JASEM ISSN 1119-8362

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

J. Appl. Sci. Environ. Manage. *September*, 2007
Vol. 11(3) 139 - 142

Degradation of methylmercaptan by crude enzyme extracts of *Thiobacillus thioparus* TK-m

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ABSTRACT: The biodegradation of methyl suphides by crude enzyme extracts of *Thiobacillus thioparus* TK-m was investigated in this work. The data revealed that crude protein contents of enzyme extracts from cells of *Thiobacillus thioparus* TK-m was influenced by the saturation levels of the EDTA and ammonium sulphate solutions. For EDTA, error levels were 3, 5, 7 and 11.0 percent at 0.005, 0.1, 0.15 and 0.2 mM EDTA respectively. For ammonium sulphate, there was an increase in recovery of total activity at the 40 and 50 percent saturation levels (122.6 and 101.1 percent respectively) but a decrease at the 65% level (90.4%). Activity of crude enzyme extracts ranged between 0.095 and 1.28 µmolmin^{-1}mg^{-1}. @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.

The biogenesis of dmethylsulphides 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). Dimethylsulphide (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).

MATERIALS AND METHODS

Influence of Tris/HCl and EDTA: Using varying aliquots of 50mM and 100mM tris/HCL buffer (pH 8.2), the influence of Tris on protein determination was examined. As a first step, calibration curves were prepared using protein albumin suspended in 0.85% NaCl solution and 50mM Tris/HCl buffer (pH 8.2). Reference blanks were 0.85% NaCl and Tris/HCl buffer. The influence of Tris was further studied by adding 100mM Tris/HCl buffer (pH 8.2) to aliquots of protein standard (2g/L) in 0.85% NaCl. The final volume was adjusted to 200ml with distilled water instead of NaCl.

To assay the influence of EDTA on protein determinations, the suspending fluid used throughout the test was Tris/HCl (no NaCl). Varying volumes of 0.4mM EDTA solution in Tris/HCl (pH 8.2) were added to 100uL of protein standard (50mg/dL) to give a range of EDTA concentrations (0, 0.05, 0.10 and 0.2 mM. These were made up to 200ul with Tris and treated as for standards to determine protein concentrations.

Influence of Ammonium sulphate: The effect of varying saturation levels of ammonium sulphate on the recovery of activity of MM-oxidase was also determined. The activity of crude extract, supernatant and ammonium sulphate precipitate fractions was determined using appropriate volumes of the test samples. Based on the sample volume used for the assay, 50mM Tris/HCl buffer was dispensed into phosphoric acid-coated vials and sealed with Teflon-coated rubber stoppers. 3ml of headspace gas was removed and replaced with 3ml of MM. These were shaken for 5 minutes and headspace gas concentration determined by injecting 50µl of gas into the gas chromatograph (GC-14A Shimadzu).

The appropriate volume of the test protein sample was then injected and headspace gas concentrations determined after shaking for 2 and 5 minutes. After 10 minutes the reaction was stopped by injecting 100µL of 4N-H_2SO_4 and shaken further for 5mins. The headspace gas concentration was then determined. Standard H_2S and MM gases were also injected for calculation of residual MM and H_2S produced.

Protein Assay: The protein concentrations of the test samples were determined by modification of the

* Correspondence

Biuret method (50mM Tris/HCl, pH 8.2 was used instead of 0.85% NaCl).

Determination of specific activity of crude enzyme extracts: Into phosphoric acid-coated serum vials (68.4 ± 0.6 ml) was put 3-x ml of 50mM Tris/HCl buffer containing 0.2mM EDTA. These were than sealed with Teflon-coated rubber stoppers. Three millilitres of headspace gas was removed and replaced by 3ml of MM gas (1950 ppm).

Vials containing methylmercaptan were shaken for 5 minutes at 25ºC and headspace gas concentration analysed by injecting 50µL of gas into the gas chromatograph. To these vials were then added xml (0.05 to 0.5ml) of the crude enzyme extract sample.

RESULTS AND DISCUSSION

Influence of Tris/HCl Buffer: Figure 1 shows the calculated mg equivalents of protein per dL when standards in Tis/HCl are read against standards with NaCl solution as blank. The values obtained show that at protein concentrations below 25mg/dL Tris/HCl had the influence of increasing the calculated protein levels. By contrast, at protein levels of 50-100mg/dL which corresponded to total Tris volumes of 100-200, calculated values were 6-22% lower than the expected theoretical.

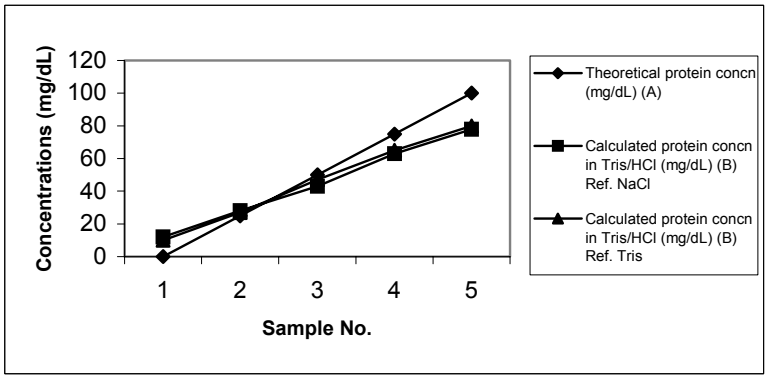

Fig 1: Influence of Tris/HCl on MM-oxidase in standard solutions

Figure 2 shows that with 50mM Tris/HCl at a constant protein concentaration of 25mg/dL, relatively wide variations were observed in the expected protein level (25mg/dL) and the levels obtained with different Tris/HCl volumes. In all cases increases were observed in calculated protein levels corresponding to an average of 18-38%.

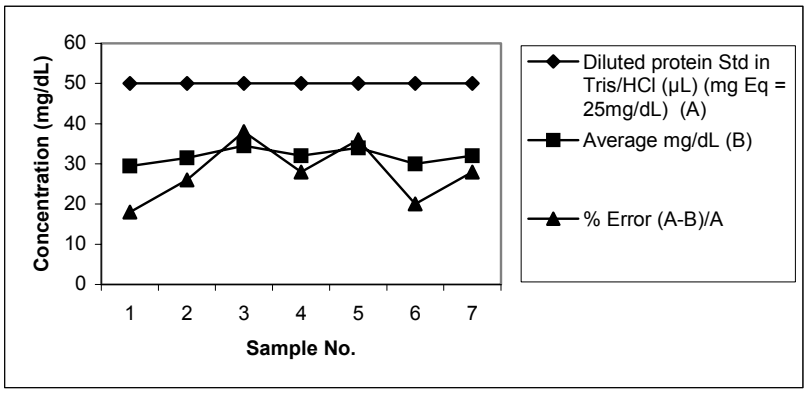

Fig 2: Influence of 50mM Tris/HCl on crude protein determination at constant protein concentration (25mg/dL)

Using 100mM Tris/HCl buffer at pH 8.2 and increasing the volume to 200µL with distilled water instead of NaCl, the results obtained are presented in Figure 3. Based on the percentage error deviation from the theoretical protein concentration of 50mg/dL, it was observed that the least errors were at Tris additions of 0, 25, 50 and 75 uL which corresponded to Tris concentrations of 0, 12.5, 25 and 37.5 mM in 200uL of diluted protein standard. Error levels were on the average 3-4%. These were

markedly lower than the average of 10-12 % recorded at 50mM and 62.5 mM Tris. Discounting experimental errors, it could be inferred that the influence of was very minimal or nil at 12.5 and 25 mM Tris. Error levels were 6 and 8% at 50 and 62.5 mM Tris respectively (1% at 37.5 mM).

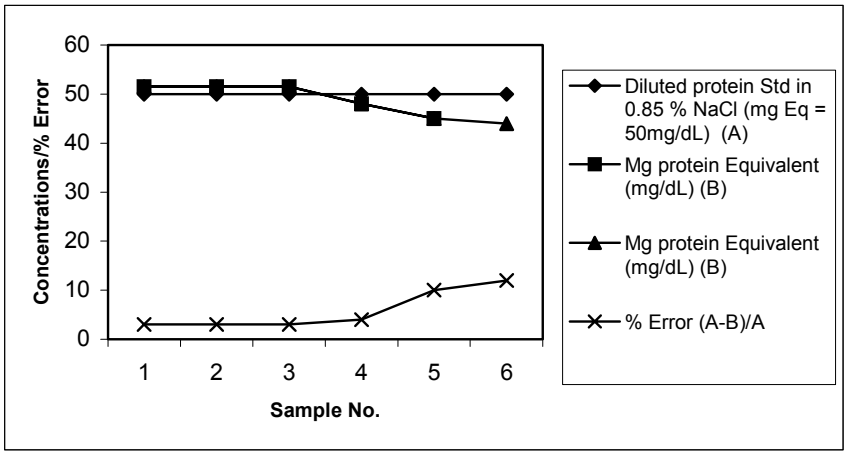

Fig 3: Influence of 100mM Tris/HCl on crude protein determination at constant protein concentration (50mg/dL)

A comparison of Figures 2 and 3 shows that the error levels at lower protein concentrations were much higher than those recorded at the higher protein levels although final Tris/HCl concentrations were identical in most cases. The differences between these two tests were that in Figure 2 protein standard was prepared in 50mM Tris/HCl buffer instead of NaCl solution and volumes made up to 200µL with 0.85% NaCl while in Figure 3 distilled water was used.

Influence of EDTA: A comparison of calculated protein concentrations at different EDTA levels (Figure 4) shows that these ranged between 50 and 56 mg/dL compared to the expected value of 50. These represent an error level of zero to 12%. A close examination of the error levels did not reveal any increase in protein concentrations as a result of progressive increase in EDTA levels. This was in contrast to the more distinct patterns recorded with Tris. When average values are used increase due to EDTA additions became more apparent with error levels being 3, 5, 7 and 11.0 percent at 0.005, 0.1, 0.15 and 0.2 mM EDTA respectively.

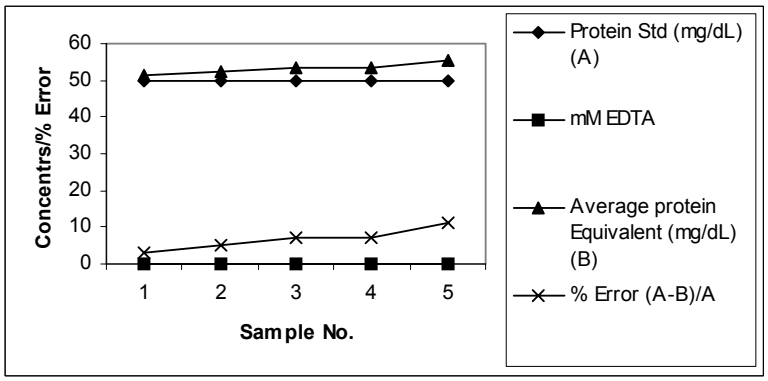

Fig 4: Influence of EDTA on crude protein determination

Influence of Ammonium sulphate on Specific activity of crude MM-oxidase extracts – Using the crude extract of MM-oxidase and ammonium sulphate precipitate fractions and supernatant it was observed that the crude extract had a protein concentration of 3.92 mg/ml (Table 1). This was reduced on treatment with $(NH_4)_2SO_4$ at saturation levels of 40, 50 and 65 percent. The highest loss was recorded at the 65% saturation (decreased to 2.32mg/ml). A slight drop in total activity was also recorded at this level. By contrast the specific activity was highest at the 65% ammonium sulphate saturation with the supernatant fraction having a more than 7-fold increase in activity compared to the crude

extract (1136.1 and 151.1 respectively). In terms of recovery of total activity, whereas there was an increase at the 40 and 50 percent saturation levels (122.6 and 101.1 respectively) there was a decrease at the 65% level (90.4%).

Table 1: *Influence of Ammonium Sulphate on recovery of activity of MM-oxidase*

% Ammonium Sulphate saturation		Protein Concentration (mg/ml)	Total protein (mg)	Total Protein (*A + B*)	Total Activity (nMol H_2S/min)	Specific Activity (nMol H_2S /min/mg protein)	% Recovery of activity
0		3.92	3./92	3.92	592.3	151.1	100
40	Precipitate	1.oo	0.88**(A)**	2.52	33.03	37.5	122.6
	Supernatant	1.62	1.64**(B)**		691.5	422.6	
50	Precipitate	1.24	1.44	2.63	60.7	42.2	101.1
	Supernatant	0.96	1.19		538.6	452.4	
65	Precipitate	1.54	2.01	2.31	194.9	96.8	90.4
	Supernatant	0.18	0.30		335.4	1136.1	

***A** = Precipitate; **B** = Supernatant*

Conclusion: From the data presented it could be observed at low protein concentrations, Tris increases the calculated protein levels. By contrast, at higher Tris concentrations colour development due to protein is suppressed resulting in lower calculated levels. The option therefore would be to have a combination of lower Tris concentrations and higher protein levels. The other alternative would be to divide the enzyme solution after purification into two portions – one suspended in sodium chloride solution (pH adjusted) and the other in Tris/HCl buffer for substrate assay. The results also show that whereas MM-oxidase with high specific activity could be extracted with high ammonium sulphate saturation, this is usually accompanied by a loss in total protein content, total activity and percentage recovery of activity.

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.

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