STUDIES IN THE SULPHUR FORMATION AT KONA, MASULIPATAM—PART II

THE isolation and general characteristics of *Vibro desulphuricans*, Konæ has previously[1] been demonstrated to be responsible for sulphate reduction in the sulphur areas at Kona. It was of interest to elucidate in greater detail the physical and physiological requirements of the organism for its growth and functioning.

1. *Temperature.*—The organism was inoculated in the stock medium and incubated under anaerobic conditions at various temperatures for 72 hours. The results are shown in Table I.

TABLE I

Temperature of incubation °C.	25	30	37	45
Blackening after ....days	5	3	No Blackening	No Blackening

2. *Thermostability.*—The culture, inoculated in the stock medium was subjected to different temperatures for measured periods; It was then immediately cooled under running tap water (temp. 25° C.) and thereafter incubated at 30° C. for 72 hours, and examined after this period. Table II gives the results,

TABLE II

Temperature °C.	55	60	60	70	75	80	80
Time of treatment in minutes	10	5	10	1	5	0·5	1
Reduction after 72 hours	+	+	nil	+	nil	+	nil

3. *Hydrogen-ion concentration.*—The organism is highly sensitive to changes of pH below 7·0. The capacity to grow and the function to reduce sulphates, were found to be abolished when the pH of the medium was slightly below 7·0 while the maximum pH tolerated was 8·5; the optimum pH was found to lie between 7·2 to 7·4.

4. *Oxygen requirements.*—The organism is a strict anaerobe and under aerobic conditions it has little effect on the sulphates. The culture tubes after inoculation are best maintained in a desiccator containing freshly prepared alkaline pyrogallol and evacuated by a filter pump.

5. *Salinity.*—The organism was inoculated in a basal medium (composition: sodium lactate 0·2 per cent., sodium sulphate 2·0 per cent., dipotassium hydrogen phosphate 0·2 per cent., ferrous ammonium sulphate 0·1 per cent. and ammonium sulphate 0·2 per cent.), containing increasing concentrations of sodium chloride. The cultures were incubated at 30° C. Results are shown in Table III.

It will be observed that optimum salinity for the organism is 6 per cent. and while the reduction can be effected in the absence of sodium chloride, the presence of as high a concentration as 13 per cent. does not affect the activity of the organisms.

6. *Sources of Nitrogen.*—A basal medium (composition: sodium chloride 6 per cent., sodium sulphate 4 per cent., sodium lactate 0·2 per cent., dipotassium hydrogen phosphate 0·2 per cent. and ferric chloride 0·1 per cent.) containing equivalent quantities of different forms of nitrogen (22 mgm. of nitrogen per 100 ml. medium) was inoculated with the culture and incubated at 30° C. The results are shown in Table IV.

It will be observed that organic sources of nitrogen as urea, casein hydrolysate and peptone are more rapidly metabolised than the inorganic forms. Nitrates and nitrites appear to be toxic to the organism.

7. *Sources of carbon.*—A basal medium (composition: ammonium sulphate 0·1 per cent., manganese sulphate 0·2 per cent., calcium sulphate 1·5 per cent., magnesium sulphate 0·2 per cent., sodium chloride 4·0 per cent., potassium dihydrogen phosphate 0·1 per cent. dipotassium hydrogen phosphate 0·2 per cent., and ferrous ammonium sulphate 0·1 per cent.) was inoculated with the culture with different salts of organic acids as sources of carbon. The results are shown below.

TABLE V

Source of carbon	Sodium formate	Sodium succinate	Sodium oxalate	Sodium lactate	Sodium acetate	Sodium citrate
Blackening in days	nil	nil	nil	3	nil	nil

Thus it was found that so far no other salt except sodium lactate was found to be utilised by the organism.

8. *Concentration of sulphates.*—A basal medium (composition: sodium chloride 6 per cent., dipotassium hydrogen phosphate 0·2 per cent., sodium lactate 0·2 per cent., ammonium chloride 0·2 per cent., ferric chloride 0·1 per cent., containing increasing concentrations of sodium sulphate was inoculated with the organisms, and inoculated as usual. The results are shown in Table VI.

TABLE VI

Concentration of sulphates	0·0	0·1	0·2	0·5	1·0	2·0	4·0	6·0	8·0
Blackening in days	nil	nil	2	2	3	3	4	6	nil

The concentrations of sulphate effective in blackening ranges between 0·2 and 2·0 per cent.

9. *Role of fixatives.*—It was found that sulphuretted hydrogen formed during the reaction, if allowed to accumulate, proved toxic to the micro-organism. It was observed that the culture maintained its activity over a longer period (upto two months) if adequate amounts of iron salts were incorporated in the medium.

Thus it is concluded that the organism has

TABLE III

Concentration of sodium chloride in the medium per cent.	0	0·5	1·0	2·0	3·0	4·0	5·0	6·0	7·0	8·0	9·0	13·0
Blackening in.....days	7	6	5	4	4	4	3	2	4	5	6	6

TABLE IV

Form of Nitrogen	No nitrogen	$(NH_4)_2SO_4$	$NaNO_2$	$NaNO_3$	Urea	Casein hydrolysate	Peptone
Blackening.....in days	No blackening	4	nil	nil	2	3	3

an optimum temperature of 30° C. and a thermal death-point at about 60° C. It requires a hydrogen-ion concentration of 7·2-7·4 and is a strict anaerobe. The optimum salinity at which the organism is most active is 6 per cent.; organic sources of nitrogen are preferred; of the sources of carbon studied, only sodium lactate was effective. The organism reduces sulphates in concentrations upto 6 per cent. The viability of the culture is enhanced by fixing the sulphuretted hydrogen released during the reaction with the aid of iron salts.

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