrically

Thiophenic compounds include thiopenes, benzothiophene (BT), dibenzothiophene (DBT), and benzonaphthothiophene (BNT). Each fraction was subjected to GC-MS analysis by using both FPD and MS detectors (GC- FPD and GC-MS, respectively for the isolation and identification of thiophenic compounds before and after their treatment with chemicals and bacteria. Selective ion monitoring (SIM) mode was applied during dibenzothiophene analyses, where the charge to mass ratio selected value was (m/z 183) for dibenzothiophene ion (DBT^+). All the three distillation fractions were analyzed for DBT in virgin state. The sulphur contents determined were 290, 415, & 450 ppm in case of light, middle and heavy fractions, respectively.

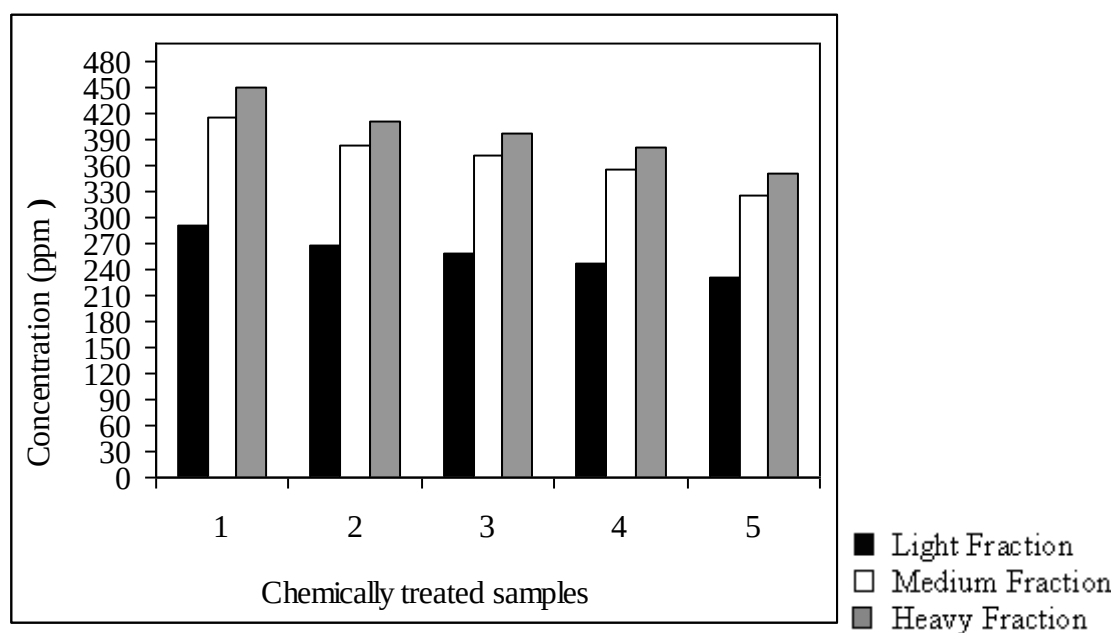
Chemical Removal

Samples under trial were scrubbed with the chemicals already mentioned in the previous sections for reduction of thiophenic sulfur compounds. The results obtained are represented in Fig.49. Determination of this sulphur analogue was done in residual light, middle and heavy oil fractions after being scrubbed with reagents under study. The sulphur contents determined were 268, 382, & 410 ppm; 259, 370, & 396 ppm; 246, 355, & 380 ppm and 230, 326, & 350 ppm in hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium arsenate (Na_2HAsO_4) and ammonium vendate (NH_4VO_3) treated samples, respectively. From the data collected, it is clear enough that ammonium vendate has reduced the maximum amount of dibenzothiophene (DBT) i.e. from 290 to 230ppm, 415 to 326 ppm and 450 to 350ppm in light, middle and heavy fractions, respectively.

Percent reduction observed in each case is shown in Fig.50, which shows a reduction of 7.95, 7.951, & 8.88 % was made by HCl in all the three samples under study. NaOH caused a reduction of 10.689, 10.843, & 12.00 %. Similarly, reduction of 15.172, 14.45, & 15.55 % was made by Na_2HAsO_4 , where as a reduction of 20.68, 21.44, & 22.22% was made by NH_4VO_3 in light, middle and heavy fractions, respectively.

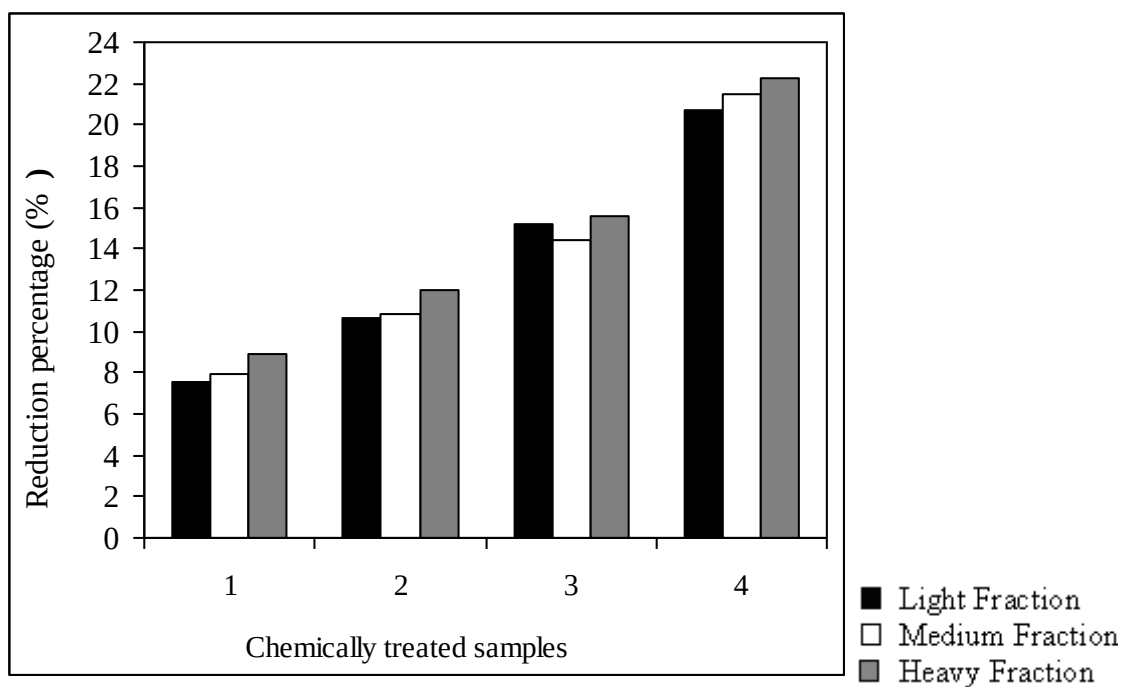
Dibenzothiophene compound was investigated among the chemical reagents used were 7.58, 7.95 & 8.88 % ; 3.11, 2.89 & 3.12 % ; 4.48, 4.33 & 3.55 % and 5.52, 5.51 & 6.67 % respectively in the samples of virgin – HCL , HCL – NaOH, NaOH- Na_2HAsO_4 and Na_2HAsO_4 - NH_4VO_3 in light , middle and heavy fractions.

Figure-49: Level of dibenzothiophene (ppm) determined in chemically treated distillate fractions



S#	Sample treated	Light fraction (ppm)	Medium fraction (ppm)	Heavy fraction (ppm)
1	Virgin	290 (± 2.00)	415 (± 300)	450 (± 2.00)
2	Hydrochloric acid	268 (± 2.00)	382 (± 2.52)	410 (± 1.52)
3	Sodium hydroxide	259 (± 1.70)	370 (± 2.0816)	396 (± 2.51)
4	Sodium arsenate	246 (± 2.00)	355 (± 2.5166)	380 (± 2.5166)
5	Ammonium vendate	230 (± 3.00)	326 (± 1.5275)	350 (± 2.000)

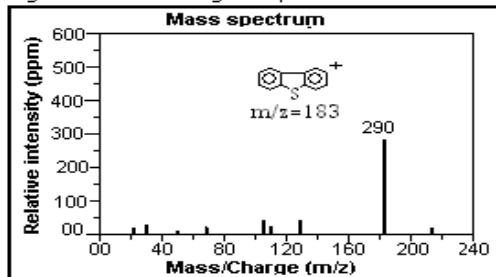
Figure-50: Reduction (%) of dibenzothiophene determined in chemically treated distillate fractions



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	7.586	7.951	8.88
2	Virgin-Sodium hydroxide	10.689	10.843	12.00
3	Virgin-Sodium arsenate	15.172	14.457	15.55
4	Virgin-Ammonium vendate	20.689	21.445	22.22

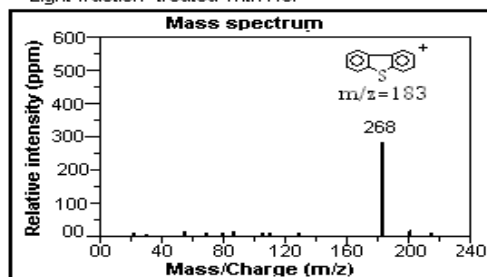
Spectrum profile-19. Dibenzothiophene GC-MS spectra of virgin and variously chemically treated light fractions.

Light fraction of the virgin sample



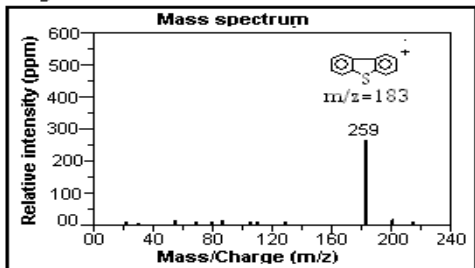
(a). L. F virgin sample.

Light fraction treated with HCl



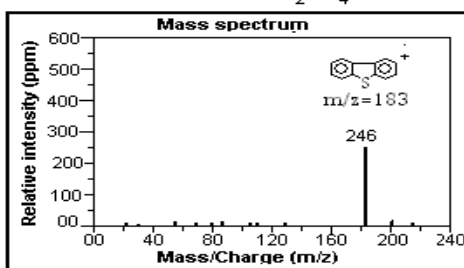
(b). L.F treated with HCL.

Light fraction treated with NaOH



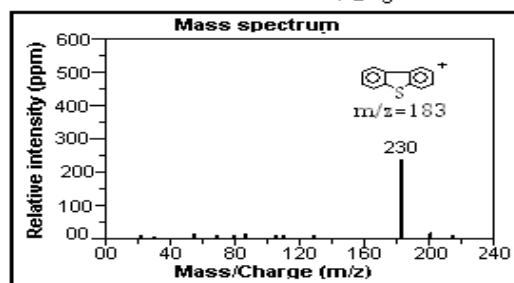
(c). L.F treated with NaOH.

Light fraction treated with NaHAsO_4



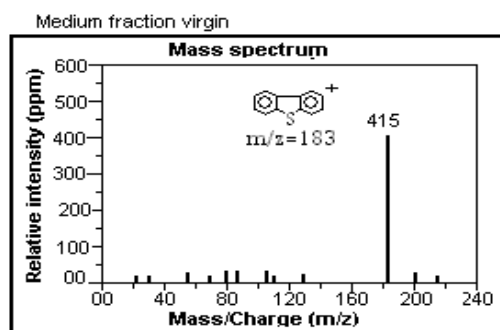
(d). L.F treated with Na_2HAsO_4 .

Light fraction treated with $(\text{NH}_4)_2\text{VO}_3$

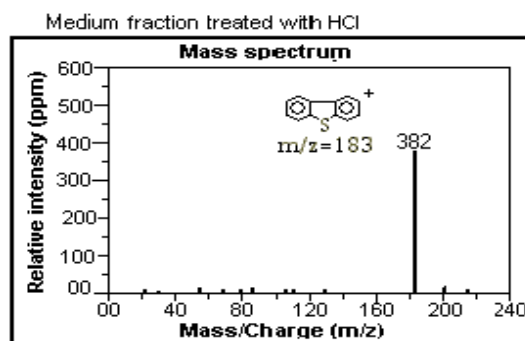


(e). L.F treated with $(\text{NH}_4)_2\text{VO}_3$.

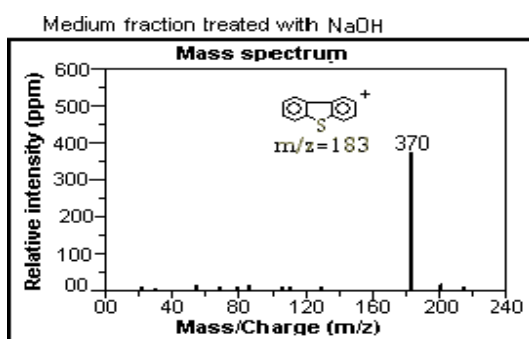
Spectrum profile-22. Dibenzothiophene GC-MS spectra of virgin and variously chemically treated middle fractions.



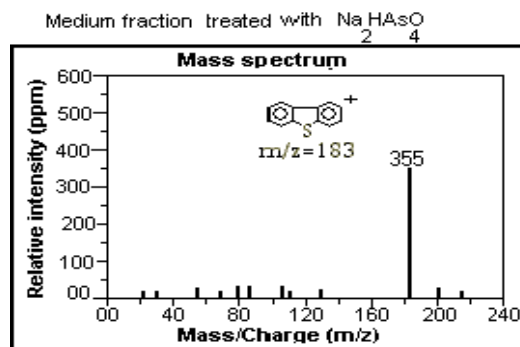
(a).M. F virgin sample.



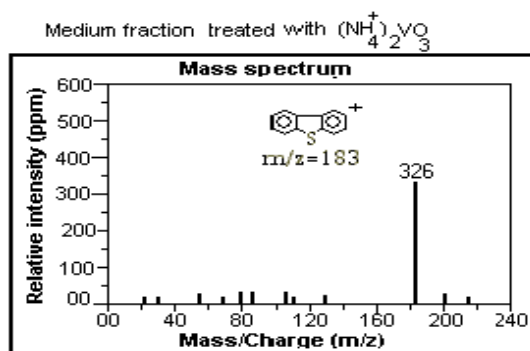
(b).M.F treated with HCL.



(c).M.F treated with NaOH.



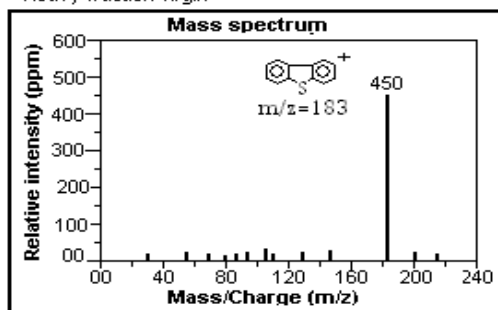
(d). M.F treated with Na_2HAsO_4 .



(e).M.F treated with $(\text{NH}_4)_2\text{VO}_3$.

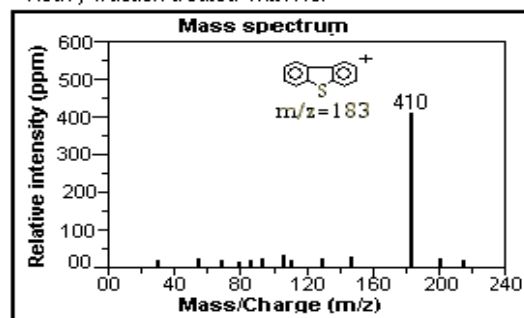
Spectrum profile-25. Dibenzothiophene GC-MS spectra of virgin and variously chemically treated heavy fractions.

Heavy fraction virgin



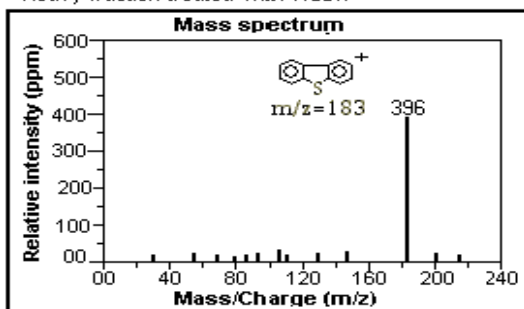
(a). H. F virgin sample.

Heavy fraction treated with HCl

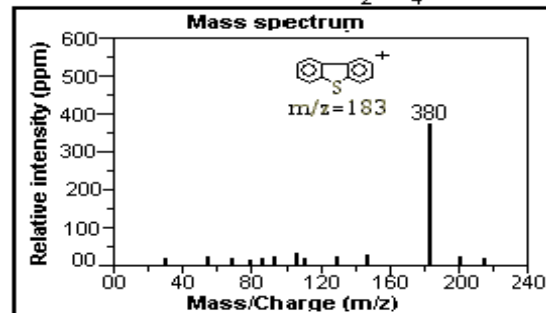
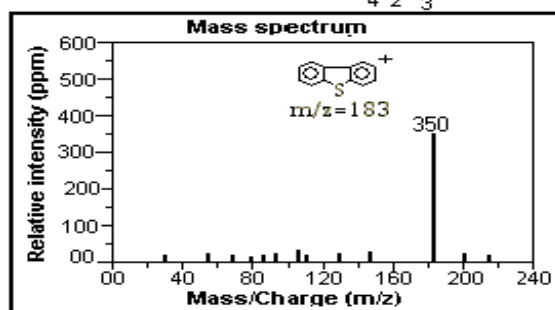


(b). H.F treated with HCL.

Heavy fraction treated with NaOH



(c). H.F treated with NaOH.

Heavy fraction treated with NaHAsO_4 (d). H.F treated with Na_2HAsO_4 .Heavy fraction treated with $(\text{NH}_4)_2\text{VO}_3$ (e). H.F treated with $(\text{NH}_4)_2\text{VO}_3$.

Removal with P.putida

The same distillation fractions were cultivated with *Ps.putida* for various inoculation times in order to check its desulphurization effect with time. Fig.51 gives values of the virgin samples and the biologically treated samples inoculated for 12 hr, 24 hr, 48 hr, one week, and one month. After each inoculation, the sulphur was determined in the residual samples. The sulphur contents determined were 290, 415, & 450 ppm in case of virgin light, middle and heavy oil fractions, respectively. These values were continuously decreased as the time of inoculation was extended and the collected data was found to be 170, 243, & 259 ppm; 157, 204, & 226 ppm; 142, 187, & 207 ppm, in case of 12hrs, 24hrs, and 48hrs inoculations, respectively. DBT is converted in to other products and hence slowly mineralize to water soluble constituents. This mineralization is assisted by bacterium under study which release certain enzymes responsible for this conversion (78).

As the inoculation time was extended beyond 48hr, DBT reduction was further increased. The sulphur contents noted in case of light, middle and heavy fractions were 129, 173, & 186 and 117, 160, & 163 ppm in one week and one month inoculations, respectively.

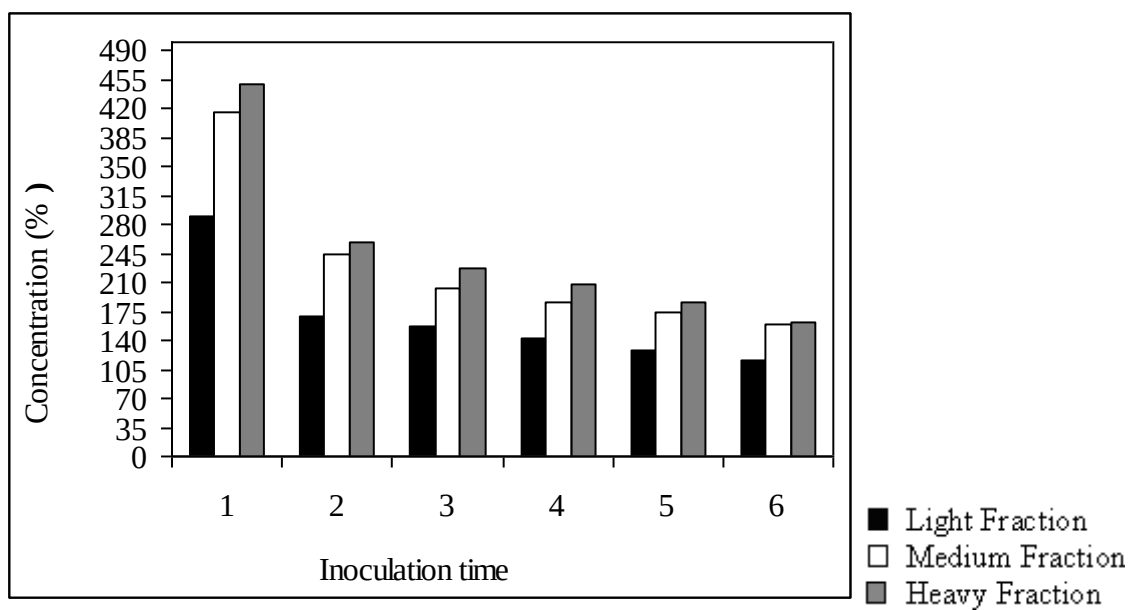
The reduction can be observed from the decrease in the intensity of the peak corresponds to DBT in GC- MS spectra profiles 19-27 for chemically treated and biologically treated samples.

Reduction percentage made by *Ps. putida* has also been determined and the values are assembled in Fig. 52, where 41.37, 41.44, & 42.44 %, 45.86, 50.84, & 49.77 % and 51.03, 54.93, & 54.00 % reduction was being determined in light, middle and heavy

fractions in case of 12 hr, 24 hr and 48 hr inoculations. In inoculations performed beyond 48 hr, reduction noted was 55.51, 58.31, & 58.66 % and 59.65, 61.44, & 63.77 % in case of one week and one month. Again, it is clear that the biological removal of DBT is comparatively more effective than the chemical method.

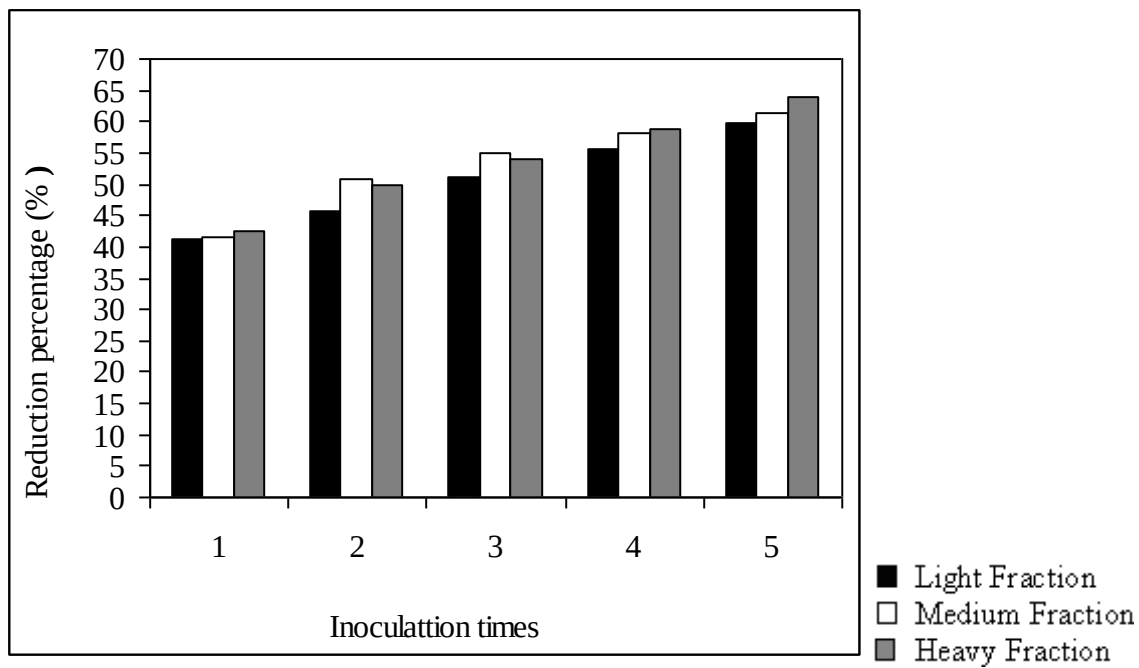
Dibenzothiophen has been tested in *P.putida* inoculated samples and the determinations were compiled as 41.37, 41.44 & 42.44 % ; 4.449, 9.40 & 7.33 % ; 5.17, 4.09 & 4.23 % ; 4.48, 3.38 & 4.66 % and 4.14, 3.13 & 5.11 % in virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

Figure-51: Level of dibenzothiophene (ppm) sulfur determined in *P.putida* treated as a function of inoculation distillate fractions.



S#	Sample treated	Light fraction (ppm)	Middle fraction (ppm)	Heavy fraction (ppm)
1	Virgin	290 (±2.00)	415 (±3.00)	450 (±2.00)
2	12 hours	170 (±4.00)	243 (±2.50)	259 (±1.527)
3	24 hours	157 (±2.00)	204 (±2.00)	226 (±2.516)
4	48 hours	142 (±2.00)	187 (±1.52)	207 (±2.08)
5	One week	129 (±4.00)	173 (±3.511)	186 (±2.516)
6	One month	117 (±2.516)	160 (±1.52)	163 (±3.00)

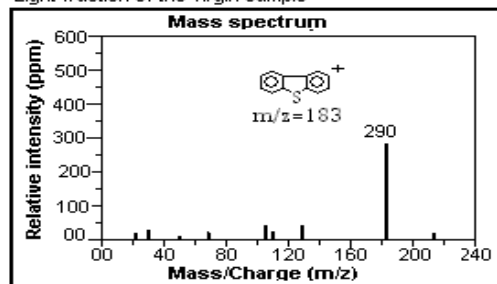
Figure-52. Reduction (%) of dibenzothiophene determined in *P.putida* treated distillate fractions as a function of inoculation time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	41.379	41.445	42.444
2	Virgin -24 hours	45.862	50.843	49.777
3	Virgin -48 hours	51.034	54.939	54.000
4	Virgin -one week	55.517	58.313	58.666
5	Virgin - one month	59.655	61.445	63.777

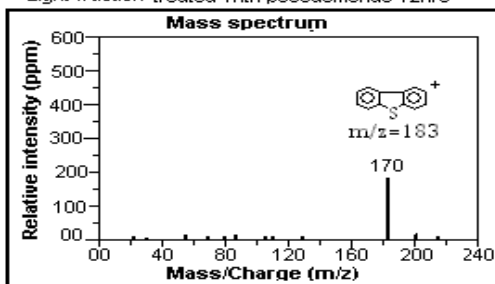
Spectrum profile-20. Dibenzothiophene GC-MS spectra of virgin and *Ps. putida* treated light fractions.

Light fraction of the virgin sample



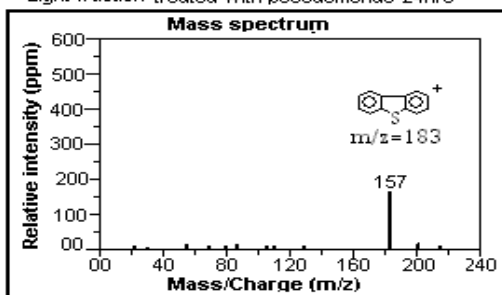
(a). L. F virgin sample.

Light fraction treated with pseudomonas 12hrs



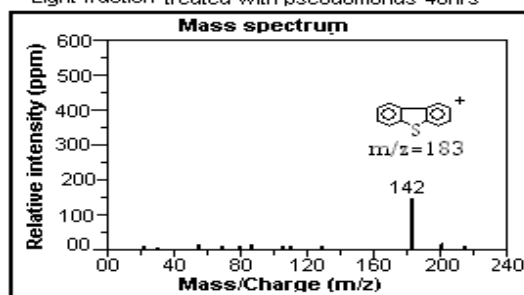
(b). L.F treated with *Ps.Putida* (12 hr).

Light fraction treated with pseudomonas 24hrs



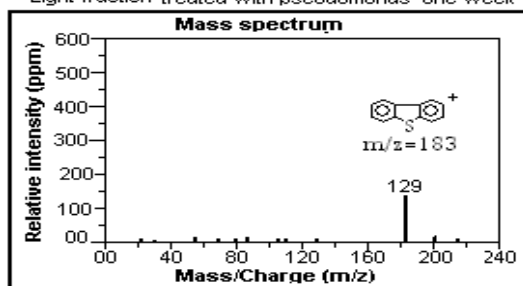
(c).L.F treated with *P.Putida* (24 hr).

Light fraction treated with pseudomonas 48hrs



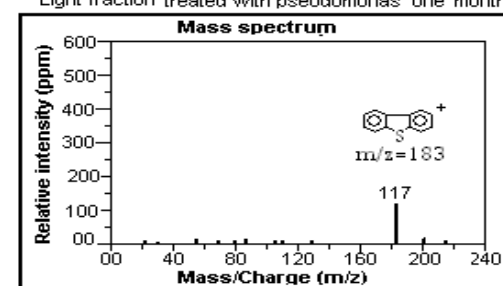
(d). L.F treated with *P.Putida* (48 hr).

Light fraction treated with pseudomonas one week



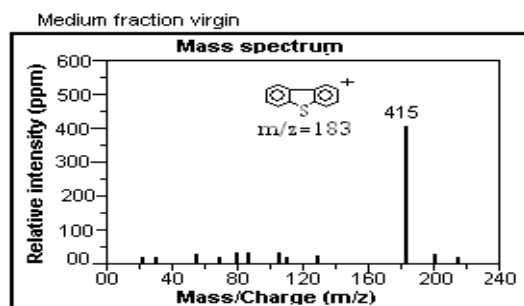
(e).L.F treated with *P.Putida* (week).

Light fraction treated with pseudomonas one month

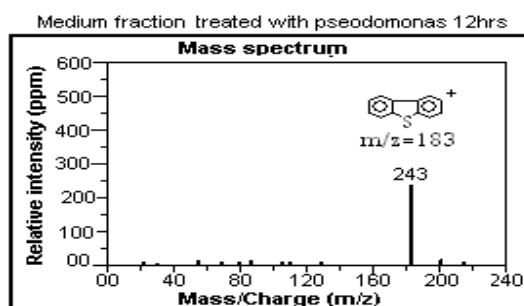


(f). L.F treated with *P.Putida* (month).

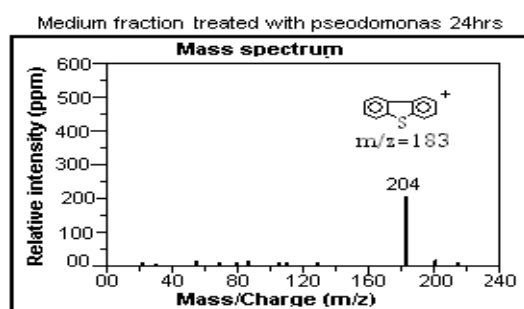
Spectrum profile-23. Dibenzothiophene GC-MS spectra of virgin and *Ps.Putida* treated middle fractions.



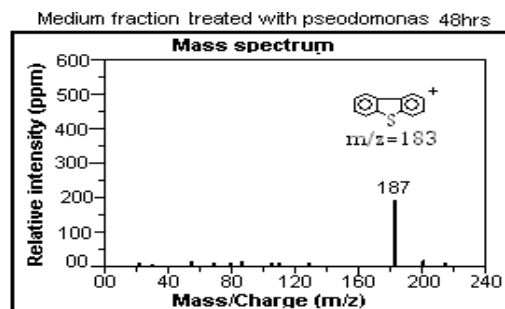
(a). M. F virgin sample.



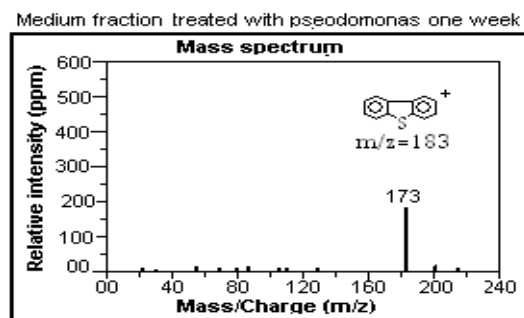
(b). M.F treated with *Ps.Putida* (12 hr).



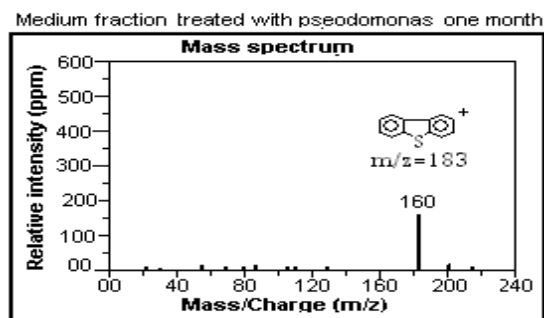
(c). M.F treated with *Ps.Putida* (24 hr).



(d). M.F treated with *Ps.Putida* (48 hr).

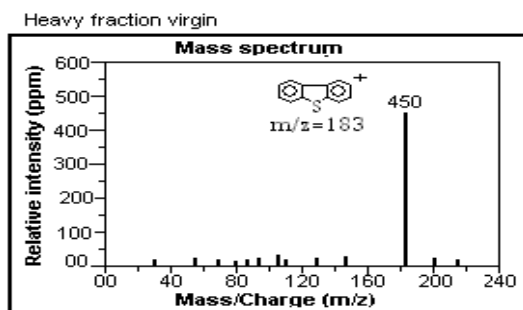


(e). M.F treated with *Ps.Putida* (week).

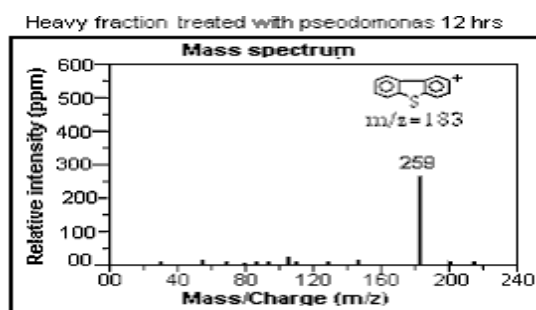


(f). M.F treated with *Ps.Putida* (month).

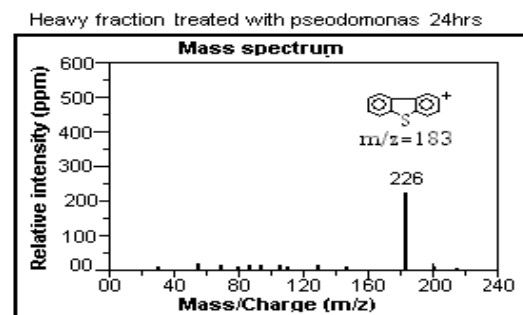
Spectrum profile-26. Dibenzothiophene GC-MS spectra of virgin and *Ps.Putida* treated heavy fractions.



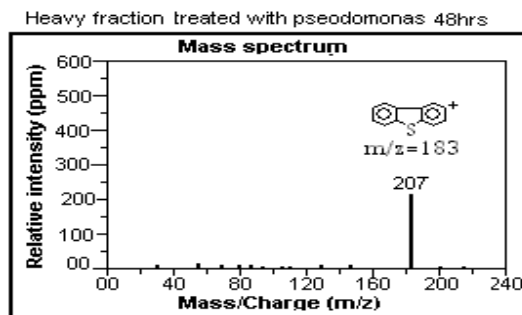
(a). H. F virgin sample.



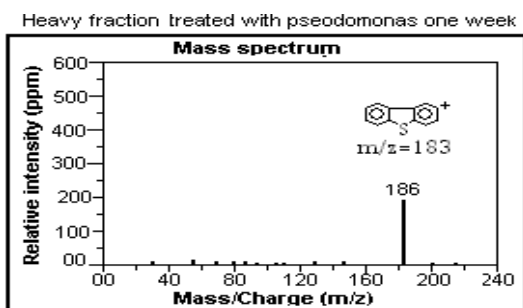
(b). H F treated with *Ps.Putida* (12 hr).



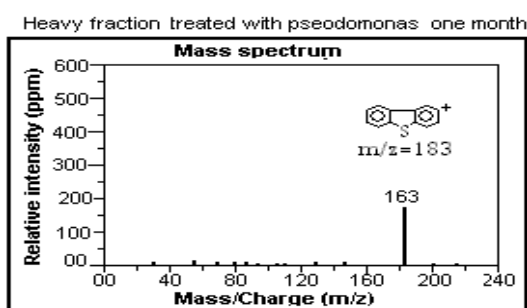
(c). H.F treated with *Ps.Putida* (24hr)



(d). H.F treated with *Ps.Putida* (48hr).



(e). H.F treated with *Ps.Putida* (week)



(f). H.F treated with *Ps.Putida* (month).

Removal with T.Thioxidanes

Crude oil distillation fractions were treated with *T. thioxidans* in order to view the ability of this microbial strain for removal of DBT. The inoculations were likewise performed for time duration selected from 00-hr to one month (720 hr). The original light, middle and heavy distillation fractions of crude oil sample were analyzed for DBT and the data collected has been shown in Fig.53. . The samples contain 290, 415 & 450 ppm DBT. The same samples were then inoculated with the bacterial strain for a contact time of 12 hr. The DBT was determined in the residual samples from this treatment, which was found to be 163, 232, & 240 ppm. In the next step, the inoculation time was extended up to 24 hr. The rate and extent of desulphurization increases with increase in time. The sulfur contents determined determined in case of 24 hr reaction time were 148, 191& 210 ppm in light, middle and heavy fractions, respectively. In order to view further depletion as a function of inoculation time, the time was extended up to 48 hr, followed by one week, followed by one month. In all these inoculations, sulphur (DBT) contents were found to be 130, 173, & 193 ppm; 117, 160, &171 ppm and 109, 148, & 152 ppm in all the above mentioned distillation fractions, respectively. Spectra profiles (19-27) show the data obtained during *T. thioxidans* treated samples by the help of GC-MS analyses. It is clear from the results obtained that depletion is quite significant in case of 12 hr contact time. This is attributed due to the fact that bacterial multiplication is so rapid in the initial time that most of the sulphur is reduced in the early stages of inoculation. In latter stages, due to the limited existence of DBT amount in the rest of the samples, a gradually minimum depletion has been noted. The depletion in DBT is quite

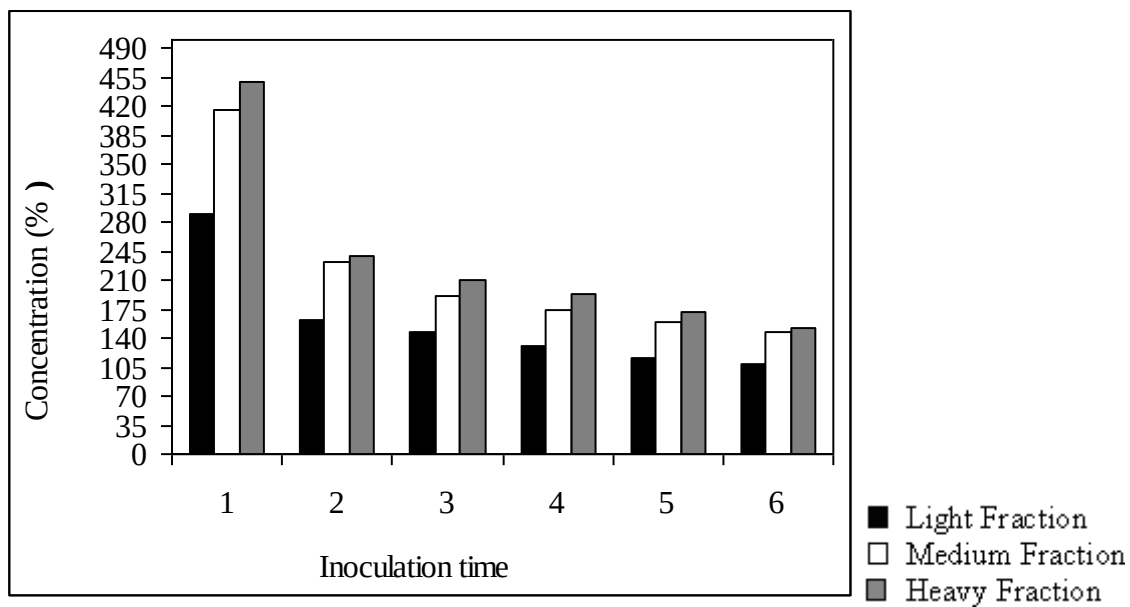
significant compared to bacterium, *Gordona* sp. strain CYKS1, which reported to transform DBT at a rate of $0.15 \mu\text{mol gDCW}^{-1} \text{ min}^{-1}$ [79].

The sulfur depletion data and the percent depletion in each case have been assembled in Fig.54. It is clear from the data that 43.73, 48.96 & 55.17 % DBT reduction has been made in first three inoculations.

The reduction observed for 24 and to 48 hr was 43.73, 44.09, & 46.66)%; 48.69, 53.97, & 53.33 % and 55.17, 58.31,& 57.11 % in light, middle and heavy fractions, respectively. This reduction is quite significant when compared with the previous studies [80]. Reduction of 59.65, 61.44, & 62.00 % and 62.41, 64.33, & 66.22 % was observed in case of one week and one-month time. Again, it is clear that most of the DBT removal occurs in the initial stage.

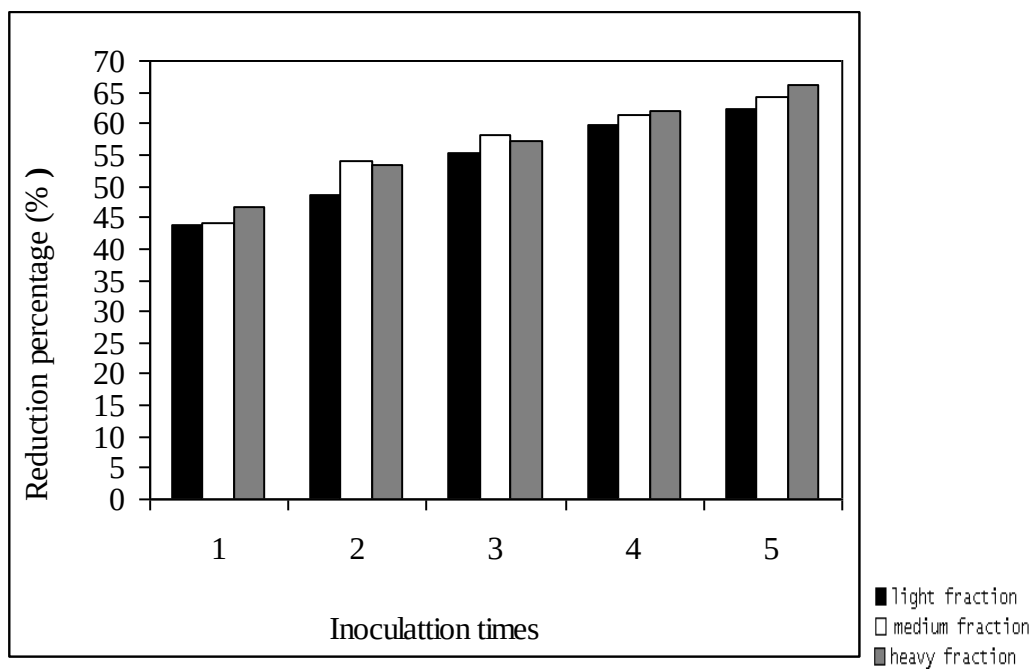
In case of *T.thiobacillus dibenzothiophen* was found to be in variously inoculated samples where the data was noted as 43.73, 44.09 & 46.66 % ; 4.96, 9.88, 10.67 % ; 6.48, 4.34 & 3.78 % ; 4.48 , 3.13 & 4.89 % and 2.76, 2.89 & 4.22 % in virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fraction

Figure-53: Level of dibenzothiophene (ppm) sulfur determined in *T. thiooxidans* treated distillate fractions as a function on of inoculation time.



S#	Sample treated	Light fraction (ppm)	Medium fraction (ppm)	Heavy fraction (ppm)
1	Virgin	290 (±2.00)	415 (±3.00)	450 (±2.00)
2	12 hours	163 (±3.511)	232 (±1.52)	240 (±2.500)
3	24 hours	148 (±3.511)	191 (±1.520)	210 (±1.520)
4	48 hours	130 (±4.00)	173 (±2.00)	193 (±1.52)
5	One week	117 (±2.00)	160 (±6.00)	171 (±3.00)
6	One month	109 (±1.00)	148 (±1.00)	152 (±2.00)

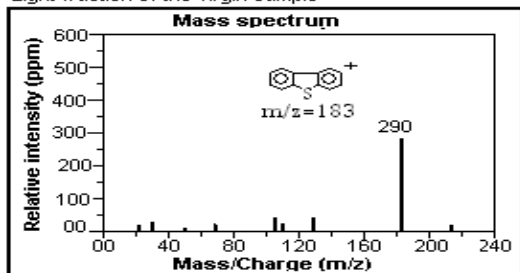
Figure-54. Reduction (%) of dibenzothiophene determined in *T.thiooxidans* treated distillate fractions as a function of time.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	43.73	44.09	46.66
2	Virgin -24 hours	48.69	53.97	53.33
3	Virgin -48 hours	55.17	58.31	57.11
4	Virgin -one week	59.65	61.44	62.00
5	Virgin - one month	62.41	64.33	66.22

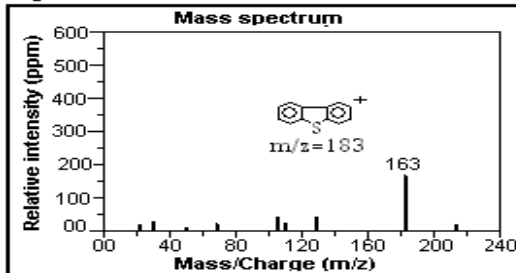
Spectrum profile-21. Dibenzothiophene GC-MS spectra of virgin and *T.thiooxidans* treated light fractions.

Light fraction of the virgin sample



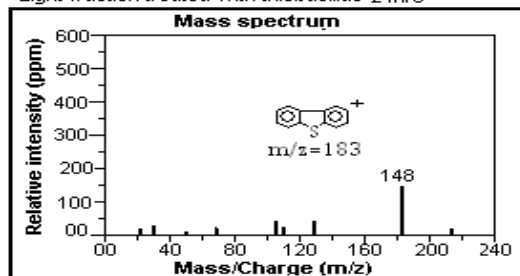
(a). L.F virgin sample.

Light fraction treated with thiobacillus 12hrs



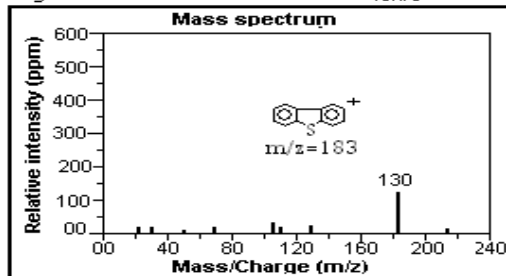
(b). L.F treated with *T. thiobacillus* (12hrs).

Light fraction treated with thiobacillus 24hrs



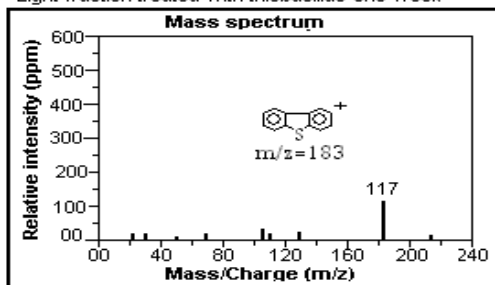
(c).L.F treated with *T.thiobacillus* (24hrs).

Light fraction treated with thiobacillus 48hrs



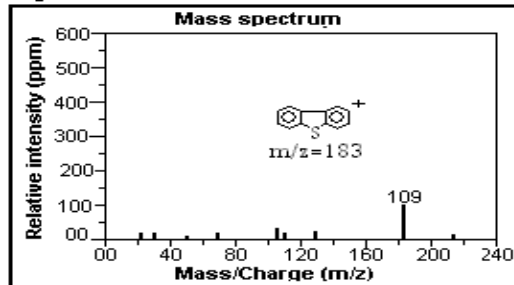
(d). L.F treated with *T.thiobacillus* (48hrs).

Light fraction treated with thiobacillus one week



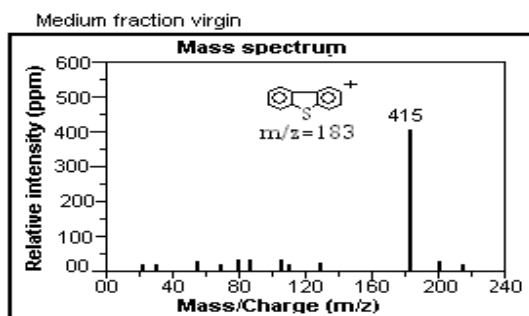
(e).L.F treated with *T.thiobacillus* (week).

Light fraction treated with thiobacillus one month

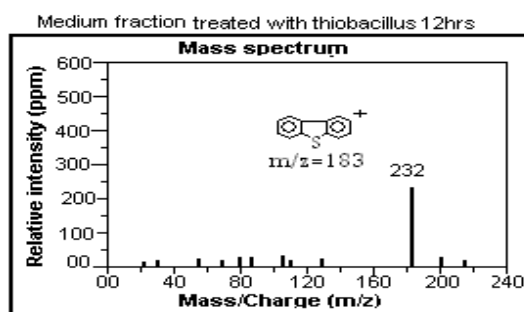


(f). L.F treated with *T.thiobacillus* (month).

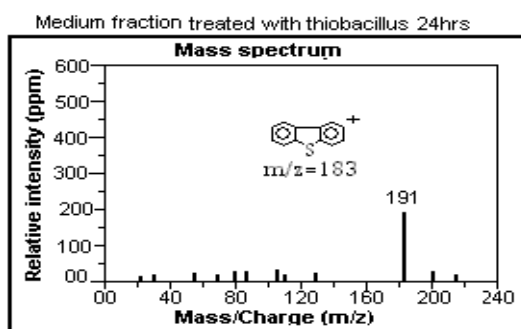
Spectrum profile-24. Dibenzothiophene GC-MS spectra of virgin and *T.thioxidans* treated middle fractions.



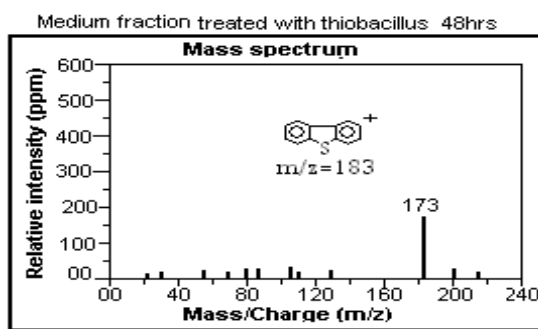
(a). M. F virgin sample.



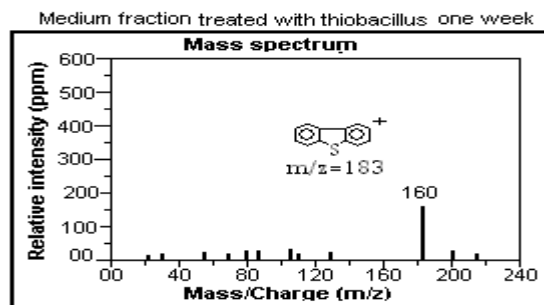
(b) M.F treated with *T.thioxidans* (12 hr).



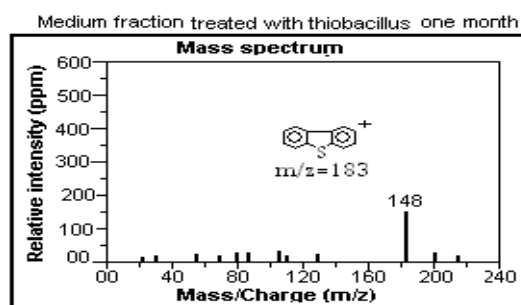
(c). M.F treated with *T.thioxidans* (24hr).



(d). M.F treated with *T.thioxidans* (48hr).

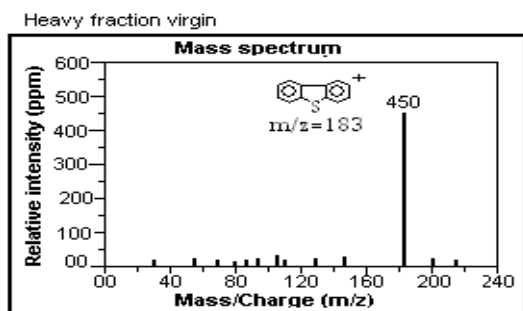


(e).M.F treated with *T.thioxidans* (week).

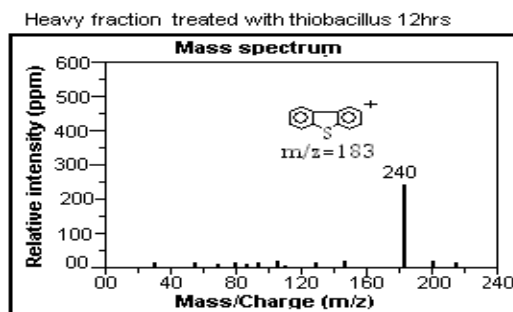


(f). M.F. treated with *T.thioxidans* (month)

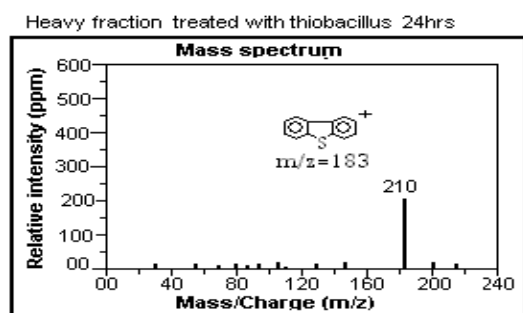
Spectrum profile-27. Dibenzothiophene GC-MS spectra of virgin and *T.thioxidans* treated heavy fractions.



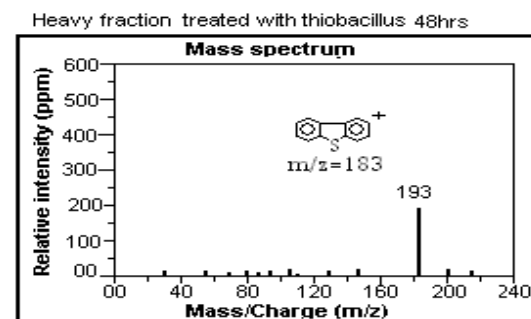
(a). H.F virgin sample.



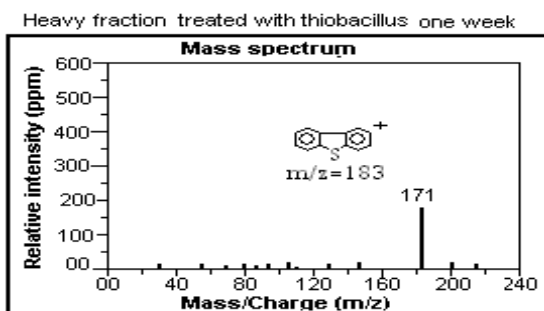
(a). H.F treated with *T.thioxidans* (12 hr).



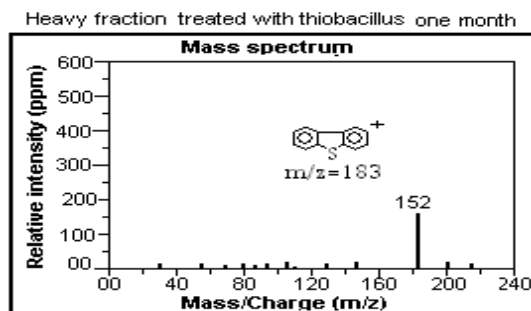
(c). H.F treated with *T.thioxidans* (24hr).



(d). H.F treated with *T.thioxidans* (48hr).



(e). H.F treated with *T.thioxidans* (week).
(month).



(d). H.F. treated with *T.thioxidans*

3.2.9. Removal of benzothiophene

Determination of benzothiophene was done Spectrophotometrically

Chemical Removal

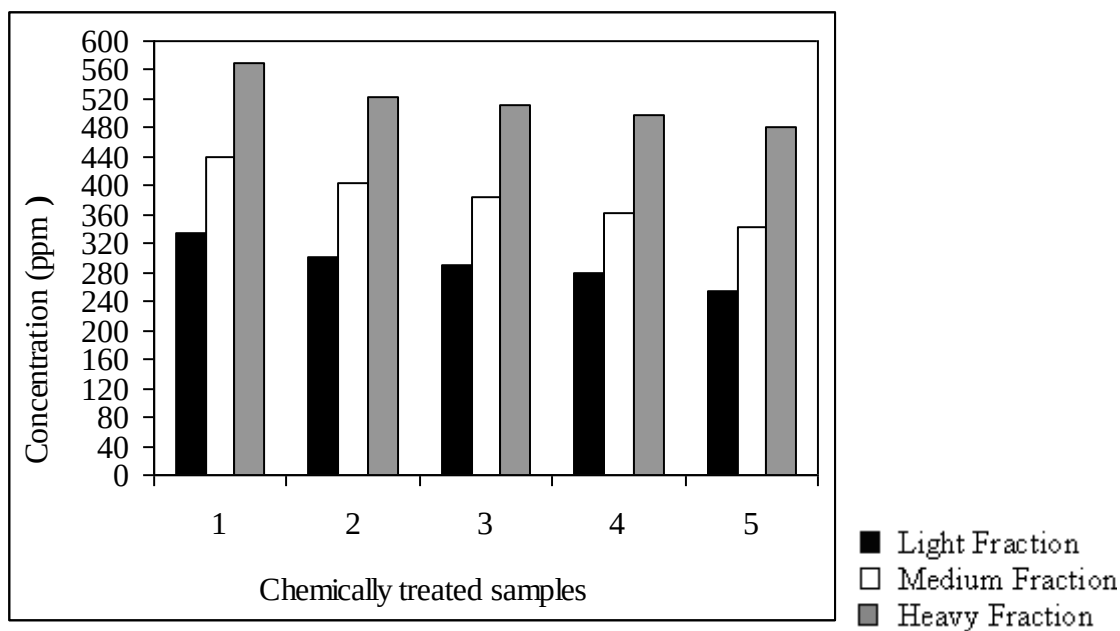
Aromatic sulfurous compounds cause series problems to the refinery and as well as to the environment because of SO_x emission, when crude oil combustions occurs in the refineries. Due to the presence of benzene ring, these compounds are carcinogenic in nature and having an unpleasant smell. An attempt was made to remove this sulphur apologue chemically as well as biologically. Oil distillation fractions were analyzed for benzothiophene first in case of original light, middle and heavy fractions. These values are given in Fig.55, which were 334, 440 & 570 ppm The same fractions were exposed to four different chemical reagents for the purpose of chemical desulphurization, and the residual samples from each chemical treatment were again tested with GC-MS for benzothiophene, where the BTH contents observed were 302, 403, & 522 ppm; 290, 384, & 511p pm; 278, 363, & 499 ppm, and 254, 342, & 480 ppm in case of hydrochloric acid (HCl), sodium hydroxide(NaOH), sodium arsenate (Na_2HAsO_4) and vanadium vendate (NH_4VO_3) treatments, respectively .The depletion can also be observed in GC-MS spectra profiles 28-36.

Percent reduction has also been determined between the chemically treated and virgin sample fractions; the complete data has been given in Fig.56. The reduction observed in light, middle and heavy fractions was 9.58, 8.40, & 8.42 %; 13.17, 12.72, & 10.35%; 16.76, 17.50, & 12.45 % and 23.95, 22.27, & 15.78 % in case of HCl, NaOH,

Na_2HAsO_4 and NH_4VO_3 treated residual samples, respectively. From the data obtained, it is concluded that ammonium vendate has been able to reduce the maximum amount of benzothiophen in the distillates understudy.

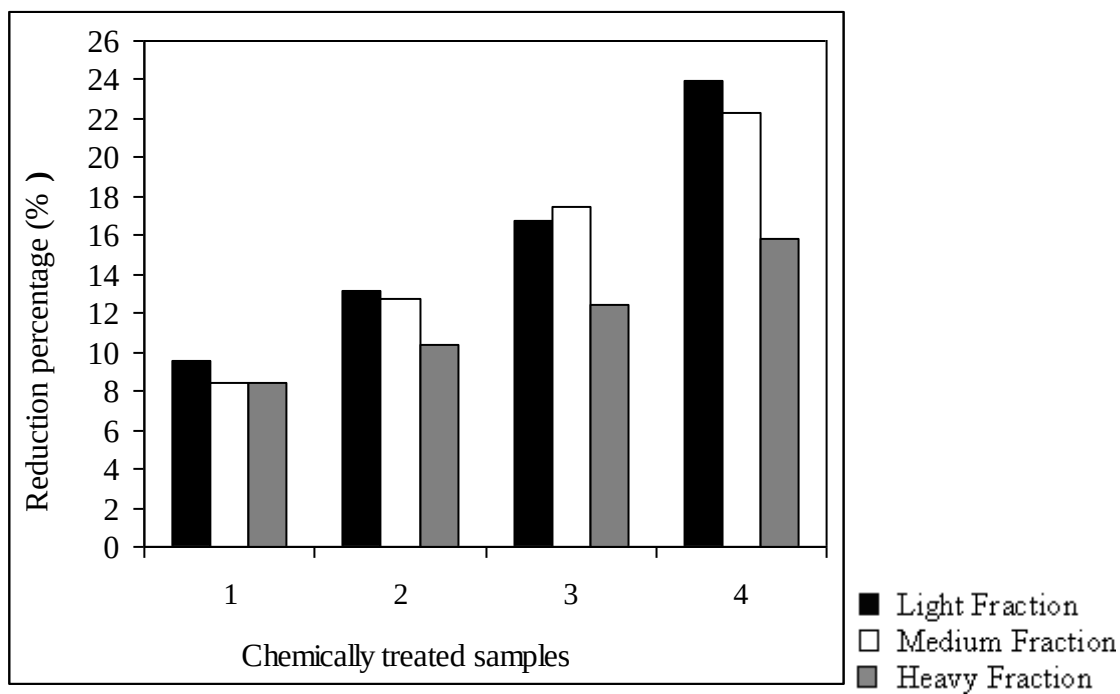
Benzothiophene has been found in chemical desulphurization among the chemicals used and the data found was 9.58, 8.40 & 8.42 % ; 3.59, 4.32 & 1.93 % ; 3.59, 4.78 & 2.1 % and 7.19, 4.77 & 3.33 % respectively in the samples of virgin – HCL , HCL – NaOH, NaOH- Na_2HAsO_4 and Na_2HAsO_4 - NH_4VO_3 in light , middle and heavy fractions.

Figure-55. Level of benzothiophene (ppm) determined in chemically treated distillate fractions



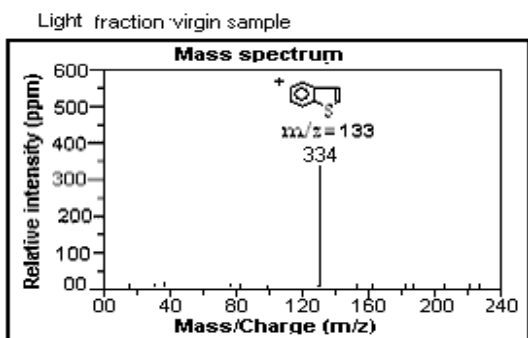
S#	Sample treated	Light fraction (ppm)	Medium fraction (ppm)	Heavy fraction (ppm)
1	Virgin	334 (± 2.510)	440 (± 2.00)	570 (± 1.520)
2	Hydrochloric acid	302 (± 5.700)	403 (± 1.520)	522 (± 1.00)
3	Sodium hydroxide	290 (± 1.00)	384 (± 1.520)	511 ($\pm 1.52.00$)
4	Sodium arsenate	278 (± 22.510)	363 (± 1.00)	499 (± 3.516)
5	Ammonium vendate	254 (± 2.5100)	342 (± 2.5100)	480 (± 1.527)

Figure-56: Reduction (%) of benzothiophene determined in chemically treated distillate fractions

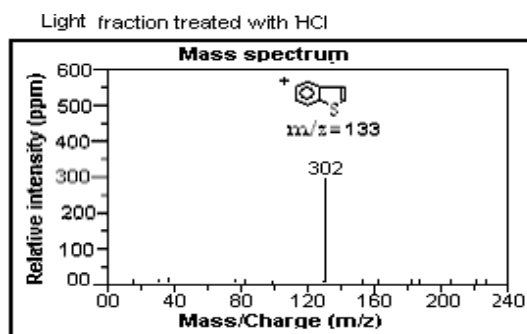


S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin-Hydrochloric acid	9.580	8.409	8.421
2	Virgin-Sodium hydroxide	13.173	12.727	10.350
3	Virgin-Sodium arsenate	16.766	17.500	12.456
4	Virgin-Ammonium vendate	23.952	22.272	15.789

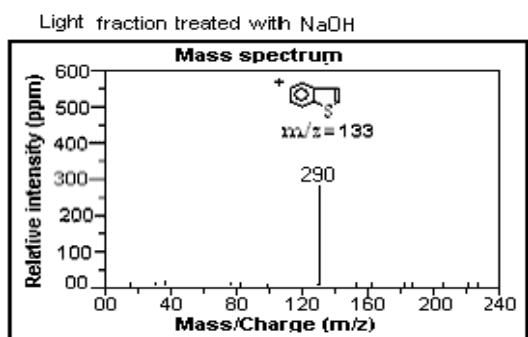
Spectrum profile-28. Benzothiophene GC-MS spectra of virgin and variously chemically treated light fractions.



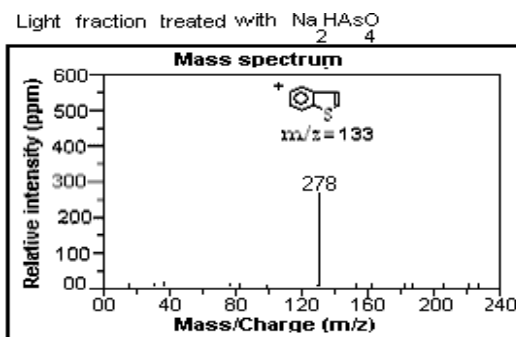
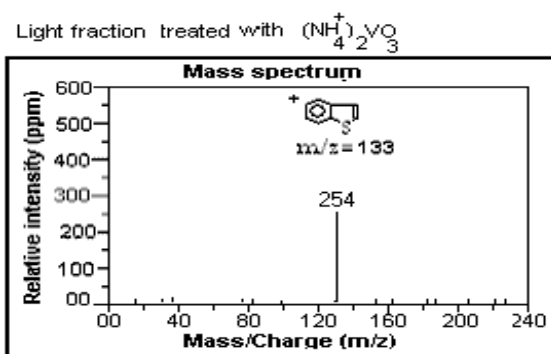
(a). L. F virgin sample.



(b). L.F treated with HCL.

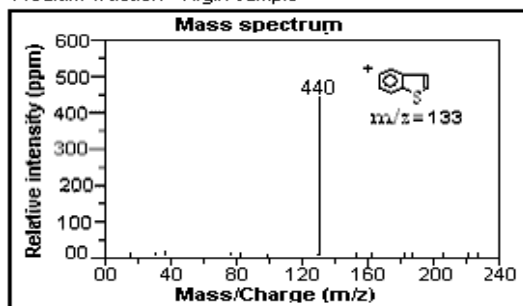


(c). L.F treated with NaOH.

(d). L.F treated with Na_2HAsO_4 .(e). L.F treated with $(\text{NH}_4)_2\text{VO}_3$.

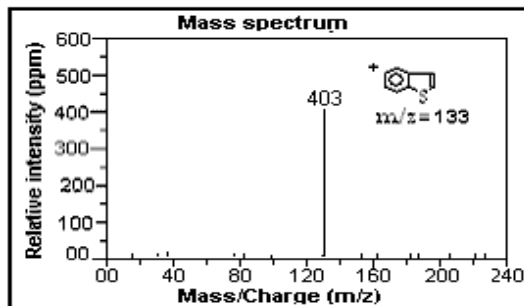
Spectrum profile-31. Benzothiophene GC-MS spectra of virgin and variously chemically treated middle fractions.

Medium fraction Virgin sample



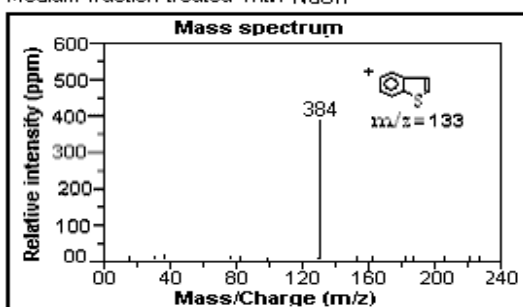
(a). M. F virgin sample.

Medium fraction treated with HCl

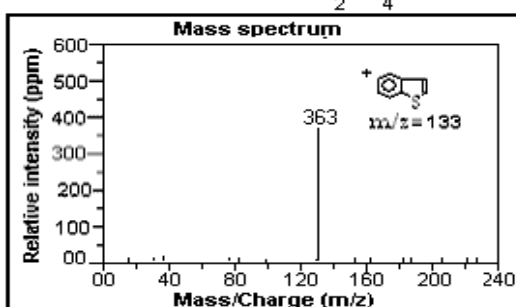
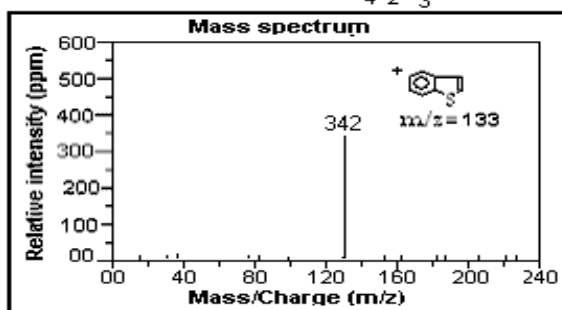


(b). M.F treated with HCL.

Medium fraction treated with NaOH

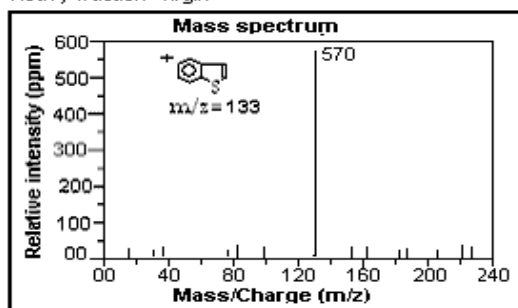


(c). M.F treated with NaOH.

Medium fraction treated with NaHAsO_4 (d). M.F treated with Na_2HASO_4 .Medium fraction treated with $(\text{NH}_4)_2\text{VO}_3$ (e). M.F treated with $(\text{NH}_4)_2\text{VO}_3$.

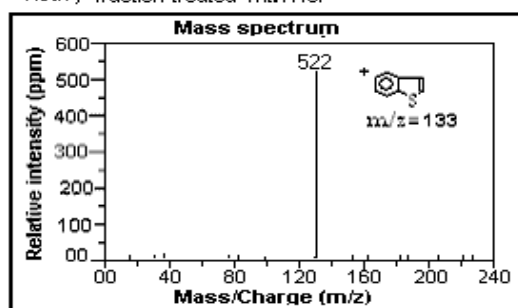
Spectrum profile-34. Benzothiophene GC-MS spectra of virgin and variously chemically treated heavy fractions.

Heavy fraction virgin



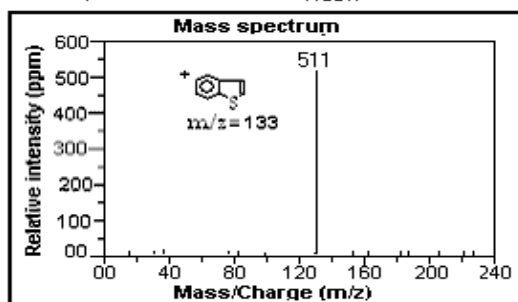
(a). H. F virgin sample.

Heavy fraction treated with HCl

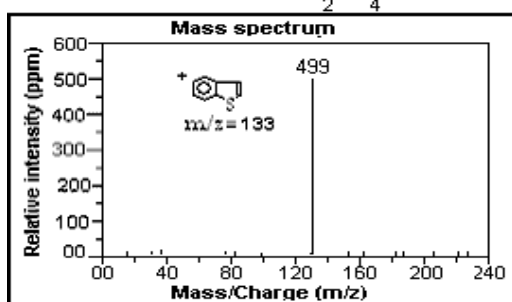
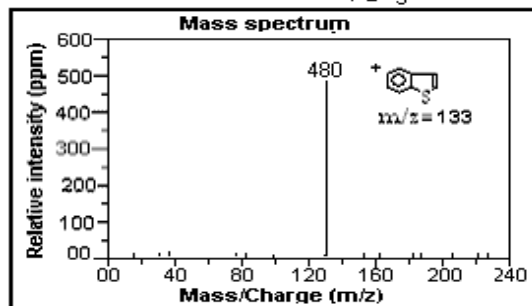


(b). H.F treated with HCL.

Heavy fraction treated with NaOH



(c). H.F treated with NaOH.

Heavy fraction treated with NaHAsO_4 (d). H.F treated with Na_2HAsO_4 .Heavy fraction treated with $(\text{NH}_4)_2\text{VO}_3$ (e). H.F treated with $(\text{NH}_4)_2\text{VO}_3$.

Reduction with P.putida

Microorganisms have naturally the potential to take aromatic sulfurous compounds from the petroleum products and to convert them to their respective aromatic alcohols, through their modified enzymatic system and causes desulphurization of the fuel based products (81). For desulphurization, *P.Putida* strain was selected for reduction of benzothiophene in the crude oil distillation fractions. The capability of the strain for BT removal was measured as a function of inoculation time. The inoculations were performed for time duration selected from 12 hr- to one month. The sulphur contents determined in the original light, middle and heavy fractions were 334, 440, & 570 ppm (Fig. 57). The same samples were inoculated with the pre cultured *P.putida* for time duration of 12 hr and benzothiophene contents determined were 180, 238, & 302 ppm. Comparing with the value obtained in case of original samples, considerable depletion can be seen, which shows that a significant amount of this sulphur moiety has been depleted by the strain during this initial inoculation time for its nourishments. In order to optimize the inoculation time, samples were kept in contact with the same microorganism beyond 12 hr. The inoculated time selected was; 24 hr, 48 hrs, one week and one month. The data of sulphur depletion is provided in Fig.57. The values of BTH determined in the residual samples from these inoculations were 162, 209, & 278 ppm , 153, 193, & 263 ppm; 144, 185, & 244 ppm, and 139, 172,& 232 ppm, respectively in light, middle and heavy fractions. .

It can be seen from the data that depletion is quite significant and rapid in case of 12 hr inoculation time. The reason for this significant reduction of BTH in the initial inoculation time is that sulphur heterocyclic compounds can serve as a sulphur source for

growth of microorganism under favorable conditions as reported elsewhere (82). Under such conditions, the bacterium releases its desulphurizing enzymes (dzs) for the rapid consumption of benzothiophene in testing distillates, and thus reproduces its self very rapidly forming colonies.

It can also be seen from the data compiled in Fig.57 that when the time was extended beyond 12 hr, sulphur depletion was in route in case of each inoculation. However, the extent is small compared to 12 hr inoculation. This dormant desulphurization is due to the fact that upon initial metabolism of sulphur configurations by the microorganism, aromatic acids are produced and hence released in to the solution middle leading to change in pH. This change in pH alters the affinity of microorganism and hence its capacity for uptake or binding with benzothiophene thereby minimizes the sulphur depletion. Aromatic acids are well-known by-products of anaerobic degradation of unsubstituted or alkylated aromatic hydrocarbons (83-85). The release of such acids in to the solution middle alters the pH, which lead to non availability of sulphur induced proteins, hence the organism lose its ability to oxidize sulphur further.

Depletion is observed in the rest of inoculations as well, however, the amount depleted is minimal with respect to initial inoculation time. The reason for the insignificant reduction in sulphur(BT) in case of 24 hr, 48 hr, one week and one month inoculation time is the poor starvation survival of bacterium as a consequence of several stresses; alteration of pH of middle, nutrients deprivation & residual metabolites accumulation. Under these circumstances, the cell viability is affected. The organisms are denourished, rendered the chances of invasion on target configurations inactive and

eventually die due to starvation. Bacterial starvation can affect the performance and cellular culturabilities; cell growth and cell survival and hence leading to cell death which stops the biological activities. Dead cells can also affect cell adhesion and biofilm attachment, hence leading to poor activity both in natural and engineering applications. In addition, formation of complex bacterial communities and some chemo repellents results in phenotypic changes in microorganisms there by rendering them inactive (86). The effect of organic solvents, pH of solution middle and nutrient deprivation on the activity of *Ps. putida* is reported elsewhere (87-89). Data obtained during pseudomonas putida treatment is given in GC-MS spectrum profiles (28-36).

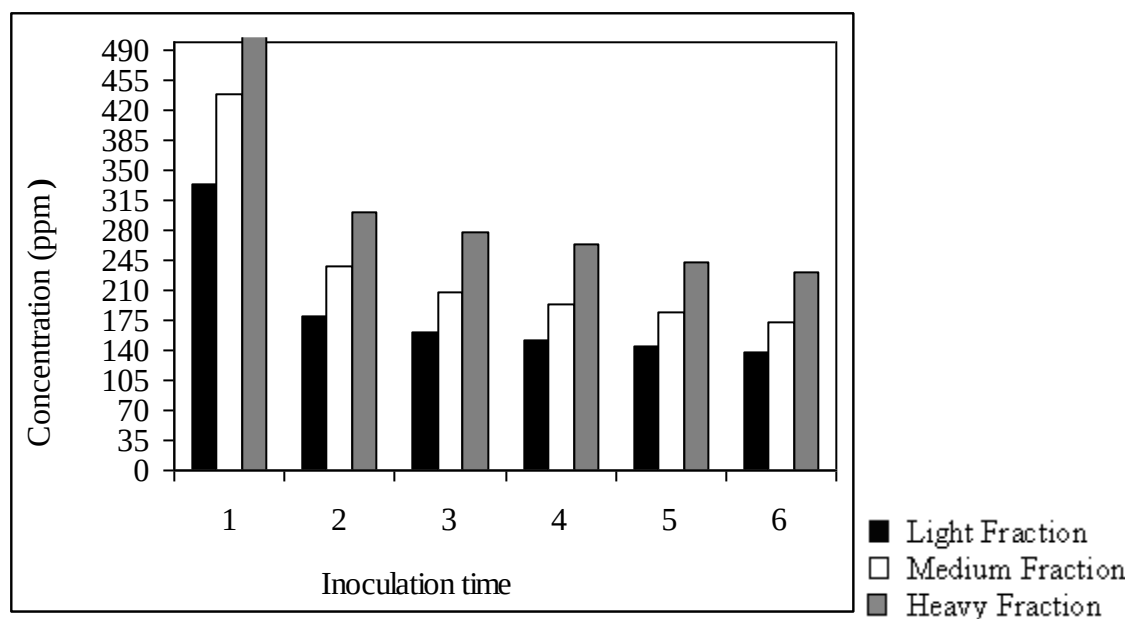
Percent reduction data has also been assembled in Fig.58. It is evident that 12 hr inoculation time has caused maximum depletion when compared with original sample i.e. 46.10, 45.90, & 47.01 %. An enhancement of further reduction percentage can be seen in case of 24 hr inoculation time, which is 51.49, 52.50, & 51.22 %, in case of light, middle and heavy fractions, respectively. Similarly, when the time was extended up to 48 hr, one week and one month, the increase in sulphur depletion observed was 54.19, 56.13, & 55.61%; 56.88, 57.95, & 57.19 % and 58.38, 60.90, & 59.29 %, respectively in the mentioned distillates. Inoculation time beyond 12 hr caused depletion but not too significant, hence, 12 hr reaction time is suggested to be an optimum time.

In case of *P.putida* treated distillate fractions benzothiophen was checked in the selected variously inoculated samples, the data obtained was noted as 46.10, 45.90 & 47.01 % ; 5.39, 6.60 & 4.21 % ; 2.7 , 3.63 & 4.39 % ; 2.69 , 1.82 & 1.58 % and 1.50, 2.95

& 2.1 % in virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

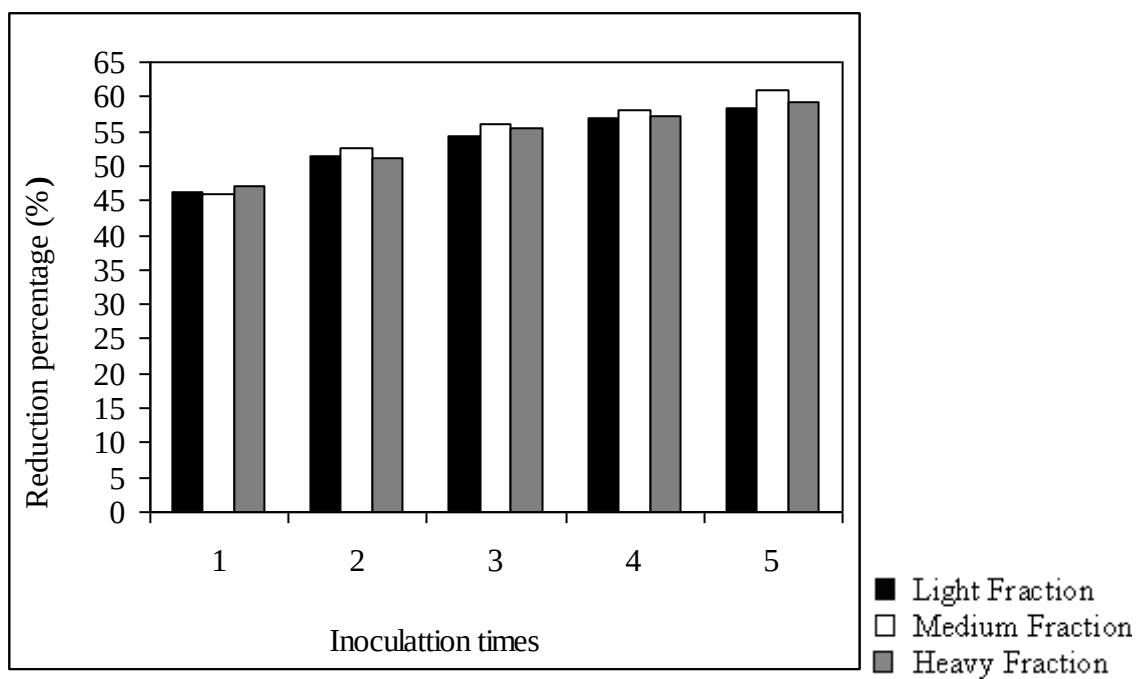
The depletion obtained in our study is quite significant compared to the previously reported microbial desulphurization particularly for middle and heavy oil fractions (90-91).

Figure-57: Level of benzothiophene (ppm) sulfur in *P.putida* treated as a function of inoculation time.



S#	Sample treated	Light fraction (ppm)	Medium fraction (ppm)	Heavy fraction (ppm)
1	Virgin	334 (± 2.516)	440 (± 2.00)	570 (± 1.520)
2	12 hours	180 (± 2.00)	238 (± 3.50)	302 (± 2.500)
3	24 hours	162 (± 4.500)	209 (± 2.500)	278 (± 1.00)
4	48 hours	153 (± 3.510)	193 (± 1.00)	263 (± 1.527)
5	One week	144 (± 1.520)	185 (± 3.00)	244 (± 1.527)
6	One month	139 (± 2.00)	172 (± 3.511)	232 (± 1.527)

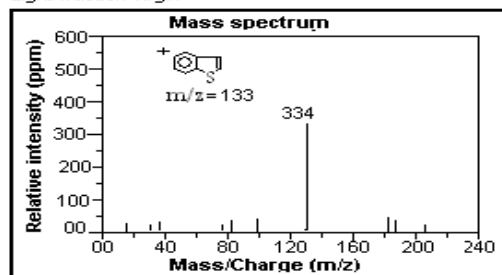
Figure-58. Reduction (%) of benzothiophene determined in *P.putida* treated distillate fractions as a function of inoculation.



S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	46.10	45.90	47.01
2	Virgin -24 hours	51.49	52.50	51.22
3	Virgin -48 hours	54.19	56.13	55.61
4	Virgin -one week	56.88	57.95	57.19
5	Virgin - one month	58.38	60.90	59.29

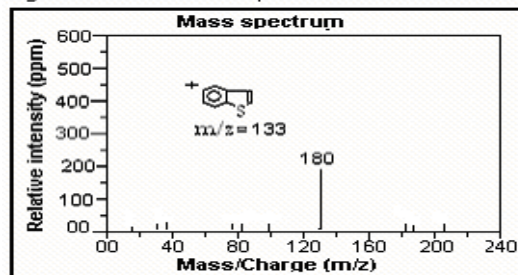
Spectrum profile-29. . Benzothiophene GC-MS spectra of virgin and *Ps.putida* treated light fractions.

Light fraction virgin



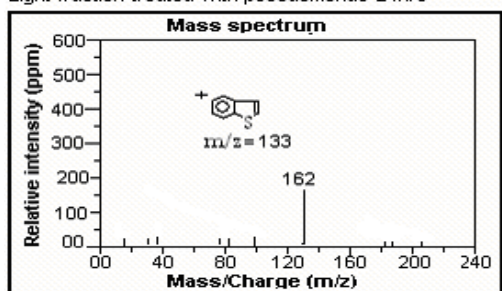
(a). L. F virgin sample.

Light fraction treated with pseudomonas 12hrs



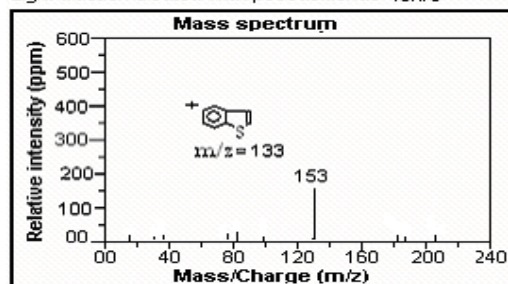
(b). L.F treated with *Ps.putida* (12 hr).

Light fraction treated with pseudomonas 24hrs



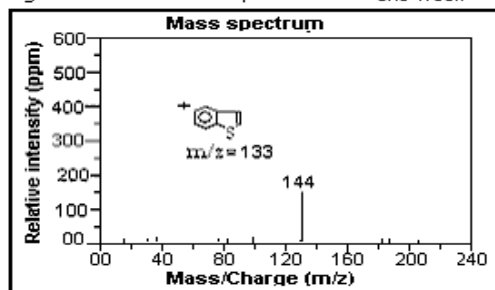
(c). L.F treated with *P.putida* (24 hr).

Light fraction treated with pseudomonas 48hrs



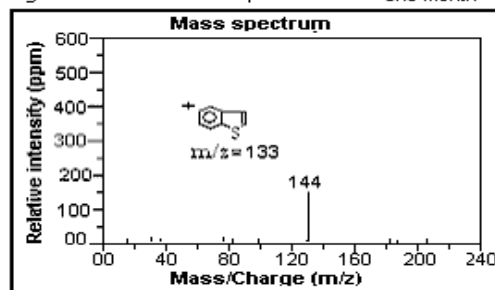
(d). L.F treated with *P.putida* (48 hr).

Light fraction treated with pseudomonas one week



(e). L.F treated with *P.putida* (week).

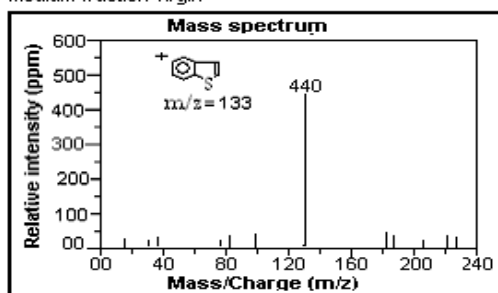
Light fraction treated with pseudomonas one month



(f). L.F treated with *P.putida* (month).

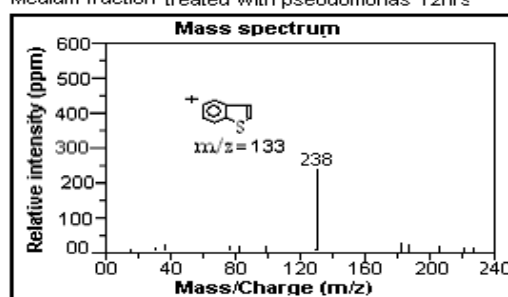
Spectrum profile-32. Benzothiophene GC-MS spectra of virgin and *P.putida* treated middle fractions.

Medium fraction virgin



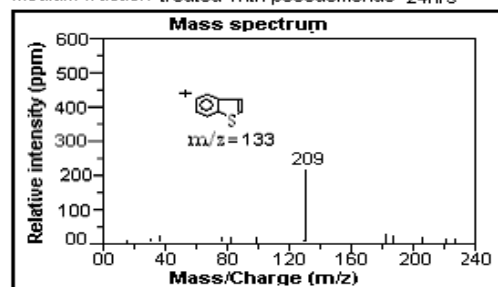
(a). M. F virgin sample.

Medium fraction treated with pseudomonas 12hrs



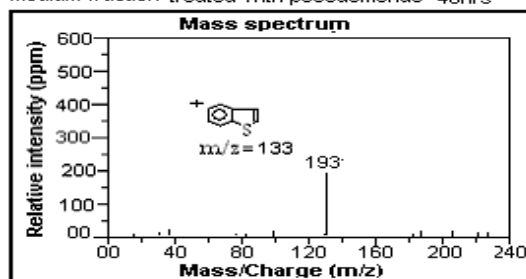
(b). M.F treated with *P.putida* (12 hrs).

Medium fraction treated with pseudomonas 24hrs



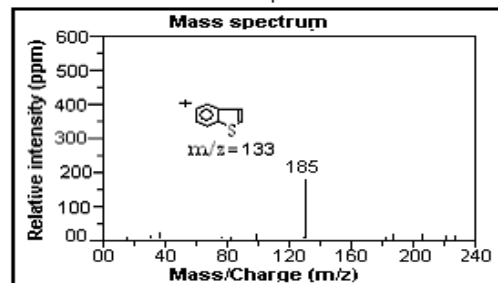
(c). M.F treated with *P.putida* (24 hr).

Medium fraction treated with pseudomonas 48hrs



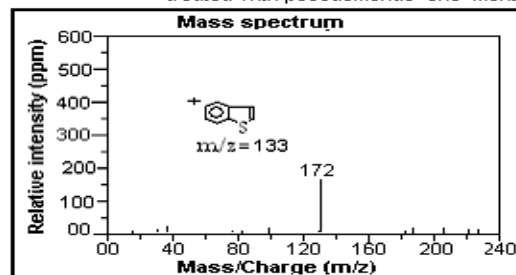
(d). M.F treated with *P.putida* (48 hr).

Medium fraction treated with pseudomonas one week



(e). M.F treated with *P.putida* (week).

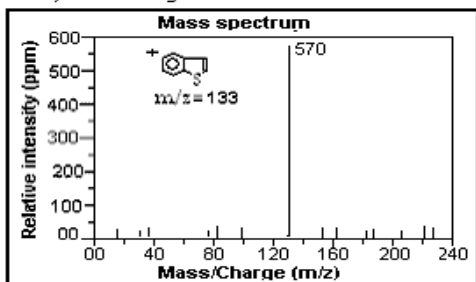
Medium fraction treated with pseudomonas one month



(f). M.F treated with *P.putida* (month).

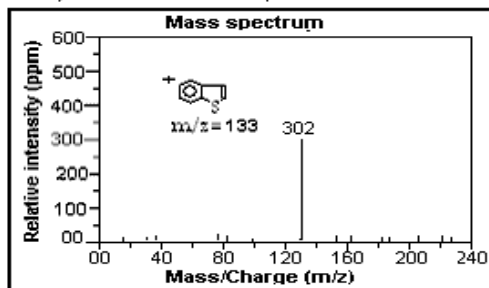
Spectrum profile-35. Benzothiophene GC-MS spectra of virgin and *Ps.putida* treated heavy fractions.

Heavy fraction virgin

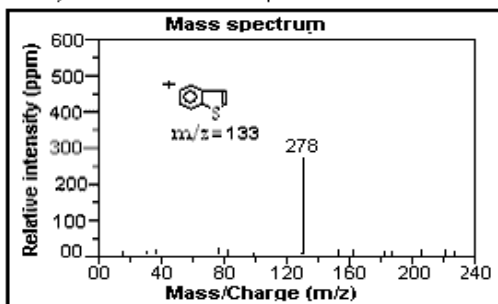


(a). H. F virgin sample.

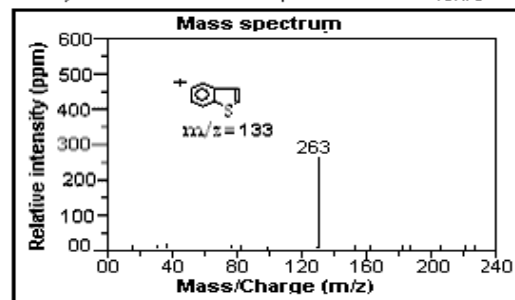
Heavy fraction treated with pseudomonas 12hrs

(b). H F treated with *P.putida* (12 hr).

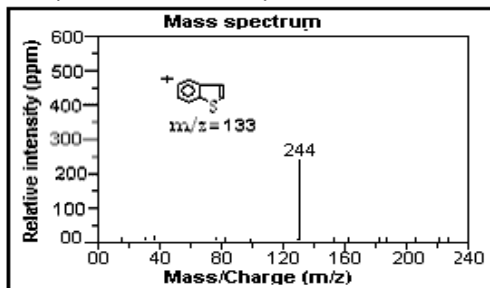
Heavy fraction treated with pseudomonas 24hrs

(c). H.F treated with *P.putida* (24hr).

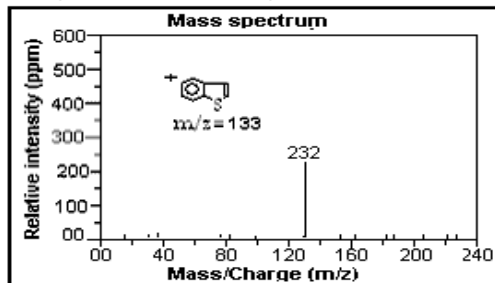
Heavy fraction treated with pseudomonas 48hrs

(d). H.F treated with *P.putida* (48hr).

Heavy fraction treated with pseudomonas one week

(e). H.F treated with *P.putida* (week).

Heavy fraction treated with pseudomonas one month

(f). H.F treated with *P.putida* (month).

Removal with T.Thioxidanes

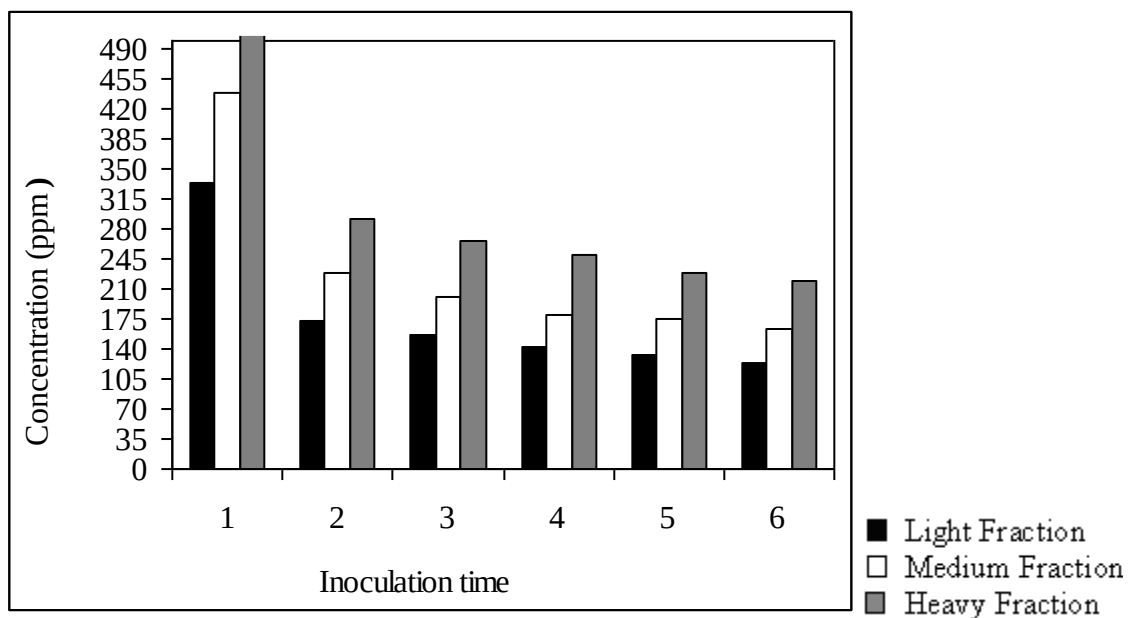
T.Thioxidans strain was inoculated for various contact time as mentioned earlier (12hr- one month) in crude oil distillates. Benzothiophene (BTH) determined with GC-MS. From the data obtained, it is clear that this bacterium has removed more sulfur than *Ps.putida* during the same inoculations, which is quite clear from the Fig. 59. It is observed that in case of original fractions, benzothiophene contents were 334, 440, & 570 ppm, in light, middle and heavy fractions, respectively. In the microbially treated samples, BTH contents determined in light, middle, and heavy fractions were 173, 229, & 293ppm; 156, 201, & 267 ppm and 142, 180, & 250 ppm in case of 12hr, 24hr and 48hr, respectively. As the inoculation time increases, *T.thioxidans* converts sulfur contents to sulfates and sulfuric acid, where the pH continuously decreased, and an acidic middle created. As the inoculation time was further extended even up to one month benzothiophene level was decreased to 134, 175, & 230 ppm and 123, 164, & 219 ppm in case of one week and one month inoculations. The reduction in sulphur can be seen in GC-MS spectra profiles 28-36.

Fig. 60 represents the percent reduction achieved in each inoculation. The reduction has been clearly shown between the virgin and *T. Thiobacillus* treated samples for various times (12hr-one month). A significant reduction has been made during all inoculations. The reduction observed in light, middle, and heavy fractions were 48.20, 47.95, & 48.59 %; 53.29, 54.31, & 53.15 %; 57.48, 59.09, & 56.14 %; 59.88, 60.22, & 59.64 % and 63.17, 62.72, & 61.57 %, respectively in case of 12 hr, 24 hr, 48 hr, one week and one month inoculations. From the data, it can be concluded that *T.thioxidans*

likes aromatic sulfurous compounds, and consume them as their nutrients to increase their reproduction.

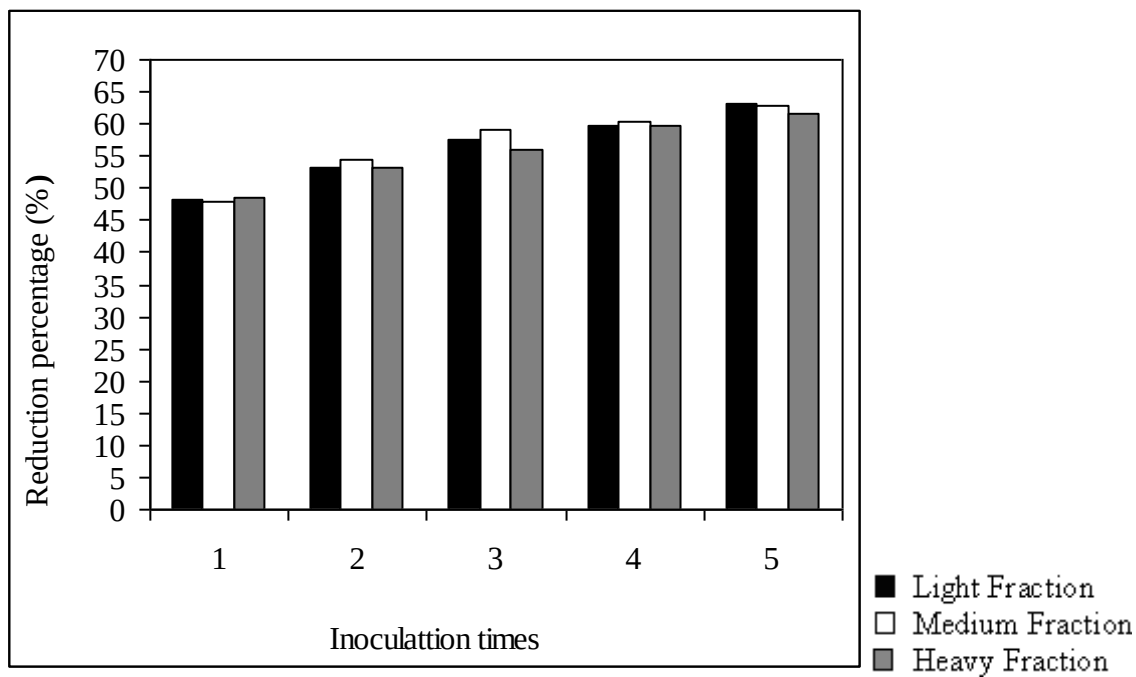
T.thiobacillus treated samples were studied for benzothiophen form contact time to time and the values collected were 48.20, 47.95 & 48.59 % ; 5.09, 6.36 & 4.56 % ; 4.19 , 4.78 & 2.99 % ; 2.40, 1.13 & 3.50 % and 3.29, 2.50 & 1.93 % in the cases of virgin – 12hr, 12hr – 24 hr, 24hr – 48hr, 48hr – one week and one week – month respectively in light, meddle and heavy fractions.

Figure-59: Level of benzothiophene (ppm) sulfur determined in *T.Thioxidanes* treated distillate fractions as a function on of inoculation time



S#	Sample treated	Light fraction (ppm)	Middle fraction (ppm)	Heavy fraction (ppm)
1	Virgin	334 (± 2.516)	440 (± 2.00)	570 (± 1.527)
2	12 hours	173 (± 2.516)	229 (± 2.5166)	293 (± 2.516)
3	24 hours	156 (± 1.527)	201 (± 1.527)	267 (± 1.527)
4	48 hours	142 (± 2.521)	180 (± 2.516)	250 (± 1.527)
5	One week	134 (± 1.527)	175 (± 1.527)	230 (± 1.527)
6	One month	123 (± 2.00)	164 (± 2.000)	219 (± 2.00)

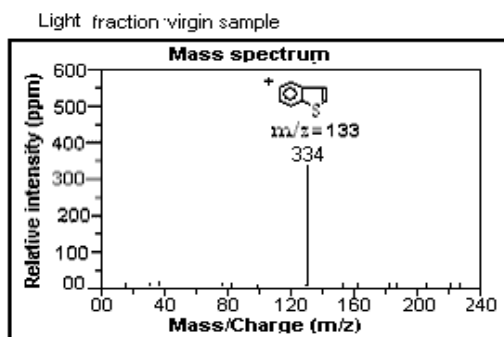
Figure-60. Reduction (%) of benzothiophene determined in *T.Thioxidanes* treated distillate fractions as a function of inoculation time.



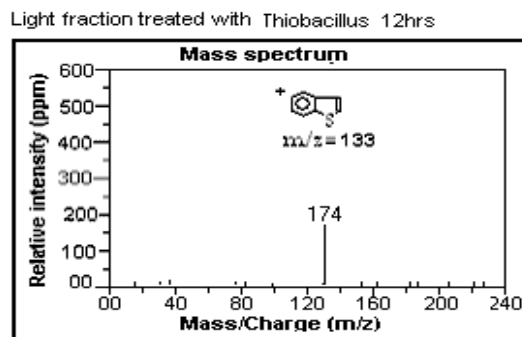
S#	Sample treated	Light fraction (%)	Medium fraction (%)	Heavy fraction (%)
1	Virgin -12 hours	48.203	47.954	48.596
2	Virgin -24 hours	53.293	54.318	53.157
3	Virgin -48 hours	57.485	59.090	56.140
4	Virgin -one week	59.880	60.227	59.649
5	Virgin - one month	63.173	62.727	61.578

Spectrum profile-30. Benzothiophene GC-MS spectra of virgin and *T.*

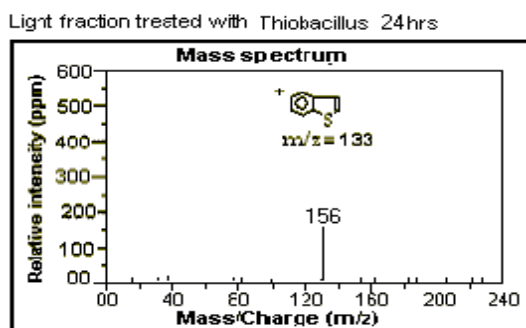
Thioxidanes treated light fractions.



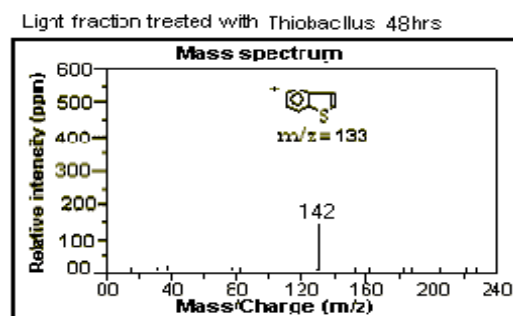
(a). L.F virgin sample.



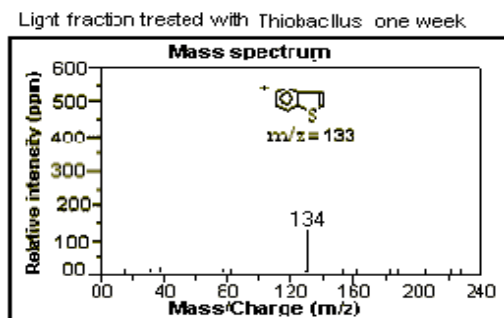
(b). L.F treated with *T.Thioxidanes* (12h).



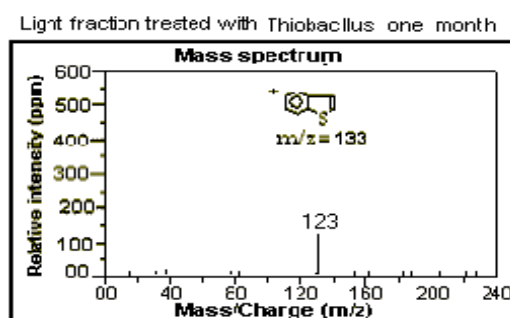
(c). L.F treated with *T.Thioxidanes* (24hr).



(d). L..F treated with *T.Thioxidanes* (48hr).



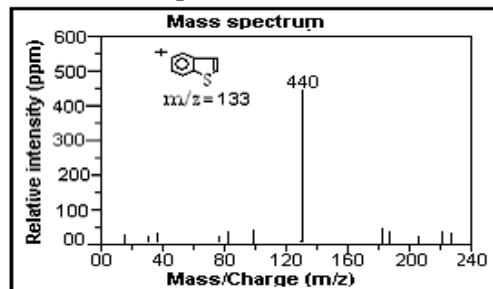
(e). L.F treated with *T.Thioxidanes* (week).



(f). L.F treated with *T.Thioxidanes* (month)

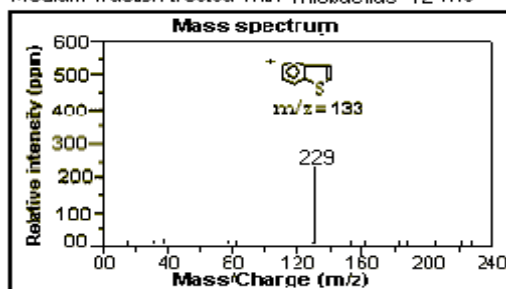
Spectrum profile-33. Benzothiophene GC-MS spectra of virgin and T.thioxidans treated middle fractions.

Medium fraction virgin



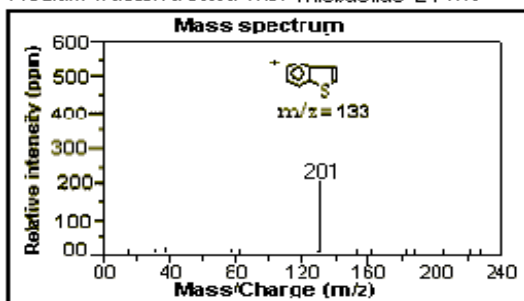
(a). M. F virgin sample.

Medium fraction treated with Thiobacillus 12 hrs



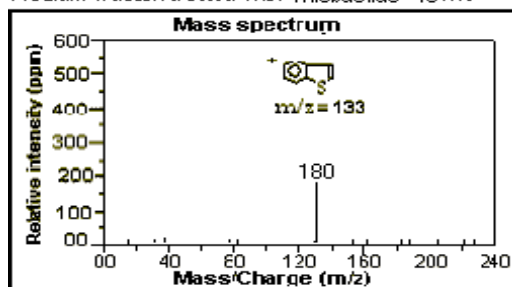
(b). M.F treated with *T.thioxidans* (12 hr).

Medium fraction treated with Thiobacillus 24 hrs



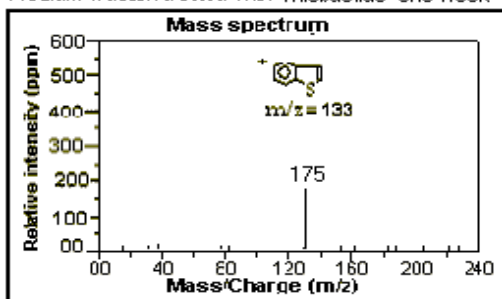
(c). M.F treated with *T.thioxidans* (24hr).

Medium fraction treated with Thiobacillus 48 hrs



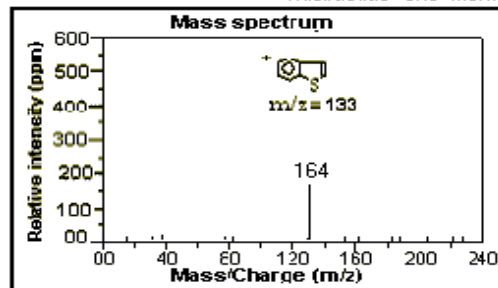
(d). M.F treated with *T.thioxidans* (48hr).

Medium fraction treated with Thiobacillus one week



(e). M.F treated with *T.thioxidans* (week).

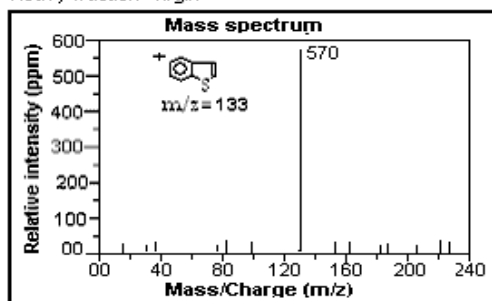
Medium fraction treated with Thiobacillus one month



(f). M.F treated with *T.thioxidans* (month).

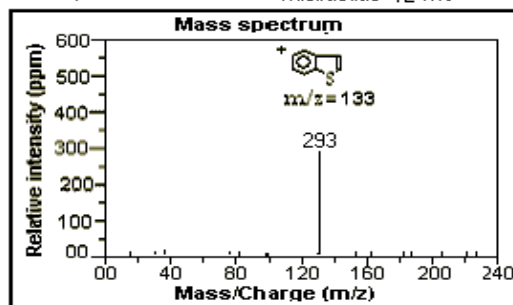
Spectrum profile-36. Benzothiophene GC-MS spectra of virgin and *T.thioxidans* treated heavy fractions.

Heavy fraction virgin



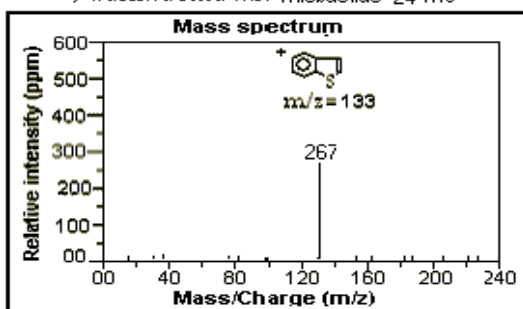
(a). H.F virgin sample.

Heavy fraction treated with *Thiobacillus* 12 hrs



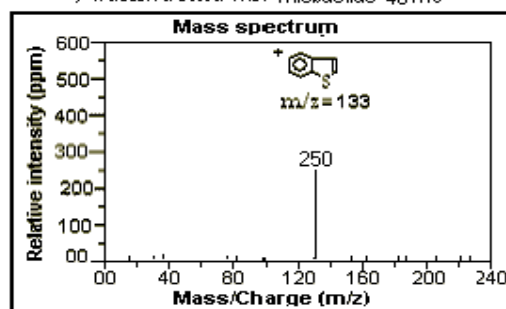
(b). H.F treated with *T.thioxidans* (12 hr).

Heavy fraction treated with *Thiobacillus* 24 hrs



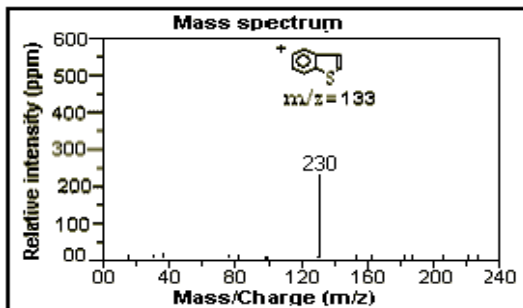
(c). H.F treated with *T.thioxidans* (24hr).

Heavy fraction treated with *Thiobacillus* 48 hrs



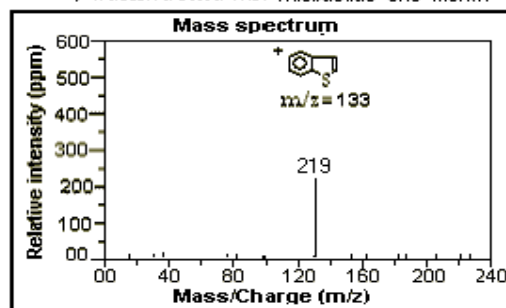
(d). H.F treated with *T.thioxidans* (48hr).

Heavy fraction treated with *Thiobacillus* one week



(e). H.F treated with *T.thioxidans* (week).

Heavy fraction treated with *Thiobacillus* one month



(f). H.F treated with *T.thioxidans* (month).

CONCLUSIONS

Two desulphurization avenues (chemical and biological) were studied and the results have been compared. The following conclusions were drawn:

- In chemical desulphurization, the effect of four chemicals was investigated. On the bases of desulphurization ability, ammonium vendate (NH_4VO_3) was found to be the best amongst the chemicals used, which has caused a significant reduction of all analogues of sulfur followed by sodium arsenate (Na_2HAsO_4), followed by sodium hydroxide (NaOH), followed by hydrochloric acid (HCl).
- In biological desulphurization, two of the bacterial strains were cultured in coal mine water and were cultivated with the distillates under study varying the time. From the data collected, it is concluded that *T. Thioxidans* has been found to be an excellent desulphurization strain, which has depleted a significant amount of sulfurous compounds. *Pseudomonas putida*, which also caused removal of enough sulfurous compounds from the crude oil distillate fractions but was found to be inferior compared to *T, Thioxidanes*.
- The effect of inoculation time was also investigated. It is inferred from the results that sulphur (each moiety) was enroute all the way with the extension in time from 00 hr till one month. However, maximum depletion was recorded in case of 12 hr inoculation time. The reduction observed when the time was extended from 12 hr-24hr, 24 hr-48 hr, 48 hr-one week, and one week to one month was not so much appreciable. Hence, 12 hr is suggested to be an optimum time for cost effective desulphurization.

REFERENCES

1. Paul Frank, Schemidth, *Fuel oil manual*, **1**, 112-113 (1985).
2. Prikey, Mike *Am.Chem.Soc. Div.Pet.Chem.* **45**, (4) 701-702 (2000).
3. ASTM, annual book of ASTM standard, *ASTM Philadelphia*, **5.01-5.04**, (1989).
4. N .B.Korchangina, Vassoyevith, *Geol.Re*, **12**, 1276 (1969).
5. Hydrocarbon processing **57** (9), 178-184 (1978).
6. B. P., Welte, Tissot, D. H “petroleum formation and occurrence” *Springer Verlag New York* (1968)
7. W. L. Nelson, “petroleum refinery processing” *Mc Graw-Hill, New York*, 910 (1958).
8. D. T., Chowdry, Rao Khthing, . *Gangadharan,s Meas Sci. Technol.*, **2** (7), 610 (1991).
9. J.S. Reid, T.A. Gearhart, E.A. Flocchini, Cahill, *Nucl. Geophys.* **6** (4) 513-516. (1992).
10. I. L. Bartle, Davies, K.D . Williams, *Anal. Chem.* **60**, 204-209 (1988).
11. J.B. McKinley, Reinhold, New Yark, *Catalysis*, **5**, 405 (1957).
12. R.A. Filinn and J.Mckinley , *U.S Patent*, **2**, 880, 171 9 (1959).
13. S.C. Schuman and H. Shalit, *Catal Rev.*, **4** (2), 245 (1970).
14. G.C .A. Schauit and B.C. *Gates, Aich*, **19** (30) 417 (1973).
15. W. F. Bland and R.L. Davison, petroleum processing Handbook, *McGraw-Hill, New Yark*, **3**, (1967).

16. W.L.Nelson, "petroleum refinery processing" McGraw-Hill, New York, **10**, (1969).
17. G.F.Ondish, J.A. Frayer, and .J.D.McKinney, *Hydrocarbon processing*, **55** (7), 105, (1976).
18. I. M. Campbell, *Chemtech*, **23**(10), 43-46 (1993).
19. J. G. Speigh, The desulfurization of heavy oil and residua. *Marcel Dekker, New Yark, N.Y.* (1989).
20. D.C.Bressler, J. A. Norman, and P. M. Fedorak.. *Biodegradation*. **8**, 297(1998)
21. A. Ishihara, Kabe, and H. Tajima.. Hydrodesulfurization of sulfur-containing polyaromatic compounds in light oil. *Ind. Eng. Chem. Res.* **31**, 1577-1580 (1992).
22. F.J.Hartdegen, J. M. Coburn, and R. L. Roberts. The microbial desulfurization of petroleum. *Chem. Eng. Prog.*, **80**, 63-67 (1984).
23. C. T Hou, and A. I. Laskin.. Microbial conversion of dibenzothiophene. *Dev. Ind. Microbiol.* **17**,351-362. 1976
24. K.Kodama, S. Nakatani, K. Umehara, K. Shimizu, Y. Minoda, and K. Yamada. 1970. Microbial conversion of petrosulfur compounds. III. Isolation and identification of products from dibenzothiophene. *Agric. Biol. Chem.* **34**,1320-1324.
25. K. Kodama, K. Umehara, K. Shimizu, S. Nakatani, Y. Minoda, and K. Yamada. Identification of microbial products from debinzothiophene and its proposed oxidation pathway. *Agric. Biol. Chem.*, **37**, 45-50 (1973)

26. K. A. Malik,. and D. Claus. Microbial degradation of dibenzothiophene, abstr. In H. Dellweg (ed.), Abstracts of the Fifth International Fermentation Symposium. *Berlin, Germany*, **23**, 421 (1976).
27. T. Halbert, A.Anderson, G. Markley, *Proc. World Petr. Congr.*, **15**, (1997).
28. M. K. Lee, J. D. Senius, M. J Grossman, *Appl. Environ. Microbiol.*, **61**, 4362-4366 (1995).
29. J. J. Kilbane, *Trends Biotechnol.* **7**(4), 97-101 (1989).
30. M. E. Gallardo, A. Ferrandez, V. De Lorenzo, J. L.Garcia, E. J. Diaz, *Bacteriol.*, **612**, 7156-7160 (1997).
31. K. A. Gray, O. S Pogrebinsky, G. T. Mrachko,, *Nature Biotechnol.*, **14** (12), 1705-1709 (1996).
32. C. S. Piddington, B. R. Kovacevich, Rambosek, *J. Appl. Environ. Microbiol.* **61**(2), 468-475 (1995).
33. S. A. Denome, E. S.Olson, K. D. Young, *Appl. Environ. Microbiol.* **59** (9), 2837-2843 (1993).
34. C. H. Monticello, Xi, L. Squires, D. J. Childs, J. D. *Biochem. Biophys. Res. Commun.* **230**, 73-75 (1997).
35. M. Z. Squires Li, C. H.; Monticello, D. J. Childs, J. D. J., *Bacteriol.*, **178** (22), 6409-6418 (1996).
36. F. Zannikos, E. Lois, S. Stournas,, *Fuel Process. Technol.* **42**, 35-45. (1995).
37. T.Ohshiro, Y. Kanbayashi, Y. Hine, Y. Izumi,. *Biosci. Biotechnol. Biochem.*, **59** (7), 1349-1351(1995).
38. D.J. Monticello, *hydrocarbon process.* **73** (2), 39-40, 42, 44-45 (1994).

39. D.J. Monticello, *proc. World petro .congr.* **15** (1997).
40. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 24/ 84 (1987).
41. F. Widdel, and F. Bak. The prokaryotes, **4**, 3352 (1992)
42. D. Cline. *Limnol. Oceanogr.* **14**, 454(1969)
43. M. J. Grossman, M. K. Lee, R. C. Prince, V. Minak-Bernero, G. N. George, and I. J. Pickering. *Appl. Environ. Microbiol.* **67**,1949-1952 (2001).
44. Honda, H., H. Sugiyama, I. Saito, and T. Kobayashi. *J. Ferment. Bioeng.* **85**, 334-338 (1998).
45. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 160/87 (1987).
46. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 71/87 (1987).
47. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 2/78 (1987).
48. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 15/67 (1987).
49. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 34/87 (1987).
50. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 4/81 (1987).
51. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 13/82 (1987).

52. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 242/83 (1987).
53. I .P standard method for Analysis and testing of Petroleum and related products, volume ASTM D 1226 -IP 107 (1987).
54. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 61/84 (1987).
55. V.Harry, Drushel, and Miller, F. James, *Anal. Chem.* **27**, 495, (1955).
56. H.S. Rohatgi and I.B. Gualatai, Agarwal, *journal of the institute of petroleum* **59**, 133-138 (1973).
57. V. Harry, Drushel, and Miller, , *Anal. Chem.* **27**, 495, (1955).
58. I .P standard method for Analysis and testing of Petroleum and related products, volume **2**, 342/79 (1985).
59. I .P standard method for Analysis and testing of Petroleum and related products, volume **1**, 103/88 (1987).

60. T. Hirata, Ohshiro, , and Y. Izumi. Desulfurization of dibenzothiophene derivatives by whole cells of *Rhodococcus erythropolis* H-2. *FEMS., Microbiol. Lett.* **142**, 65-70. (1996).
61. H. T. Thompson, C J. Coleman, H J.; Hopkins, R L. *Bulletin.* **659**, (1972).
62. R. Quintero, Borngre, Biotechnological processes for the refining of petroleum. *Fuel Processing Technol.* **81**,155-169 (2003).
63. M. V. Landau, M.Herskowitz, D. Givoni, S.Laichter, D. Yitzhaki, *Fuel.* **75** (7), 858-866 (1996).
64. Fei Tao, Bo Yu, Ping Xu, and Cui Qing Ma *J. Applied and Environmental Microbiology* **72**, (7) . 4604-4609 (2006).
65. Lee, M. K., J. D. Senius, and M. J. Grossman. *J. Appl. Environ. Microbiol.* **61**, 4362-4366 (1995)
66. Sylvia A. Denome, Edwin S. Olson and Kevin D. Young *J. Appl Environ Microbiol.* **59**(9): 2837-2843 (1993)
67. Buck, J. W., and J. H. Andrews. *J. Appl. Environ. Microbiol.* **65**, 465-471. (1999).
68. Wang, P., and S. Krawiec. *J. Arch. Microbiol.* **161** , 266-271 (1994).
69. D.Yitzhaki, M. V Landau, D. Berger, M. Herskowitz,. deep desulfurization of heavy atmospheric gas oil with Co-Mo-Al catalysts, effect of sulfur adsorption", *Appl. Catal. A*, **122**(2), 99-110 (1995).
70. G Voordouw, SM Armstrong, MF Reimer, B Fouts, AJ Telang, Y Shen and D Gevertz . *J.Appl. Environ. Microbiol.* **62** (5), 1623-1629.

71. ME Gallardo, A. Ferrandez, V De Lorenzo, JL Garcia and E Diaz *J. Bacteriol.*, **179** (22) 7156-7160 (1997).
72. R. M .Borichewski, *J. Bacteriol.* **93** (2):597-599 (1967).
73. D.Berger, M.V.Landau, M.Herskowitz, Z.Boger, "Deep hydrodesulfurization of atmospheric gas oil. Effects of operating conditions and modeling by artificial neural network techniques", *Fuel*, **75**(7), 907-911. (1996).
74. M. V. Landau, D.Berger , M. Herskowitz, *J. Catal.*, **159** (1), 236-45 (1996).
75. Metin Gürü, Meltem Cubuk , Sukru Dursun , Ayhan Demirbas *j. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects.*, **28**, 559 – 565 (2006).
76. M. Kertesz and C. Wietek *j. Applied Microbiology and Biotechnology* **57**, 460-466 (2001).
77. I .Hoff Rebecca, Z., 1993, *marine pollution bulletin*, **29**, 476-481.
78. Fedorak, M. F., and D. Grbic-Galic. Aerobic microbial cometabolism of benzothiophene and 3-methylbenzothiophene. *Appl. Environ. Microbiol.* **57**, 932-940 (1991).
79. Sung-Keun Rhee, Je Hwan Chang, Yong Keun Chang, and Ho Nam Chang *J. Appl Environ Microbiol* **64** (6), 2327-2331 (1998)
80. Lei, B., and S. C. Tu.. Gene overexpression, purification, and identification of a desulfurization enzyme from *Rhodococcus* *Rhodococcus* sp. strain IGTS8 as a sulfide/sulfoxide monooxygenase. *J. Bacteriol.* **178**, 5699-5705 (1996)
81. Gallagher, J. R., E. S. Olson, and D. C. Stanley.. Microbial desulfurization of dibenzothiophene: a sulfur-specific pathway. *J. FEMS Microbiol. Lett.* **107**, 31-36 (1993)

82. Tanaka, Y., T. Matsui, J. Konishi, and K. Maruhashi. Biodesulfurization of benzothiophene and dibenzothiophene by a newly isolated *Rhodococcus* strain. *J. Appl. Microbiol. Biotechnol.* **59**, 325-328 (2002.)
83. Cozarelli, I. M., R. P. Eganhouse, and M. J. Baedeker. Transformation of monoaromatic hydrocarbons to organic acids in anoxic groundwater environments. *J. Environ. Geol.* **16**, 135-141 (1990).
84. Heider, J., A. M. Spormann, H. R. Beller, and F. Widdel. *J. Anaerobic bacterial* metabolism of hydrocarbons. *FEMS Microbiol. Rev.* **22**, 459-473 (1999).
85. Zhang, X., and L. Y. Young.. Carboxylation as an initial reaction in the anaerobic metabolism of naphthalene and phenanthrene by sulfidogenic consortia. *J. Appl. Environ. Microbiol.* **63**, 4759-476 (1997)
86. Meckenstock, R. U., E. Annweiler, W. Michaelis, H. H. Richnow, and B. Schink.. Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. *J. Appl. Environ. Microbiol.*; **66**:2743-2747 (2000).
87. GuoBin Shan, JianMin Xing, HuaiYing Zhang, and HuiZhou Liu *J. Applied and Environmental Microbiology* 71 (8) 4497-4502 (2005).
88. Antje Kahnert, Paul Vermeij, Claudia Wietek, Peter James, Thomas Leisinger, and Michael A. Kertesz *Journal of Bacteriology* **182** (10) 2869-2878 (2000).
89. Grimberg, S. J., W. T. Stringfellow, and M. D. Aitken. 1996. Quantifying the biodegradation of phenanthrene by *Pseudomonas stutzeri* P16 in the presence of nonionic surfactant. *Appl. Environ. Microbiol.* **62**:2387-2392 (1996).