JASEM ISSN 1119-8362

Full-text Available Online at www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *September*, 2007
Vol. 11(3) 53 - 58

Factors Influencing Degradation of Mercaptans by *Thiobacillus thioparus* TK-m (1)

ADOKI, A

Shell Petroleum Development Co. of Nigeria Ltd, P.O. Box 263, Old Aba Road, Port Harcourt, Nigeria
E-mail: akuro.adoki@shell.com

ABSTRACT: Degradation of methylmercaptans by *Thiobacillus thioparus* TK-m was influenced by pH of the reaction medium. Ratios of headspace concentrations in empty vials and those of acidified buffer solutions were less than 1.0. 95% of the H_2S was in headspace with the remaining 5% in solution upon acidification. The values for MM were 80% in headspace and 20% in solution. Different buffer solutions also influence the rate of removal of mercaptans from reaction vessels. For methylmercaptan oxidase, using Tris/HCl buffer, the highest level of oxygen uptake was recorded at pH 8.5. Mild changes in the pH levels were recorded at the end of the reactions. Compared to Tris/HCl buffer, phosphate buffer supports a significantly lower reactivity of MM-oxidase towards methylmercaptan. @JASEM

Thiols and hydrogen sulphide (H_2S) are malodorous compounds which exceed the odour threshold at low concentrations (Leonardos *et al*, 1969). They are produced by the wood-pulping industry, manure and sewer systems as exhaust gases. They constitute health problems as some are known to be toxic to both man and animals at very low concentrations.

Thiols are among the major volatile organic sulphur compounds produced in natural environments (Krouse and McCready, 1979). They can arise from the breakdown of sulphur-containing amino acids and lignin, and can be produced by algae, and in soils, freshwater, anaerobic lagoons and oceans (Loveluck *et al*., 1972; Sivela and Sundman, 1975; Zinder and Brock, 1978; Banwart and Bremner, 1976; Young and Maw, 1958). Thiols also present a major odour problem in the wood-pulping industry (Sivela and Sundman, 1975). Little work has been published on the microbial degradation of other thiols with the exception of methanethiol.

The biogenesis of methylsulphides provides a principal input of volatile sulphur to the atmosphere. This contribution has significant effects on the sulphur cycle and on global geochemistry (Taylor and Kiene, 1989). Dimethysulphide (DMS) is photochemically oxidized in the atmosphere to methanesulphonic and sulphuric acids. These strong acids contribute, along with nitric and organic acids, to the natural acidity of precipitation (Taylor and Kiene, 1989). Recent problems with acid rain have aroused interest in the anthropogenic and natural sources of volatile sulphur compounds. In addition to affecting the pH of precipitation, the emission of DMS has been linked with the regulation of global climate. There is at present little knowledge available on the microorganisms involved in the biodegradation of methylsulphides. DMS has been reportedly degraded by *Thiobacillus* sp. strain MS1 (Sivela, 1980), *Thiobacillus thioparus* TK-m (Kanagawa *et al.,* 1982; Kanagawa and Kelly, 1986; Kanagawa and Mikami, 1989; Tanji *et a*l, 1989; Gould and Kanagawa, 1992) *Hyphomicrobium* sp. strain S (deBont *et al.*, 1981) and *Hyphomicrobium* sp. strain EG (Suylen and Kuenen, 1986; Suylen *et al.,* 1986; Suylen *et al* 1987; Smith and Kelly, 1988).

This study aims at ascertaining the degradation of higher chain-length thiols by intact cells of *Thiobacillus thioparus* TK-m.

MATERIALS AND METHODS

Enzyme: Purified methyl mercaptan oxidase used for this assay was obtained from cells of *Thiobacillus thioparus* TK-m and supplied by Dr. T. Kanagawa (National Institute of Bioscience and Human-Technology, Tsukuba, Japan) and maintained as slants on medium C (Kanagawa and Kelly, 1986).

Stability of mercaptans in acid solutions: Into each of phosphoric acid coated serum vials (68.8 ± 0.6ml vol) was put 3ml of Tris/HCl buffer (pH 8.2) containing 0.2mM EDTA. These were then sealed with Teflon-cated rubber stoppers and 3ml headspace gas replaced by 3ml of the following gas combinations (in duplicates): 3ml methylmercaptan (MM); 2.5ml of MM + 0.5ml of H_2S; 1.5ml of MM + 1.5ml of H_2S; 0.5ml of MM + 2.5ml of H_2S; 3ml of H_2S

The serum vials containing the gases were shaken at 25°C and the headspace gas concentrations determined after 5, 10 and 15 minutes to ascertain equilibrium between gas and liquid phases. After 15 minutes, 100 µl of 4N-H_2SO_4 was added to one vial of a set and shaken by hand for 5 seconds. The headspace gas was then analysed. The vial was further shaken and headspace gas reanalysed after 5, 10, 20, 30 and 60 minutes. Two empty phosphoric acid-coated vials were sealed with Teflon-coated

Correspondence: E-mail: akuro.adoki@shell.com

rubber stoppers and 3ml of headspace gas replaced with 3ml of MM in one vial and the other with 3ml of H_2S. These were then shaken for 10 minutes and the gas concentrations determined.

Influence of pH on MM oxidase from Thiobacillus thioparus: Buffer solutions: For this assay two sets of buffers were used. Phosphate buffers (pH 6.0, 6.5, 7.0, 7.5, 8.0) were prepared by mixing 50mM K_2HPO_4 and 50mM KH_2PO_4 and the pH of the resulting solutions determined by the use of a pH meter (D-12 Horiba). One hundred millilitres of each set was prepared and 1ml of 20mM EDTA solution (pH 8.1) added. Tris buffers (pH 7.5, 8.0, 8.5 and 9.0) were prepared by mixing 5ml of 1M Tris and 1ml of 20mM EDTA (pH 8.1). To the resulting solution was added 85ml distilled water and then 1M HCl dropwise to make buffers of the pH range stated earlier. The resulting buffers were made up to 100ml with distilled water.

Potassium biphthalate buffer was prepared by first making a 0.1M solution of $C_6H_4(COOH)(COOK)$ in distilled water. Fifty millilitres (50ml) of the biphthalate solution was then put in 100ml beakers and 1N NaOH added dropwise to make buffers of pH 4.0, 4.5, 5.0, 5.5 and 6.0. The resulting solutions were then made up to 100ml with distilled water. Glycine buffer was prepared by first making 50mM each of glycine plus sodium chloride and sodium hydroxide. The 50mM NaOH solution was then added to the 50mM glycine + NaCl solution to give buffers of pH 8.5, 9.0, 9.5, 10.0, 10.5 and 11.0.

Preparation of methylmercaptan solution: Into each of clean serum vials was put 50ml of 50mM phosphate buffer, pH 7.5, containing 0.2mM EDTA. The vials were then sealed with Teflon-coated rubber stoppers and N_2 gas bubbled into the solution for 30 mins. Methylmercaptan (MM) gas (2,000ppm in N_2) was then bubbled for 30 minutes also.

Analysis: The influence of pH on methylmercaptan oxidase was determined by measuring oxygen uptake during degradation of methylmercaptan in an oxygen electrode chamber using a digital DO/O_2/Temp meter (UC-12, Central Kagaku Co. Ltd, Japan). The reaction was initiated by first pipetting 2ml each of the sets of buffers and 8.2µl of methylmercaptan solution. To the reaction vessel was then added aliquot of the MM-oxidase. Increased concentrations of enzyme were also applied. Reaction was stopped after 6 minutes.

Stoichiometry of methylmercaptan oxidation: Serum vials (68.8 ±0.6ml vol.) were soaked in 0.1N HCl for 24h, washed with distilled water and dried at 105°C. The inside of each vial was then coated with phosphoric acid using 150mM H_3PO_4 in acetone. To each vial 3.0ml of the appropriate buffers containing 0.2mM EDTA (pH 8.1) was added and the vials sealed as described earlier (vial volume reduced by 0.4ml). To the sealed vials 239 nMol MM gas was added by replacing 3.0ml of headspace gas with 3.0ml MM gas (2mlL^{-1} of nitrogen) and then the vials shaken for more than 10 minutes at 25°C. The amount of MM in headspace was then determined. The reaction was initiated by the injection of 10µl of enzyme solution and the vials shaken at 25°C for 10 minutes. The reaction was terminated by the injection of 0.1ml 2N H_2SO_4. Methylmercaptan and H_2S in headspace were then determined.

RESULTS AND DISCUSSION

Stability of mercaptans in acid solutions: Analysis of final headspace gas concentrations before and after injecting 100ul of 4N- H_2SO_4 revealed that both MM and H_2S were stable in acid solutions. However, when headspace concentrations in empty vials and those of acidified buffer solutions were compared, it was observed that ratios of vials containing solutions were less than 1.0 (see Table 1). For H_2S, a ratio (B/A where A = theoretical concentration in vial, and B = determined in vial containing buffer) of about 0.95 was obtained while for MM the corresponding value was 0.80. The results obtained indicated that only about 95% of the H_2S was in headspace with the remaining 5% in solution upon acidification. The values for MM were 80% in headspace and 20% in solution.

Table 1: Stability of mercaptans and hydrogen sulphide in acid solutions

Gas Combination	% Headspace Gas Concentrations				Ratio (B/A)	
	Methylmercaptan		H_2S		Methylmercaptan	H_2S
	Theoretical (A)	Determined (B)	Theoretical (A)	Determined (B)		
Empty Bottle + 3ml Substrate		94.18		99.36		
Buffer + 3ml Substrate + Acid	94.18	74.19	99.36	93.23	0.79	0.94
Buffer + 2.5ml H_2S + 0.5ml MM + Acid	15.70	13.58	82.80	79.9	0.86	0.97
Buffer + 1.5ml H_2S + 1.5ml MM + Acid	47.09	38.02	49.68	48.13	0.81	0.97
Buffer + 0.5ml H_2S + 2.5ml MM + Acid	78.48	63.13	16.56	16.97	0.81	1.0

Influence of pH on MM oxidase from Thiobacillus thioparus: Figures 1 to 3 show the uptake of oxygen by methylmercaptan oxidase at varying pH levels using tris/HCL and phospahate buffers. From the figures it could be deduced that in the absence of substrate or enzyme there was a slight oxygen uptake in Tris buffer at pH 8.0. This was a sharp contrast to the more than 6-fold increase in oxygen uptake recorded when substrate was added at the same pH. This was due to auto-oxidation of MM, a reaction that takes place even in the absence of MM-oxidase. Also, the concentration of the enzyme in the reaction mixture was found to influence the level of O_2 uptake. Due to reduced activity of the enzyme extract, an enzyme volume of 100µl was found to have the highest O_2 uptake when compared with other levels tested.

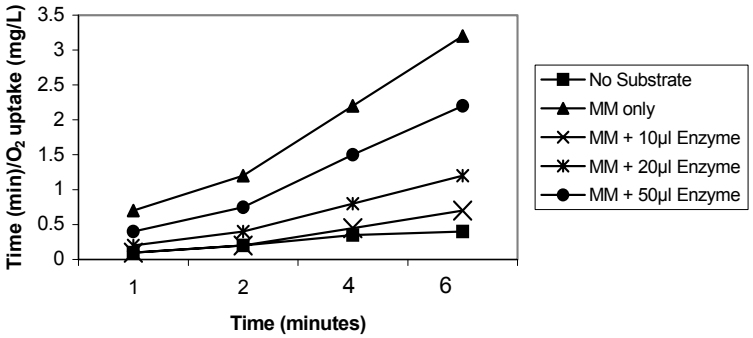

Fig 1: Influence of varying methylmercaptan (MM) oxidase titres on degradation of MM at pH 8.0

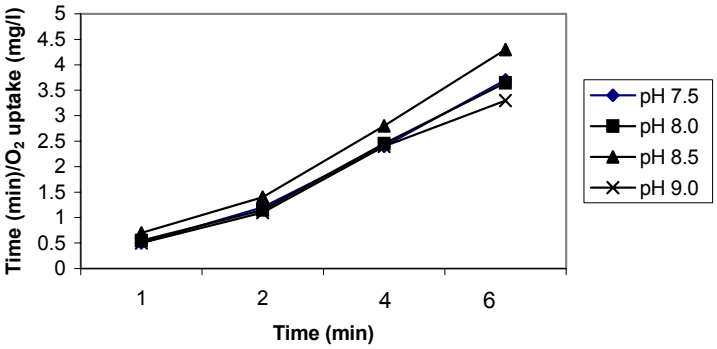

Fig 2: Influence of pH levels on degradation of methylmercaptan (MM) by MM-oxidase

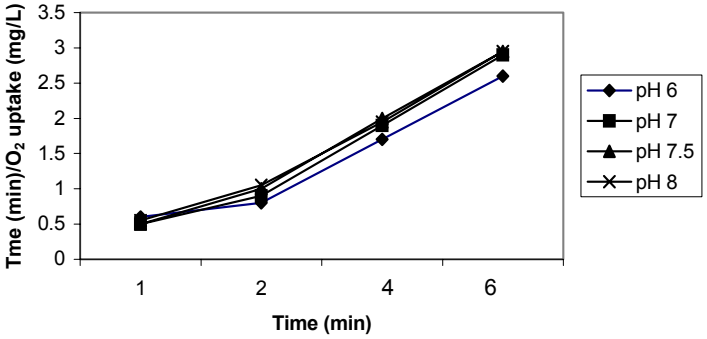

Fig 3: Influence of pH on methylmercaptan oxidase using phosphate buffer

For methylmercaptan oxidase, using Tris/HCl buffer, the highest level of oxygen uptake was recorded at pH 8.5. Mild changes in the pH levels were recorded at the end of the reactions. Compared to Tris/HCl buffer, Figure 3 shows that phosphate buffer supports a significantly lower reactivity of MM-oxidase towards methylmercaptan. The highest level of O_2 uptake recorded for phosphate buffer (2.95 mg/l) is less than the levels recoded for Tris/HCl buffer (3.3 to 4.3 mg/L). The results generally show that the choice of buffer and the pH level are crucial factors influencing the degradation of methylmercaptan by MM-oxidase.

Figure 4 shows that at the pH levels tested, m ole ratios of MM consumed and H_2S produced were stoichiometrically equivalent. Similarly, Figure 5 also shows that at all enzyme titres used, concentrations of MM consumed corresponded to H_2S production.

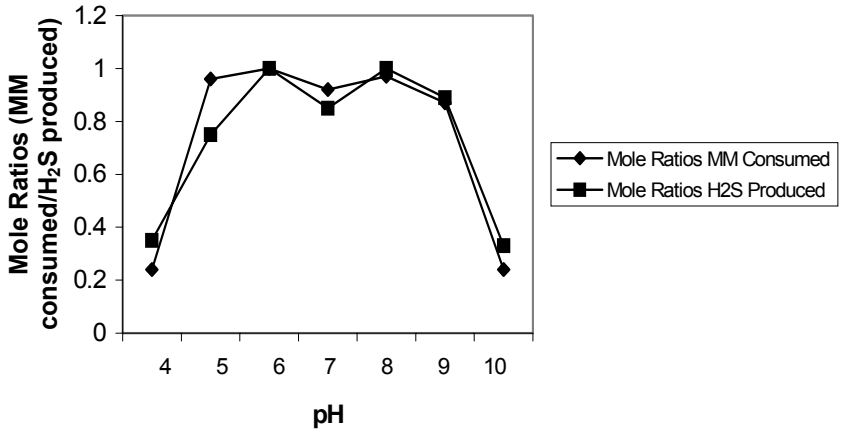

Fig 4: Comparison of methylmercaptan (MM) consumption and H_2S production at varying pH levels

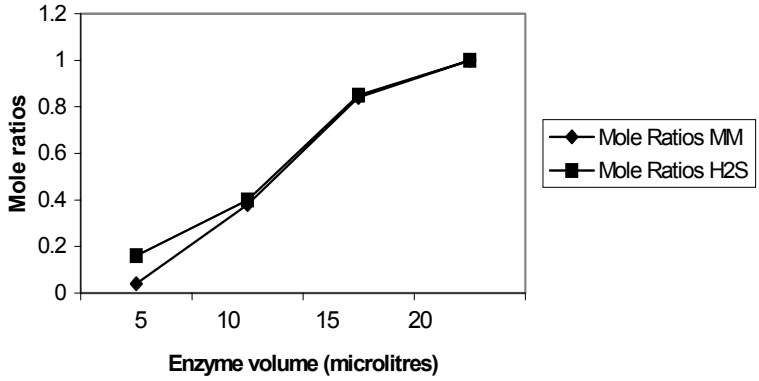

Fig 5: Influence of methymercaptan (MM) oxidase volume on degradation of methylmercaptan

Preliminary results on the degradation of different thiols by enzyme extracts of *Thiobacillus thioparus* TK-m in Liquid Medium are shown in Figure 6. The data show that enzyme extracts of the test strain could degrade other thiols apart from C_1 and C_2.

As expected, the degradability rates depended on the chain-length of the thiol, a consistently higher rate was recorded for C_1 compared to C_2. Much lower activities were recorded with propanethiol, butanethiol, pentanethiol and haxanethiol (0.069, 0.068 and 0.090 $\mu molsmg^{-1}proteinmin^{-1}$ respectively). Only very minimal degradation of secondary thiols was recorded. The degradation of thiols by a strain of *T. thioparus* has been reported previously (Smith and Kelly, 1988).

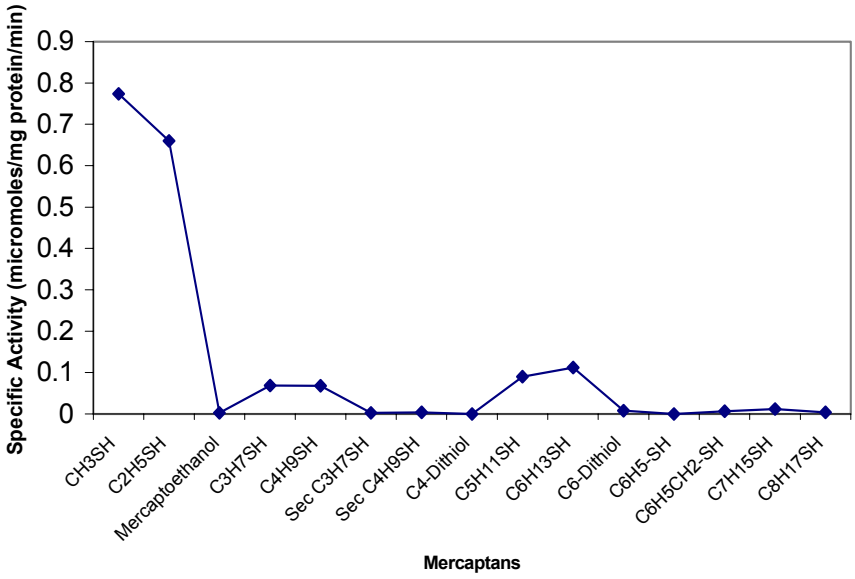

Fig 6: Specific activity of methylmercaptan (MM) oxiadse on various mercaptans

Conclusion: Results obtained show that mercaptans and their biodegradation product, hydrogen sulphide, are fairly stable in acid solutions. However, the degradation rate of mercaptans by MM-oxidase is influenced by the pH of the reaction medium and choice of buffer solution. Also, lower chain length primary mercaptans are more easily degraded than their corresponding secondary counterparts.

Acknowledgement: Dr. A. Adoki thanks the Science and Technology Agency of Japan for the Fellowship and financial support for this work to. Dr. T. Kanagawa of the National Institute of Bioscience and Human-Technology is also acknowledged for his supervision.

REFERENCES

Banwart, W L; Bremner, J M (1976). Evolution of volatile sulphur compounds from soils treated with sulphur-containing organic materials. *Soil Biol. Biochem*. 6, 113 - 115.

deBont, J A; van Dijken M; Harder, W (1981). Dimethylsulphoxide and dimethylsulphide as a carbon, sulphur and energy source for growth of *Hyphomicrobium* S. *J. Gen Microbiol*., 127: 315 - 323.

Gould, W D; Kanagawa, T (1992) Purification and properties of methylmercaptan oxidase from *Thiobacillus thioparus* TK-m. *J. Gen. Microbiol*. 138, 217 – 221

Kanagawa, T; Kelly, D P (1986). Breakdown of dimethylsulphide by mixed cultures and by

Thiobacillus thioparus. *FEMS Microbiol. Lett*. 34: 13 - 19.

Kanagawa, T; Mikami, E (1989). Removal of methanethiol, dimethylsulphide, dimethyldisulphide and hydrogen sulphide from contaminated air by Thiobacillus *thioparus* TK-m. *Appl. Environ. Microbiol.*, 55 (3), 555 - 558.

Kanagawa, T ; Dazai, M ; Kukuoka, S (1982) Degradation of O,O-dimethyl phosphodithioate by Thiobacillus thioparus TK-1 and Pseudomonas AK-2. Agr. Biol. Chem. 46, 2571-2578

Krouse, H R; McCready, R G L (1979). In *Biogeochemical Cycling of Mineral-forming Elements* (Trudinger, P. A. and Swine, D. J., Eds), pp. 401 - 430. Elsevier, Amsterdam.

Leonardos, G; Kendall, D; Barnard, N (1969). Odor threshold determinations of 53 odorant chemicals. *J. Air Pollution Control Assoc.*, 19: 91 – 95.

Loveluck, J E; Maggs, R; Rasmussen, R A (1972). Atmospheric dimethylsulphide and the natural sulphur cycle. *Nature*, London, 237, 452 – 453

Sivela, S (1980). Dimethylsulphide as a growth substrate of an obligately chemolithotrophic *Thiobacillus*. Commentations Physico-Mathematical Dissertations 1: 1 – 69.

Sivela, S; Sundman, V (1975). Demonstration of *Thiobacillus*-type bacteria which utilize methyl sulphides. *Arch. Microbiol.*, 103, 303 - 304.

Smith, N A; Kelly, D P (1988). Isolation and physiological characteristization of authotrophic sulphur bacteria oxidizing dimethyldisulphide as sole source of energy. *Journal of General Microbiology*, 134, 1407 - 1417.

Suylen, G M H; Kuenen, J G (1986). Chemostat enrichment and isolation of *Hyphomicrobium* EG, a dimethylsuphide oxidizing methylotroph and reevaluation of *Thiobacillus* MS1. *Antonie van Leeuwenhoek*, 52, 281 – 293.

Suylen, G.M.H., Large, P.J., van Dijken, J.P. and Kuenen, J.G. (1987). Methylmercaptan oxidase, a key enzyme in the metabolism of methylated sulphur compounds by *Hyphomicrobium* EG. *J. Gen. Microbiol.*, 133, 2989 – 2997.

Suylen, G M H; Stefass, G C; Kuenen, J G (1986). Chemolithotrophic potential of *Hyphomicrobium* species, capable of growth on methylated sulphur compounds. *Arch. Microbiol.*, 146: 192 – 198.

Tanji, Y; Kanagawa, T; Mikami, E (1989). Removal of dimethyl sulphide, methyl mercaptan, and hydrogen sulphide by immobilized *Thiobacillus thioparus* TK-m. J. Ferment. Bioeng., 67 (4), 280 – 285.

Taylor, B F; and Kiene, R P (1989). Microbial metabolism of dimethylsulphide In Biogenic sulphur in the environment, 203 – 221. Amer. Chem. Soc.