

Samples of these soils were inoculated into Van Deldens' medium (Composition  $\text{KH}_2\text{PO}_4$  grams; Na-lactate 0.5 grams; Asparagin 0.1 gram;  $\text{MgSO}_4$  0.1 gram;  $\text{FeSO}_4$  traces; Tap-water 100 ml.; and agar 2 grams). pH was adjusted to 7.2, and stab cultures were made to secure the growth of the anærobes. Only one sample—collected at 6 ft. in the sulphur-bearing area—showed distinct blackening after four days' incubation at 30° C.

TABLE I  
Number of bacteria per gram of soil

Level from surface	Aerobic		Anærobic	
	Sulphur-bearing area	Sulphur non-bearing area	Sulphur-bearing area	Sulphur non-bearing area
0-1'	3,45,000 2,80,000	5,22,000 6,00,000	very few	very few
1½'	65,000 80,000	1,92,000 2,09,000	3000 2200	"
2½-3'	72,000 79,000	2,56,000 3,14,000	2500 3100	"
4-4½'	70,000 81,000	2,58,000 3,00,000	6000 7600	3000
6'	30,000 18,000	25,000 29,000	25000 33200	8000 6300

**STUDIES IN THE FORMATION OF  
SULPHUR AT KONA (MASULIPATAM)  
PART I**

IN continuation of our studies<sup>1</sup> of the sulphur formation at Kona near Masulipatam, the soil and the subsoil water in the sulphur-bearing area were found to contain sulphuretted hydrogen, both bound and free; its presence was confirmed by (1) evolution of hydrogen sulphide on acidification of the soils; (2) strong smell of hydrogen-sulphide; and (3) the formation of a heavy black precipitate of lead sulphide on the addition of a solution of lead acetate to the subsoil water.

It was of interest to determine if the production of this sulphuretted-hydrogen was attributable to the direct or indirect activity of micro-organisms.

Samples of soils at various depths from surface up to 6 feet were collected from (a) sulphur area and (b) non-sulphur area. Weighed quantities of the samples were triturated with measured amounts of sterile water under aseptic conditions and dilutions ranging from 1:10 to 1:100,000 were effected. These dilutions were used for plating out on nutrient agar fortified with the essential inorganic salts. The plates were incubated at 30° C. both under aerobic and anærobic conditions. Plates were counted in duplicates and only those giving a colony count between 30 and 300 were used for the calculation of bacterial populations. The results are shown in Table I.

It may be observed that anærobic population is much larger (2 to 5 times) in the sulphur-bearing area than the corresponding population of the non-sulphur area.

This culture, which apparently included the sulphate-reducing organism, was transferred to a stock medium devised to simulate the environmental conditions of its natural habitat. The composition was as follows:—

$(\text{NH}_4)_2\text{SO}_4$  0.1 gm.;  $\text{MnSO}_4 \cdot 3\text{H}_2\text{O}$  0.2 gm.;  $\text{CaSO}_4$  (Gypsum) 1.5 gm.;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 gm.; NaCl 4.0 gm.;  $\text{KH}_2\text{PO}_4$  0.2 gm.;  $\text{Fe.Am.SO}_4$  0.1 gm.; distilled water 100 ml.

The pH was adjusted to 7.2. After inoculation and incubation at 30° C. under anærobic condition the yellow precipitate turned black in three to four days.

A systematic study of the micro-organism responsible for the reduction of sulphates has been made and the organism characterised as *Vibrio desulphuricans*, Konæ.

*Vibrio desulphuricans*, Konæ

**Isolation and habitat.**—Occurs in the hydrogen sulphide-forming areas at 6 feet below the surface, in ground water and in the sand, occurring at the same depth.

**Morphology and staining.**—The organism is a spore-bearing short curved rod with rounded ends, roughly 1-2  $\mu$  in length, sometimes forming an S-shaped curve, occurring in groups and rarely singly. It stains well with ordinary aniline dyes like Carbofuchsin, and is decolourised by Gram's method (Gram-ve). Methylene blue stains the organism quite distinctly when kept for 3-5 minutes. The organism is actively motile and appears to possess a single terminal flagellum.

**Cultural and Biological.**—The organism is a strict anærope and prefers a specialised media,

The optimum temperature is 30° C., growth being very poor at lower or higher temperatures. The optimal pH is 7.0-7.4. The thermal death point is 60° C.

The organism grows well in liquid media containing sulphates, sodium chloride, phosphates, sodium lactate, and ammonium salts. Presence of traces of iron salts facilitates growth and renders the visual observation of the reduction easy.

The organism does not reduce nitrates. It accomplishes the reduction of sulphites, thio-sulphates and free sulphur.

#### REDUCTION OF SULPHATES IN SOILS OF KNOWN COMPOSITION

Pure acid-washed sand, moistened with the nutrient solution containing all the essential salts, was intimately mixed and dried at 90°C.; this facilitated an even distribution of the salts in the entire mass of the sand.

Cylindrical jars of uniform size (12" × 1½") were filled first with about one-third its height with the treated sand; 10 ml. of a uniformly suspended active culture added and then covered with an other batch of the same sand. Each jar contained 250 gms. of the sand. The sand in each jar was wetted with compounded sea-water until a head of 1" of water remained at the top. A control jar with the sand, identically treated, but with no inoculum, was maintained.

Distinct black bands were visible on the fourth day and these bands gradually developed in width upto about the fifteenth day.

It thus seems justifiable to conclude that *Vibrio desulphuricans*, Konæ is responsible for the production of sulphuretted hydrogen formed in the sulphur-bearing area. Further experiments with a view to initiate the process of sulphate reduction in areas other than the sulphur-bearing areas are in progress. We wish to tender our grateful thanks to the Government of Madras for the generous support of a scheme, of which these studies form a part. Our thanks are also due to Sir J. C. Ghosh for his kind interest in the course of these investigations.

K. K. IYA.

M. SREENIVASAYA.

Section of Fermentation Technology,  
Indian Institute of Science,  
Bangalore,  
August 31, 1945.

---

1. Iya and Sreenivasaya, "A preliminary study of the Bacterial flora associated with sulphur deposits on the East Coast (Masulipatam)", *Curr. Sci.*, 1944, **13**, (12), 316-17.