Biological Removal of Hydrogen Sulfide from Various Distillates of Crude Oil with *Pseudomonas putida*

[1]MUHAMMAD ISHAQ*, [1]MUHAMMAD NAEEM, [1]IMTIAZ AHMAD,
[1]MOHAMMAD SHAKIRULLAH AND [2]MUHAMMAD ARSALAN KHAN
[1]*Institute of Chemical Sciences, University of Peshawar, N.W.F.P, 25120, Pakistan.*
[2]*Department of Chemical Engineering, YIC, Yanbu AL Sinayah, KSA.*

(Received 17th March 2007, revised 9th July 2007)

Summary: Removal of hydrogen sulfide (H_2S) has been attempted from various distillation fractions with bacterium *Pseudomonas putida*. Biodesulfurization technique has been found to be more effective than conventional desulphurization methods. Tremendous amount of hydrogen sulfide (H_2S) has been removed in all the distillation fractions under study i,e. 64.94 %, 65.83 % and 66.66 % has been reduced in case of light (bp 200 ^{0}C), medium (bp 300 ^{0}C), and heavy (bp 360 ^{0}C) distillate fractions, respectively. Because of the volatile nature, hydrogen sulfide (H_2S) was found to be concentrated mostly in light distillate (0.0170 %) next in medium (0.0120 %) and then in heavy (0.0090 %) distillate fractions.

Introduction

Anthropogenic activities plays a vital role in the degradation of various environmental media mainly through SO_x and NO_x emissions These include mineral and ore processing and fossil fuel combustion. Every one has the right to live in a clear and clean environment. In order to keep the environment clean, there should be a constant check on emanation of such pollutants and toxins. There is a general understanding that if these pollutants are not controlled, the life survival will be impossible due to the global environmental problems like acid rain, green house effect and ozone depletion. In addition, corrosion of the industrial plants, poisoning of the catalyst etc need attention. Governmental regulations are in vogue for the permissible release of sulfur contents in petroleum products [1-3]. The refineries have to produce markeTable-petroleum with minimum or no atmospheric pollution [4].

Sulfur contents may be kept as low as possible to over come all those problems which are mainly due to sulfurous emissions [5]. Some conventional methods have been used for sulfur depletion. Amongst these, chemical methods have been tried in the past to remove the sulfur from petroleum based oils. Sulfur has been catalytically converted to hydrogen sulfide (H_2S) in the presence of hydrogen gas at a high pressure and at elevated temperature [6]. Mercaptane and hydrogen sulfide have been removed by treating with silver nitrate solution up to a great extent [7].

Biodesulfurization is an emerging method for the removal of sulfurous compounds from the petroleum products. A number of various bacteria have been used for biodesulfurization. Microbes can oxidize soluble organo sulfur compounds [8]. Specific enzymes e.g. D_{SZC} and D_{SZA} has been isolated from microbes and specially used for bodesulfurization [9-15] which converts sulfur to their sulfides and sulfuric acids [16] and other products [17].

The present work describes microbial sulfur depletion in some crude oil distillates. The samples were inoculated with *P. putida* for the removal of hydrogen sulfide, with the objectives to make the samples lean in hydrogen sulfide and to make the environment clean for the survival of biota.

Results and Discussion

First Distillate Light Faction (bp 200 ^{0}C)

Hydrogen sulfide contents were observed to be depleted in the light distillate of crude oil when it was inoculated with *p. putida* bacteria. Original sample was analyzed for the determination

*To whom all correspondence should be addressed.

of hydrogen sulfide and the value of hydrogen sulfide was found to be 0.0170 %. The original sample was then inoculated for time duration of 12 hr, the significant reduction in H_2S content (0.0097 %) was noticed. For further extensive removal of hydrogen sulfide, the inoculation time was chosen beyond 12hrs, ranges from 24 hrs up to one month (720 hr). Reductions made by *p. putida* were observed and found to be 0.0090 %, 0.0086 %, 0.0075 % and 0.0063 %, respectively in case of 24 hr, 48 hr, one week and a one month contact times (Fig. 1 and Table-1). 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. *P. delafieldii* R-8 was immobilized on magnetic polyvinyl alcohol beads and a diesel oil

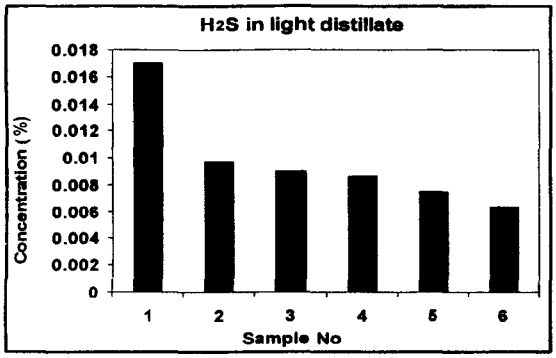

Fig. 1: Levels of Hydrogen Sulphide (%) in original and variously inoculated light oil samples

1. Original sample
2. 12 hours inoculated sample
3. 24 hours inoculated sample
4. 48 hours inoculated sample
5. One week inoculated sample
6. One month inoculated sample

Table-1: Percent Reduction in hydrogen sulphide as a function of Inoculation time in case of Light distillate.

Sample	H_2S level (%)
Original sample	0.0170
12 hours inoculated sample	0.0097
24 hours inoculated sample	0.0090
48 hours inoculated sample	0.0086
One week inoculated sample	0.0075
One month inoculated sample	0.0063

desulfurization reaction was performed. After desulfurization, immobilized cells could be easily separated magnetically from the BDS reactor [18].

Second Medium Fraction (bp 300 ^{0}C).

Fig. 2 graphically represents the contents of hydrogen sulfide in virgin and in variously inoculated fraction, which was found to be 0.012 % in case of virgin sample. Later on, the effect of pseudomonas on sulfur depletion was investigated. The data is presented in Table-2. The concentration of hydrogen sulfides content was dropped to 0.0070, 0.0060, 0.0054, 0.0048 and 0.0041 % in case of all inoculated times; 24 hr, 48 hr, one week and one month, respectively. In the initial stage, significant depletion was observed which continued till the last inoculated time. It has been reported that IGTS8 cannot utilize aromatic sulfurous compounds as a sole sulfur source but X7B may carry a novel system for the desulphurization of heterocyclic sulfur-containing xenobiotics. More detailed analyses of the purified enzymes and genes involved in sulfur-carbon bond degradation by strain X7B would provide a further explanation for the biodesulfurization of sulfurous compounds by a bacterial strain [19].

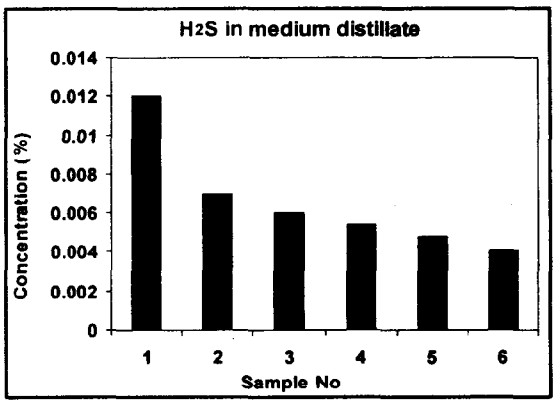

Fig. 2: Levels of Hydrogen Sulphide (%) in original and variously inoculated middle oil samples

1. Original sample
2. 12 hours inoculated sample
3. 24 hours inoculated sample
4. 48 hours inoculated sample
5. One week inoculated sample
6. One month inoculated sample

Table-2: Percent Reduction in hydrogen sulphide concentration as a function of Inoculation time in case of Middle distillate.

Sample	H_2S level (%)
Original sample	0.0120
12 hours inoculated sample	0.0070
24 hours inoculated sample	0.0060
48 hours inoculated sample	0.0054
One week inoculated sample	0.0048
One month inoculated sample	0.0041

Third Heavy Fraction (bp 360 ^{0}C).

Heavy distillate was also treated in a similar way like the other two fractions. First of all, hydrogen sulfide (H_2S) was quantified in virgin sample and its concentration was noted to be 0.0090 %. Then, by adopting the same fashion of inoculation, it was interacted with *P. putida* for contact time selected from 12 hr to one month. The values obtained were found to be 0.0055, 0.0048, 0.0042, 0.0039 and 0.0030 % in case of 12 hr, 24 hr, 48 hr, one week and one month inoculation time, respectively. The data has been presented in Fig. 3 and assembled in Table-3.

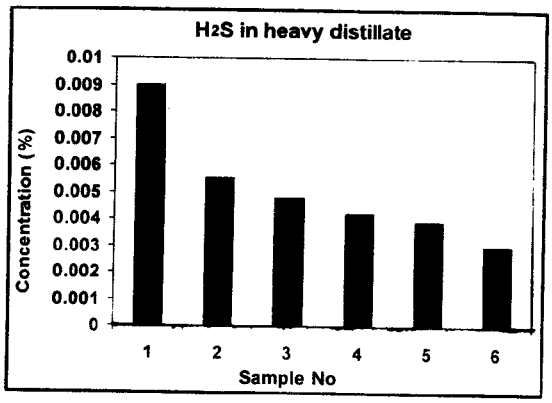

Fig. 3: Levels of Hydrogen Sulphide (%) in original and variously inoculated heavy oil samples

1. Original sample
2. 12 hours inoculated sample
3. 24 hours inoculated sample
4. 48 hours inoculated sample
5. One week inoculated sample
6. One month inoculated sample

Hydrogen sulfide (H_2S) content has been found to be decreased across the boiling point of the fractions used, maximum content was observed in light distillate, then in medium distillate and minimum in heavy distillate, which may be due to its volatile nature.

Table-3: Percent Reduction in hydrogen sulphide concentration as a function of Inoculation time in case of Heavy distillate

Sample	H_2S level (%)
Original sample	0.0090
12 hours inoculated sample	0.0055
24 hours inoculated sample	0.0048
48 hours inoculated sample	0.0042
One week inoculated sample	0.0039
One month inoculated sample	0.0030

Experimental

Collection of Sample

Sample was collected from the Attok Oil Refinery, Ltd. (ARL) Rawalpindi (Pakistan) in crude form and was preliminary converted into three distillates i.e., light fraction (bp 200 ^{0}C), medium fraction (bp 300 ^{0}C) and heavy fraction (bp 300 ^{0}C).

Chemicals

All the chemicals used were of analytical grade.

Sampling of Bacteria

Coal mine water was sampled from Much coal mine (Baluchistan) and incubated for 24 hr at 30 ^{0}C not to damage the microorganisms. Many slides were prepared for the confirmation of *p. putida*, which were studied under microscope. Physiological and structural studies showed the existence of *p. putida*.

Bacterial Strains and Medium

P. putida was primarily isolated as a bacterial strain capable of degrading sulfur carbon bond in sulfurous compounds.

Estimation of Sulfur Compounds

P. putida was shaken at 28 °C at 200 rpm on a rotary shaker. During the time course of

bacterial growth, aliquots of the culture were removed, cultivated with hydrogen sulfide as the sole sulfur source in 50 ml conical flasks containing hydrogen sulfides.

Cultured microorganisms were inoculated with distillates sample in small conical flasks and incubated for various periods selected from 12h to one month time, each residual sample was analyzed for hydrogen sulfide contents. Samples were tested before and after bacterial treatment by adopting a well-established I.P. standard method used for the determination of hydrogen sulfide [20].

Hydrogen Sulfide Determination.

A suitable-quantity of the sample was taken in a separating funnel containing 35 ml of the cadmium sulfate solution and shaken vigorously for 5 minutes. The contents of the separating funnel were filtered. The precipitate was washed and transferred along with the filter paper to a 250 ml titration flask containing 25 ml 0.1N iodine solution. 10 ml of hydrochloric acid solution (18 %) was added and titrated the excess iodine with 0.1N sodium thiosulfate solution using starch as indicator. The data obtained before and after treatment with *p. putida* was recorded.

Conclusions

It is concluded from the study that depletion of hydrogen sulphide is possible with the strain of microorganism used in current study. It is further noted that the appropriate time of contact is 12 hr. Extension of contact time beyond 12 hr is helpful to minimize sulphur depletion further; however, the decline is not so much significant as in case of 12 hr contact. It has been further observed that hydrogen sulphide mostly concentrates in light fraction compared to heavy fraction.

References

1. R. M. Atlas, *Mar. Pollution Bulletin.* **31**, 178 (1995).
2. E. Berman and L. T. M. Bui, *The Review of Economics and Statistics.* **83**, 498 (2001).
3. A. Pilkington *Technology, Analysis and Strategic Management.* **10**, 211 (1998).
4. J. H. Gary and G. E. Handwerk, *Petroleum Refining: Technology and Economics* , CRC; 4th edition, 2001.
5. U. S. Environmental Protection Agency (EPA). **402**, 99 (1990).
6. T. Kabe, A. Ishihara and H. Tajima, *Ind. Eng. Chem. Res.*, **31**, 1577 (1992).
7. C. Yin, D. Xia, Fuel. **80**, 607 (2001).
8. R. P. Kiene and D. G. Capone, *Microbial Ecol.*, **15**, 275 (1988)
9. I. M. Cambell, *Am Chem. Soci. Div. Petroleum*, **38**, 275 (1993).
10. S. A. Denome, C. Oldfield and L. J. Nash, *J. Bacteriol.* **176**, 6707 (1994).
11. S. A. Denome, E. S. Olson and K. D. Young, *Appl. Environ. Microb.* **59**, 2837 (1993).
12. B. Lei and S. C. Tu, *J. Bacteriol*, **178**, 5699 (1996).
13. F. Li, P. Xu, C. Q. Ma, L. L. Luo and X. S. Wang, *Microbiol. Lett.* **223**, 301 (2003).
14. M. Z. Li, C. H. Squires and D. J. Monticello, *J. Bacteriol.* **178**, 6409 (1996).
15. C. S. Piddington, B. R. Kovacevich and J. Rambosek., *Appl. Environ. Microbiol*, **61**, 468 (1995).
16. K .A. Gray, O, S. Pogrebinsky, G. T. Mrachko, L. Xi, D. J. Monticello and C. H. Squires, *Nat .Biotechnol.* **14**, 1705 (1996).
17. R. Z. Hoff, *Marine Pollution Bulletin.* **26**, 476 (1993).
18. G. B. Shan, J. M. Xing, M. F. Luo, H. Z. Liu and J. Y. Chen, *Biotechnol. Lett.* **25**, 1977 (2003).
19. K. J. Kayser, B. A. Bielaga-Jones, K. Jackowski, O. Odusan and J. J. Kilbane, *J. Gen. Microbiol.* **139**, 3123 (1993).
20. I. P. Standard Methods for Analyses and Testing of petroleum and related products, ASTM. **1**, 103 (1987).