P A R T - II

Page No.

FOURTH ADDITIONAL PAPER — 397 - 431

" BACTERIAL PHOTOSYNTHESIS IN THE OXIDATION PONDS OF AHMEDABAD ".

C O N T E N T S.

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I. INTRODUCTION

In nature solar energy is being utilized in two types of photosynthetic processes called green plant photosynthesis and bacterial photosynthesis. The chief point of difference between the two types is that in the case of green plant photosynthesis the gross conversion of CO_2 to cell material requires a source of reducing power which is ultimately provided by water, "through a special reaction linked with the photochemical process, which results in the oxidation of water to molecular oxygen;" while in the case of bacterial photosynthesis, "water is not used as an ultimate reductant but reduced sulphur compounds and molecular hydrogen". So Stainer (1961) concludes that green plant photosynthesis will be an aerobic process while bacterial photosynthesis an anaerobic process and strict anaerobiosis entails as a corollary obligate phototrophy.

The phenomenon of bacterial photosynthesis has been recognised as a distinct form of photosynthesis from the 19th century and has been attributed to two selective groups of the eubacteria which depend primarily and exclusively upon light as an energy source and carry on a photosynthetic mode of metabolism. They are Thiorhodaceae, the purple coloured sulphur bacteria and Athiorhodaceae, the purple coloured sulphur-less bacteria. There are 13 genera under Thiorhodaceae of which Thiopedia is one genus, the type species being *Thiopèdia rosea Winogradsky*. Breed et al (1957) describe the type species as follows:

" Individual cells spherical to short rod-shaped, the latter shortly before cell division. Arranged in flat sheets with typical tetrads as the structural units. These arise from division of cells in two perpendicular directions. Cell aggregates of various sizes, ranging from single tetrad to large sheets composed of thousands of cells. Non-motile, non-spore forming. Contain bacteriochlorophyll and carotenoid pigments. Capable of photosynthesis in the presence of hydrogen sulphide, then storing sulphur granules, Anaerobic . The cells measure 1 to 2 μ often appearing as slightly elongated cocci regularly arranged in platelets. Colonies are red to nearly black, depending upon the amount of sulphur stored. Red colour is visible only with large cell masses and not in individuals. A valuable distinguishing feature is the common presence of a relatively large pseudovacuole, or aerosome in the cells of plankton samples. It is widely distributed in mud and stagnant bodies of fresh, brackish, and salt water containing H_2S and exposed to light. It is very common in sulphur springs where it frequently gives rise to extensive mass developments".

Fig. 45. Photo-micrographs of Thiopedia rosea from the oxidation ponds of Ahmedabad.

2. RESULTS

Thiopedia rosea Winogradsky, the purple coloured sulphur bacterium has been found to occur in the pilot-plant oxidation pond and in the series of seven ponds more or less as a constant plankton form throughout the period of our investigations and under a variety of conditions. The origin, development and ecology of this species are discussed in this paper which describes " bacterial photosynthesis" while the first three papers of Part III describe green plant (algal) photosynthesis in the oxida-ponds of Ahmedabad.

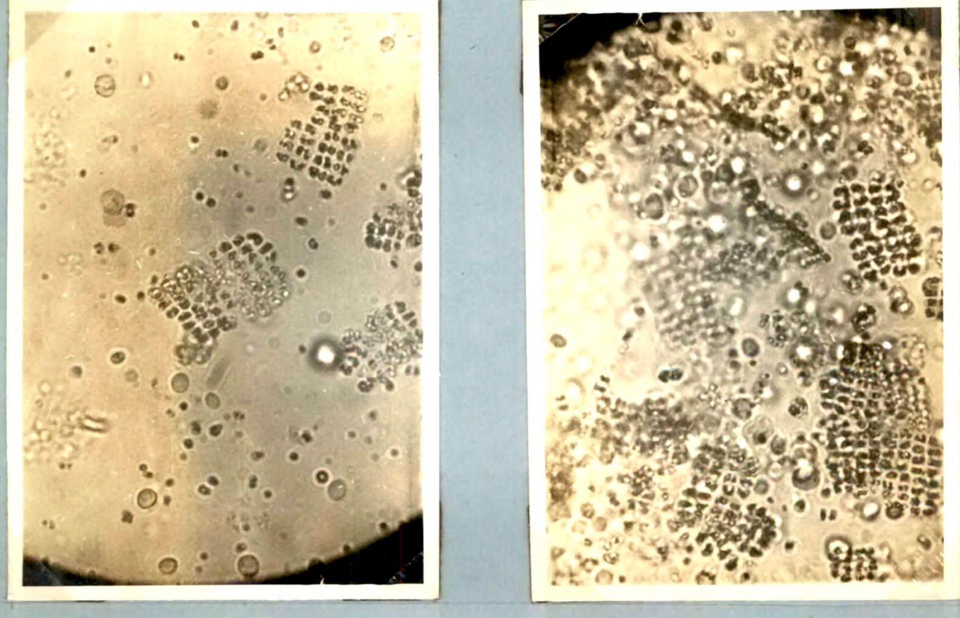
Conditions under which this organism has been found to occur in the two types of oxidation ponds are shown in Tables 1 and 2 (Appendix). From a study of these tables it will be seen that it has been found to occur as a plankton: (i) when the ponds were greenish; (ii) when the pond water contained dissolved oxygen or no dissolved oxygen; (iii) when the pond water contained H_2S or no H_2S; (iv) when the pond was entirely rose-red, pink or purple coloured; when it was floating as a scum and washed ashore depending upon the direction of the wind; and (v) at the bottom of the pond or as pink spots all along the margin of the ponds.

It was found in massive populations on three important occasions which require detailed consideration.

(a) 29th and 30th April 1962:

On 25th April there was a breach in the main sewage carrier and so there was no flow of sewage into the series of seven ponds when each pondbegan to function independently. A few days later all the ponds developed a rose-red colour. As this was an unusual phenomenon tests were made round the clock on 29th and 30th April at four hourly intervals, to find out if there was any dissolved oxygen. The results are shown in Table 3 (Appendix) from which it will be seen that the maximum amount of 17.30 mg./l. was recorded at 3 p.m. in the surface layers which were rose-red. On throwing stones into each of the ponds it was found that the layers about 6" below the rose-red surface layer were greenish due to an algal bloom of *Chlorella* and *Ankistrodesmus*. Microscopic examination of the centrifuged sediments of the rose-red surface layer revealed the presence of rose-red coloured organisms in single tetrads or multiples of tetrads in large platelets exactly resembling colonies of *Merismopedia*. At first it was mistaken for the blue-green alga *Merismopedia tenuissima* on the basis of its morphological structure, but the pinkish colouration of the colonies and black sulphur droplets inside the cells could not be explained. Also the presence of supersaturated

Fig. 46. Another set photo-micrographs of <u>Thiopedia rosea</u> from the oxidation ponds of Ahmedabad.



dissolved oxygen discounted the possibility of the occurrence of purple coloured sulphur bacteria. It was, therefore, thought first that it was a case of chromatic adaptation of the blue-green alga, Merismopedia tenuissima (Fritsch 1952). Very striking cases of red or purple coloration due to the mixing of the hypolimnetic water containing Oscillatoria rubescens occur during circulation periods in some Swiss lake, where the phenomenon is termed the appearance of "Burgunderblut". But Euglena sanguinea and Haematococcus pluralis may produce " blood lakes " in the mountains of Central Europe (Klausener 1908; Huber-Pestalozzi 1936).

(b) 14th November 1962:

Again the first of the seven series of oxidation ponds developed a pinkish-violet scum which was slowly drifting to the other six ponds. The scum was collected in a Winchesterquarts bottle for further examination in the laboratory. There was H_2S in the bottle as was shown by the lead paper test. Also, there was no dissolved oxygen in the sample collected. The coloured bloom continued for a week more. On microscopic examination, the organisms was exactly resembling Merismopedia but differed from it in its red coloured pigmentation, and containing somewhat black sulphur droplets and growing in the presence of H_2S. Another interesting point was that the tetrad colonies

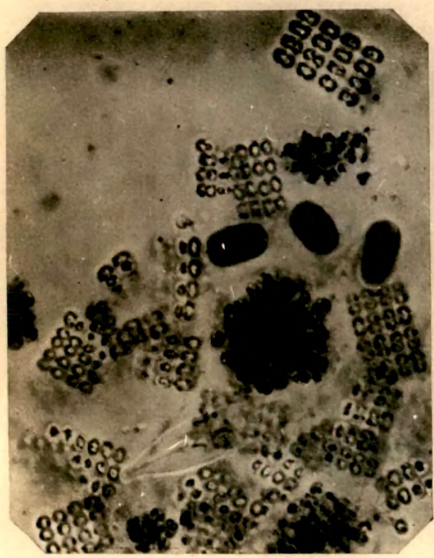
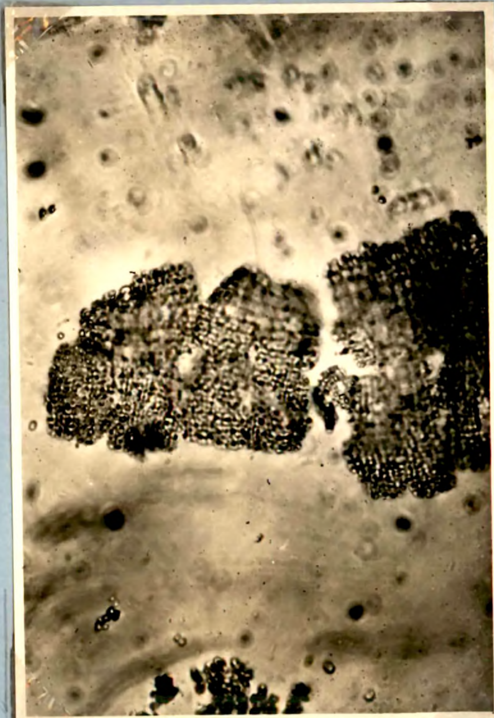


Fig. 1. Formen von roten Schwefelbakterien: Chromatium, Thiocystis und Thiopedia, nebeneinander am gleichen Standort aus einem verunreinigten Stadtgraben. Übergangsformen fehlen. Alle enthalten S-Tröpfchen.



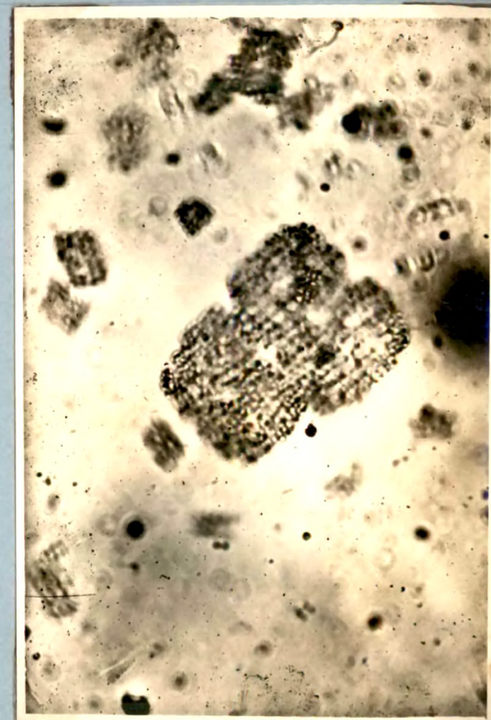


Fig. 47. A Photo-micrograph of the red sulphur bacteria by Pringshein; and two magnified views of Thiopedia rosea collected from the oxidation ponds of Ahmedabad.

were exactly resembling the coloured organisms noted on the former occasion and the photomicrograph by Pringsheim (1932) of *Thiopedia rosea*. Ellis (1932) has stated that it was once called *Merismopedia littoralis* and has added; "*Oerstedt* named it *Erthroconis littoralis*. Warming was of the opinion that it was either a variety of *Bacterium sulfuratum* or a small colony of *Clathrocystis.* In Migula's classification *Merismopedia littoralis* was placed among the sulphur bacteria. It was regarded as identical with the *Thiopedia rosea* described by Winogradsky and so the term *Merismopeida* was dropped and the term *Thiopedia* took its place. The genus Merismopedia of to-day belongs to the Cyanophyceae ....".

Specimens of the organisms were sent for correct identification to Dr. J.W.G.Lund F.R.S. of the Fresh Water Biological Association of the British Empire, England who had confirmed it as *Thiopedia rosea*. The only difficulty was that this organism was found in the presence of plenty of dissolved oxygen on the former occasion and in the presence of H_2S on this occasion. How to reconcile these vital differences noted on both the occasions?

Professor William, J. Oswald of the University of California to whom the matter was also referred replied in his letter dated 27-12-62 as follows:

" From your description, it appears that the dissolved oxygen, producing bloom of April 29th and April 30th, 1962 differed from the bloom of november 1962. The April bloom was definitely algal, because of the dissolved oxygen present. The presence of H_2S in November was probably due to an acid producing fermentation occurring in the bottom sludge of the initial pond.

$$C\,H_2O \xrightarrow{\text{Bact.}} C\,H_3COOH \xrightarrow[\text{Base}]{\text{Acid}} H^= + CH_2COOH^-$$

Such acid fermentation if not fllowed immediately by methane fermentation ----

$$C\,H_3COOH \text{ ---------- } CH_4 + CO_2$$

leads to evolution of H_2S in accordance with the equation

$H^+ + H\,S^- \xrightarrow[\text{Alkaline}]{\text{Acid}} H_2S$. The H_2S, then provides substrate for photosynthetic bacteria which carry out the reaction.

$$CO_2 + 2H_2S \xrightarrow{\text{Light}} CH_2O + H_2O + 2S$$

I surmise that the presence of dissolved oxygen in the ponds during the summer was unfavourable to the establishment of Methane fermentation; whereas, low temperature in the fall would prevent its occurrence. I feel that once methane fermentation is established the ponds will perform satisfactorily. Without specific data on design characteristics of the ponds and on your climate, it is impossible to suggest any remedies for the improvement of pond action".

There is no doubt that there was green plant (algal) photosynthesis on 29th and 30th April due to the bloom of Chlorella and Ankistrodesmus, formed below the surface layer of rose-coloured purple sulphur bacteria. The point for consideration was as to how the latter was seen to exist in the presence of so much of dissolved oxygen when it is known that it is strictly anaerobic and that it grows only in the presence of sulphuretted hydrogen according to Breed et al (loc. cit)".

(c) 22nd April, 1964:

On this date, conditions almost similar to that found on 29th and 30th April 1962 were found in one of the series of ponds adjacent to the Pilot Plant Oxidation Pond at 10.00 a.m. The surface of the entire pond was rose-red in colour. Near the inlet the bank was covered with brownish deposits while at the out-let end there was greenish scum interspersed with brownish scum. The middle portion of the pond was more or less similar. On throwing a stone at a spot where there was a brownish surface floating scum, a green layer was seen coming up to the surface from the bottom.

Parker (1962) has recorded the occurrence of *Chromatium*, a purple coloured sulphur bacterium in the presence of oxygen in the first two ponds of his aerobic eight-cell ponds in summer in Australia. His results are shown below only for 4 ponds.

Item	Raw sewage	POND I	POND II	POND III	POND IV
Detention time(days)	-	3.8	8.0	8.0	18
H_2S (mg./l.)	6.7	8.6	2.7	0	0
Dissolved oxygen(mg./l.)	0	0	0.8	1.0	2.0
B.O.D. (mg./l.)	521	100	46	22.7	22.7
Organic N (mg./l.)	26.3	12.5	14.5	11.2	13.7
Ammonia N (mg./l.)	32.4	46.4	52.5	48.7	45.0
Nitrate N (mg./l.)	0	0	0	0.30	0.49
Nitrite N (mg./l.)	0	0	0	0.01	0.17
Total bacteria at 22°C (no/ml)	7.0×10^6	7.9×10^5	2.6×10^5	1.6×10^5	1.0×10^5
Algae (no.ml.)	Nil	7.0×10^3	7.1×10^6	2.4×10^7	1.4×10^7
Predominant algae	Nil	Chroma-tium	Chroma-tium	Eugle-na etc.	Euglena etc.

In pond I, there was no dissolved oxygen but in Pond II there was 0.8 mg./l., when the purple coloured sulphur bacterium was dominant in both the ponds. Also, the BOD reduction was respectively 80.8% and 91.1% in Ponds I and II.

3. DISCUSSION

Before reconciling the conflicting conditions under which *Thiopedia rosea* was found to occur in the oxidation ponds of Ahmedabad it may be worth while to compare the conditions under which purple coloured sulphur bacteria in general and *Thiopedia rosea* in particular have been reported to occur in lakes of the world and in oxidation ponds in other parts of the world.

OCCURRENCE OF THIOPEDIA ROSEA AND OTHER PURPLE SULPHUR BACTERIA IN LAKES

The growth of *Thiorhodaceae* is reported to proceed in the bottom layers of shallow lakes when H_2S is produced as a result of the activities of sulphate-reducing bacteria in the deoxygenated muds. Hutchinson (1957) has recorded the extraordinary case of Son-Sakesar-Kahar, a lake situated at the western end of Son plateau in the Salt Range of Pakistan, Panjab in which the entire lake became dull pink in 1932 owing to a dense bloom of the purple sulphur bacteria Lampro-cystis roseopersicina. The colonies in the surface and in 3-m samples which contained small amounts of H_2S were pink, the colonies in the deeper water were white and probably dead. The Lamprocystis bloom was not always seen in this lake. He has also recorded the occurrence of massive populations of purple sulphur bacteria at just above the level of the chemocline in the lake Ritom.

Utermohl (1925) found the colourless bacterium Macromonas bipunctatus in narrowly defined layers in the hypolimnion of north German lakes, where the concentration of dissolved oxygen lay between 2.0 and 3.0 mg./l. No, H_2S was detected. In a number of other lakes studied by him he found also Thiopedia rosea which was seen generally at or near the bottom. In the Krummensee, he found the colourless sulphur bacterium Macromonas bipunctatus at 11m, its maximum at 9 m depth and larger number of Thiopedia rosea at 11 m though it might have been possible that even more cells of the purple bacteria were found between the 11m layer and the bottom at 12 m. In september 1921, he found a well developed zone of Thiopedia rosea between 32m and 36 m in the Hemmelsdorfersee. None was seen below that stratum. The H_2S required for the organism must have passed, either by molecular diffusion or very turbulent diffusion from a depth of several meters below the maximum. He had also found the organism at or near the bottom.

Ruttner (1937) had noted the presence of coloured sulphur bacteria when there was no analytically detectable H_2S in the following lakes. In the Lunzer Obersee *Chromatium Weissei* formed a rose-red layer at the limits of detectable oxygen scarcely 50 m thick. In the Krotensee he observed a stratification of purple bacteria on 24th June 1933 near the Chemocline as detailed below:

Depth (m)	Temp (C)	O2 mg/l.	PH	Chromatium weissei	Sp.	Lamprocystis per ml.
20	4.6	0.79	7.46	0	0	0
21	-	-	-	0	5800	8300
22	-	-	-	140	90	-
22.5	-	-	-	-	10	-
25.0	4.7	0.0	7.38	20	0	100
30.0	4.9	0.0	7.10	-	0	0

In another lake called Toplitzsee he had recorded the vertical distribution of the purple coloured sulpher bacterium *Lamprocystis* on 18.6.1933.

Depth (m)	Temp (C)	O2 (mg/l.)	PH	Lamprocystis (per ml)
10.0	6.10	7.46	7.62	0
12.5	5.60	3.01	-	-
15.0	5.60	0.76	-	0
17.5	-	0.24	-	-
20.0	5.65	0.25	7.28	0
22.5	5.80	0.14	-	-
25.0	5.90	0.15	-	80
30.0	5.90	0.11	7.28	650
50.0	5.90	0.11	7.30	240

Vetter (1937) found practically little difference in the vertical distribution of <u>Thiopedia rosea</u> in the Schleinsee. This organism was found in great abundance in the lower layers of water where the oxygen content fell below 2.5 mg/l; and at greater concentrations only when the content of dissolved oxygen increased after a previous low value. During late June and throughout July when the organism was multiplying, H_2S was always detectable at or above 10 m and was usually absent from the 11 m level just above the deepest mud. In August and abundance of <u>Thiopedia rosea</u> was seen just above the strata where H_2S was detectable; but later on massive populations of the purple bacterium was seen at 9 m and 10 m where 0.5 to 0.8 mg./l. of H_2S was present. The maximum developments of the organism were seen towards the end of October at 10 m depth when dissolved oxygen was found to increase from0.8 to 2.3 mg./l; and at complete turn-over when the oxygen content increased further to 7.5 mg./l. the purple coloured organism began to decrease in numbers.

Collins (1958, 1963) has stated that the growth of <u>Thiorhodaceae</u> proceeds in the bottom layers of shallow lakes where H_2S is produced as a result of the activities of sulphate-reducing bacteria in the deoxygenated muds. In the circulating layers above, members of <u>Athiorhodaceae</u> are to be found, where in the presence of oxygen and light, they sometimes form a pink layers at the interface of the oxygenated and deoxygenated zone. Determination of the activity of these selective groups of bacteria is of vital importance in elucidating their function in the natural medium of lake water.

OCCURRENCE OF PURPLE COLOURED SULPHUR BACTERIA IN SEWAGE OXIDATION PONDS

Photosynthetic bacteria have been observed in anaerobic ponds (Oswald 1960a). He has reported about the surface of primary anaerobic pond becoming deep pink due to *Thiopedia rosea*. The secondary pond receiving the effluent was deep green due to a bloom of *Euglena*. Odours from the primary anaerobic pond were largely controlled by the *Thiopedia* which used up H_2S (Oswald 1960b). He found the BOD removal in each pond to vary between 75% and 80% and the overall reduction was greater than 90%.

Green (1960) found a one-cell oxidation pond deep red and about one-eighth of the surface was covered with a floating scum of the same. Parker (1960) had stated that photosynthetic bacteria and not algae were sometimes present in the Australian anaerobic lagoons. Parker et al (1963) found *Chromatium* spp. and other related organisms in large numbers and on those occasions ~~in~~ the sulphide content was reduced to zero by their metabolic activities. Hunt and Westenberg (1964) found a lagoon at the first stage of filling with sewage to become deep carmine pink probably due to a culture of Thiopedia and it remained practically odourless. A few days after the pond had been filled up the colours changed to green. Williams (1964) found the colour of his oxidation pond turning pink when the discharge of septic tank effluent into the pond was stopped. The organism causing the pink colour passed through ordinary filter paper but could be separated by centrifuging. Shaw (1962) has mentioned a shimmering red colour in his experimental ponds which were fed with night soil; and the colouration was caused by a species of Moina, a crustacean. Abbott (1962) has mentioned the interesting case of the pond at Muizenberg turning pink due to Thiopolycoccus, a purple coloured sulphur bacterium when one of the ponds became heavily contaminated with sea water and so had a high sulphate content. For nearly a month thereafter both the ponds continued to be pink and dissolved oxygen was absent, but there were broad streaks of green on the surface. After a further two weeks the dissolved oxygen content was about 0.35 mg./l. and still there was a pink colour. Two weeks later again, the pond turned into a bright green colour.

A very interesting feature about the treatment of wastes from creameries in Kansas state was that the liquid in the pond was of the colour of diluted blood instead of green. Organisms responsible for purification were believed to be purple sulphur bacteria (Venkataramanan 1961-62). Smallhorst (1963) has reported about the odixation ponds of Texas U.S.A. turning from green to pink and in a few cases almost red.

Neel (1963) is perhaps the only one who made a fairly detailed study of 'pink' ponds in the vicinity of Kansas, Missouri city in 1955. In his studies of sporadic cases of pink colouration in oxidation ponds he found that this phenomenon occurred in those ponds which had developed thermal and chemical stratification. He found the top 6" of the ponds to be sparsely populated with algae which increased in numbers in the next lower 6" depth. This layer was generally greener and the green colouration was generally found to extend further downwards to about 24".

The next lower 6" stratum was pinkish to dark purple, contained H_2S and very dense populations of the two purple coloured sulphur bacteria, Chromatium sp. and Thiospirillum sp. The amount of oxygen dissolved was greater at 12" to 18" depth than at the surface, and it disappeared at the junction of the green and pink strata. So, the photosynthetic purple sulphur bacteria were found below the green algal stratum.

The oxidation ponds which were thermally stratified gave no indication of the presence of purple sulphur bacteria in the lower layers. The ponds turned pink or red only when there was a sudden turn-over as a result of sudden drop in temperature and the resultant cooling of the surface layer and overturning. A return to the stratified condition was reached if there was a spell of calm, sunny weather. The pink colour at the surface then disappeared when thermal stratification was restored.

Neel has mentioned another case of three ponds receiving wastes from a milk processing and cheese plant in Missoori turning pink for a brief period in the early spring and for several weeks in the autumn of 1956. The spring colouration was caused by *Chromatium* sp. and *Thiospirillum* sp. and the fall colour by *Thiopedia*.

He has cited a third instance of a two-celled lagoon facility receiving treated washing, spillage, and sanitary sewage from a dairy near Kansas city in 1960-61 becoming pink for nearly five months under ice cover in one of the ponds. *Thiopedia*, small rods and spirals were the dominant organisms on one occasion, and *Chromatium* and *Thiospirillum* on another occasion. Soon the coloured sulphur bacteria were replaced by ~~the~~ algae. He also noted the presence of small amounts of dissolved oxygen even when the pond was pink. There were intermittent green and pink periods and the BOD reduction was nearly 92%. He has also recorded a fourth case of purple coloured sulphur bacteria appearing in some north Dakota lagoons which were receiving organic wastes from potato starch and potato flake industries in the summers of 1960 and 1961. He did not identify the purple organisms.

From all these studies he concluded that in all thermally stratified oxidation ponds of about 3 feet in depth, the photosynthetic purple coloured sulphur bacteria were thriving below the upper algal layers of 1-2 ft. and that the algae utilised the visible rays and the purple bacteria the far and short infra red rays of the solar spectrum. The entire pond became pink only when there was complete circulation due to sudden climatic changes. Also, there was considerable reduction in BOD when the ponds were pink.

In almost all the cases cited above purple coloured sulphur bacteria have been found to flourish in regions in which not only H_2S is absent but also oxygen is present; and to colour the water rose-red, pink or deep red as in the oxidation ponds of Ahmebabad. But no one has recorded the presence of a member of the purple coloured sulphur bacteria in lakes or oxidation ponds either as a constantly occurring form or in the presence of a large amount of dissolved oxygen as in Ahmedabad. These facts would strongly suggest that these organisms are not strictly anaerobic as has been stated by Breed et al (1957). The discovery of the nature of bacterial photosynthesis by Van Niel (1931) was followed by studies on the photosynthetic metabolism of organic substrates by the purple bacteria which showed that ~~the~~ under anaerobic conditions in light these organisms could perform a very effective assimilation of organic

Carbon (Gaffron 1933, 1935; and Muller 1933).

The most recent studies of the physiology of these organisms indicate that such distributions as recorded in this paper are not entirely unexpected. They may be using instead of H_2S simple organic compounds which have been elaborated by bacterial action and/or the simple organic acids and polysaccharides which are among the extra-cellular products released during algal synthesis (Fogg 1960) in the oxidation ponds of Ahmedabad. Recently Stainer (1961) has reviewed this aspect of the problem admirably and has shown the major physiological distinctions between green plants and purple bacteria as follows:

Description	Green Plants	Green bacteria	Purple bacteria
a) Source of reducing power	H_2S	H_2S, other reducing inorganic compounds	H_2S, other reduced inorganic compounds. Organic compounds.
b) Photosynthetic oxygen evolution	Yes	No	No
c) Principal source of carbon	CO_2	CO_2	CO_2 or organic compounds
d) Relations to oxygen	Aerobic	Strictly anaerobic	Strictly anaerobic or facultatively aerobic

So, purple coloured sulphur bacteria including Thiopedia rosea can flourish in the presence of oxygen also. This has been confirmed by Professor F.E. Fogg, of the Westfield College, University of London in a personal communication dated 6.12.1965, when the occurrence of Thiopedia rosea in the presence of oxygen on 29th and 30th was referred to him. He stated:

" These bacteria normally grow under anaerobic conditions but might appear sometimes in Water with a high oxygen concentration as on 30th April. The occurrence of a similar rose-red bloom on 14th November in association with hydrogen sulphide again suggests purple sulphur bacteria".

In the oxidation ponds of Ahmedabad Thiopedia rosea has been found to be a constantly occurring planktonic form. It appears to form at first at the bottom of the ponds and slowly rises upto the surface. When the production of H_2S in the bottom layers is high and continues for a long period then it forms a bloom and colours the entire ponds as in the case of the series of ponds from January to May 1963; or if the bloom is periodical, the organisms rise up in the hydrosphere after sometime and covers the entire surface layer forming a thick floating scum due to their death and decay . Then the cells are washed ashore depending upon the direction of the prevailing wind. On such occasions green coloured algal organisms develop below the surface layer and gradually convert the rose-red or pink or purple coloured ponds into green ponds. Otherwise the organism is still being formed in sufficient number at the bottom so as to be dominant or subdominant in the Plankton (Vide Tables 1, 2 and 3 Appendix).

Thiopedia rosea and BOD reduction

When there was the occurrence of *Thiopedia rosea* in the ponds there was also considerable reduction in BOD as shown in Table 1, 2 and 3 (Appendix). Also, in the series of seven ponds (Paper No. 2, Part II of this thesis) the entire ponds were reddish during January through April, 1963. The BOD reduction at that time is shown below:

5-day BOD at 20°C PPM, 1963.	Raw sewage	Effluents from Pond No.							Final % Red
		1	2	3	4	5	6	7	
January	180	155	122	90	118	82	75	64	64.4
February	153	162	152	130	72	82	55	51	66.7
March	257	127	140	103	80	78	70	68	73.5
April	175	135	127	97	83	70	66	74	57.7

The maximum reduction was 73.5%; and the minimum reduction 57.7%. Parker (1962) found a reduction of 91.1% in the effluent from the second pond of the series of eight ponds when another purple bacterium, Chromatium sp. was the dominant organism in the pond. So, it would appear that the occurrence of purple coloured sulphur bacteria in oxidation ponds does not affect the efficiency of the ponds.

Since Thiopedia rosea is an obligate phototroph radiant energy must be reaching the bottom of oxidation ponds for its development. This radiation cannot but be short infra-red as has been pointed out by Hutchinson (1957) and Rabinowitch (1955) who states:

" Photosynthesis requires visible light. About 50% of sunlight reaching the earth's surface belong to infra red and have quanta too small for photosynthesis. Therefore, they are wasted as far as utilisation by plants is concerned".

" To get better results, perhaps purple bacteria could help. They can make photosynthesis in the far red and near infra red, an important segment of the solar spectrum".

" Bacteria cannot make true photosynthesis. They do not produce oxygen from water. However, they do photosynthesis with some rather hydrogen donors such as organic compounds and can thus perhaps store one-third of the energy stored in true photosynthesis".

" Therefore, I think, that in addition to growing algae, one should also investigate the possibility of growing purple coloured bacteria on a large scale perhaps in light filtered through a suspension of green cells".

What Robinowitch has suggested is really taking place naturally in the oxidation ponds of Ahmedabad. The purple coloured sulphur bacterium *Thiopedia rosea* is found to develop ordinarily in the bottom layers when the surface layers are greenish due to the presence of algal organisms. Then the green plant (algal) photosynthesis goes on in the surface layers and bacterial photosynthesis in the bottom layers so that the entire solar spectrum is being utilized in sewage purification at Ahmedabad almost throughout the year.

S U M M A R Y

In the oxidation ponds of Ahmedabad periodical blooms of the purple coloured sulphur bacterium *Thiopedia rosea* are seen to develop colouring the entire pond rose-red, pink or purple. On other occasions the bacterium is seen as a plankton along with green and blue-green algae, On one occasion it was found to proliferate in the presence of a considerable amount of dissolved oxygen and on other occasions in the presence of absence of sulphuretted hydrogen

All these observations though seemingly contradictory can be reconciled on the basis of the most recent studies on the physiology of the organisms as reviewed by Professor Stainer and as stated by Dr. Fogg.

When it is occurring as plankton the visible and invisible rays of the solar spectrum are being utilised in the form of green plant (algal) photosynthesis on the top layers and bacterial photosynthesis in the bottom layers of oxidation ponds. Then there is also considerable reduction in BOD.

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T A B L E NO. I

ENVIRONMENTAL CONDITIONS OF THE OCCURRENCE OF THIOPEDIA ROSEA ON SOME IMPORTANT DATES IN THE PILOT PLANT OXIDATION POND IN THE PIRANA SEWAGE FARM AT AHMEDABAD DURING -(1962-1964).

Date	Colour	H_2S +or-	Temp (C)	D.O mg./1	5-day BOD mg./1	Total No.of organisms per $ml \times 10^3$	Thiopedia rosea.% of the total	Other algal organisms(remaining % of the Total)				
1962												
16-7	Red-patches floating	-	31.2	Nil	90	841	9.0	Chlorella,	Ankistrodesmus,	Pyrobotrys,	Arthrospira,	Euglena
20-7	Red	-	28.0	2.34	50	514	40.3	"	"	"	"	"
23-7	Y .Green	-	29.2	Nil	60	422	16.3	"	"	"	"	"
3-8	Green	-	32.0	6.00	40	19	21.0	"	"	"	"	"
6-8	Green	-	30.2	1.74	-	17	8.2	"	"	"	"	"
10-8	Red	H_2S	29.2	Nil	210	54	94.4	"	"	"	"	"
31-8 3.0 P.M.	Pale Green	Nil	34.5	10.14	30	144	39.6	"	"	"	"	"
14-9 3.0 P.M.	Pink	H_2S	35.7	Nil	190	282	76.6	"	"	"	"	"
17-9	Pale Green	Nil	31.5	Nil	120	864	27.0	"	"	Chlorococcum	"	"
14-11	"	"	28.0	"	25	213	56.3	Chlamydomonas	"	Chroococcus	Oscillatoria	"
16-11	Greyish	"	29.6	"	45	108	47.2	Arthrospiria	"	"	"	"

TABLE NO. I (Contd.)

Date	Colour	H_2S +or-	Temp. ^oC	D.O. mg./l.	5-day BOD mg./1	Total No.of orga-nisms per ml. $x10^3$	Thiopedia rosea % of the total	Other algal organisms(remaining % of the total)			
19-11	Brown	Trace	29.0	Nil	85	222	40.6	Ankistro-desmus	Chlamy-domonas,		
21-11	"	"	26.5	"	120	220	50.0	"	"	Euglena, Chlorococcum	Phacus Euglena
23-11	Red	Nil	25.6	3.18	-	228	61.8	Chlorella,	Ankistro-desmus	Chlamydomonas,	Chroococcus
26-11	Red	"	26.8	2.24	60	252	40.4	"	"	"	Arthrospira
13-12 (3PM)	Green	"	26.6	2.51	100	993	27.8	"	"	"	"
1 9 6 3					1 9 6 3					1 9 6 3	
14-1	Y.Green	"	19.5	1.83	85	270	10.0	"	"	"	"
11-2	Grey		21.5	-	110	211	34.9	"	"	Chlorococcum	
18-2	Pink	"	21.0	Nil	95	362	38.7	-	"	"	
22-2 (3PM)	Violet scum	"	28.0	2.84	75	439	48.7	Chlorella	"	"	
8-4	Y.Green	"	27.1	0.77	130	279	12.7	"	"	"	Eugelena

TABLE NO. I (Contd.)

Date	Colour	H_2S +or-	Temp. (C)	D.O mg./1	5-day B O D mg./1	Total No.of organisms per ml.x10^3	Thiopedia rosea % of the total	Other algal organisms(remaining % of the Total)
18-6	Green	Nil	29.7	5.37	50	111	48.7	Euglena Ankistrodesmus, Arthrospira, Oscillatoria
6-7	P. Yell.	"	27.7	Nil	70	686	53.3	" " " "
25-10 3 P.M.	Purple	H_2S	32.5	Nil	90	65	100	- N i l -
28-10	Rose-Red	H_2S	29.0	Nil	-	53	100	- N i l -
Bottom	Intense Red	H_2S	-	-	-	88	100	- N i l -
4-11	Pink	Nil	28.1	0.50	60	252	32.0	Chlorella
22-11	Green	"	29.5	8.52	40	403	32.3	Chlorella
1 9 6 4					1 9 6 4			1 9 6 4
23-3	Purple	Nil	31.7	1.87	70	58	45.6	Chlorella, Arthrospira, Euglena
1-4	Purple	Nil	30.5	Nil	140	636	27.0	Crucigenia, Arthrospira, Euglena

TABLE NO. 2

ENVIRONMENTAL CONDITIONS OF THE OCCURRENCE OF THIOPEDIA ROSEA WINOGRADSKY ON SOME IMPORTANT DATES IN THE SEVEN CELL OXIDATION POND IN THE PIRANA SEWAGE FARM AT AHMEDABAD DURING 1962-63

Date	Cell No.	Colour	H_2S	Temp.	D.O. mg./l	5-day BOD mg./l.	Total No. of organisms per ml $\times 10^3$	Thiopedia rosea % of the Total	Other algal organisms (remaining % of the total)
1962							1962		1962
10-1	1to4	Green	Nil	25.0	0.90to 6.41	65-10	521	1.9	Arthrospira, Ankistrodesmus, Oscillatoria, Chlorella
18-4	1to4	"	"	30.2	Nil	100-70	241	24.0	Chlorella, Euglena, Oscillatoria, Navicula
25-4	3to7	Red	"	28.6-30.1	0.64to 2.94	-	322	17.1	Chlorella, Oscillatoria, Pandorina, Ankistrodesmus
30-5	3to7	Y.Green	"	31.3-30.5	1.71to 2.48	102-30	196	28.5	Chlorella, Oscillatoria, Ankistrodesmus, Euglena
4-7	3to7	"	"	31.0-32.1	1.22to 1.80	130-40	356	10.1	Chlorella, Oscillatoria, Ankistrodesmus, Euglena
19-7	3to7	Red	"	31.5-30.3	Nil	120-41	168	32.1	Chlorella, Ankistrodesmus
1963							1963		1963
2-1	1to7	Red	Present	20.0-19.5	Nil	130-75	322	14.0	Chlorella, Ankistrodesmus, Chlamydomonas
9-1	"	Brown	Nil	18.2-19.6	0.35	120-60	527	22.8	Chlorella, Pyrobotrys, Chroococcus, Ankistrodesmus
23-1	"	Purple	"	20.1-23.4	Nil	-	500	41.6	Chlorella
7-2	"	Yellow	H_2S	23.8-25.0	"	165-55	1810	40.7	Chlorella, Ankistrodemus, Chlamydomonas, Chroococcus
13-2	"	Purple	"	23.0-25.5	"	120-46	1830	85.8	Ankistrodesmus, Chlorella, Chlorococcum
20-3	"	Purple	"	27.1-24.7	"	90-40	1780	57.8	Chlorella, Ankistrodesmus, Chlorococcum
22-5	"	Green-Yellow	Nil	30.0-29.2	1.24to 0.48	70-20	6220	25.0	Chlorella, Ankistrodesmus, Chlorococcum

TABLE NO.2 (Contd.)

Date	Cell No.	Colour	H_2S	Temp.	D.O. mg./1	5-day BOD mg./1	Total No. of organisms per ml x 10^3	Thiopedia rosea % of the Total	Other algal organisms (remaining % of the Total)
15-6	1to3	Yellow	H_2S	20.0	.30to 3.80	150-80	3200	25.4	Chlorella, Ankistrodesmus, Euglena
10-7	1to7	Green	Nil	28.5-29.0	0.70to 0.95	90-70	1830	24.7	" " Euglena
7-8	2to7	Rose-Red	"	29.2	0.70to 0.90	170to 30	1360	50.1	" " " Oscillatoria
30-10	1to4	Green	"	28.0	Nil	70-40	166	70.3	" Arthrospira, Oscillatoria, Euglena
13-11	1to7	Brown	"	27.0-28.0	"	120-30	1270	73.7	Chlamydomonas, Chlorella, Arthrospira
4-12	1to12	Rose-Red	"	25.7-26.2	"	105-90	1400	26.9	Chlorella only.

TABLE NO. 3

DIURNAL VAIRATIONS IN THE SERIES OF SIX OXIDATION PONDS IN THE PIRANA SEWAGE FARM AT AHMEDABAD ON 29TH AND 30TH APRIL 1962.

	Temp. C	Colour	D.O. mg./1	5day B O D mg./1	Total orga-nisms per ml. x 10^3	Thio-pedia rosea % of the Total	Other algal organisms(remaining % of the Total
10 A.M.				10.00 A.M.(29.4.62)			10.00 A.M.
Inlet to Pond No.I	35.0	Black	Nil	250	-	-	-
outlet from Pond No.I	31.5	Brown	"	100	-		Chlorella
" " " II	29.5	"	"	-	247	18.2	Chlorella,Ankistrodesmus,Oscillatoria,Netzschi
" " " III	20.5	Yellow	0.52	-	123	31.7	Chlorella,Ankistrodesmus,Pyrobotrys,Oscillator
" " " IV	30.0	"	3.35	75	402	22.4	" " " "
" " " V	29.8	Brown	0.75	66	405	16.3	" " " "
" " " VI	29.5	Yellow	1.64	60	414	12.1	" " " "
3.00 P.M.				3.00 P.M.			3.00 P.M.
Inlet to Pond No.I	36.5	Black	Nil	205	-	-	- - - - -
Outlet from Pond No.I	32.0	"	"	110	-	-	- - - -
" " " II	-	-	-	-	-	-	- - - -
" " " III	35.5	Red-Green	9.64	60	927	12.6	Chlorella,Ankistrodesmus,Arthrospira,Oscillator -e
" " " IV	34.7	Red	17.30	75	894	4.7	" " " Euglena
" " " V	32.5	Brown	8.75	70	882	9.9	" " " "
" " " VI	32.6	Brown	6.70	50	966	10.0	" " " "

TABLE NO. 3 (Contd.)

SOURCE	Temp. C	Colour	D.O.	5-day BOD mg./1	Total organ -isms per ml X 10^3	Thiopedia rosea % of the Total	Other algal organisms(remaining % of the total)
10.00 P.M.					10.00 P.M.		10.00 P.M.
Inlet to Pond I	31.2	Black	-	-	-	-	--
Outlet from Pond I	31.0	Grey	Nil	-	-	-	--
" " II	30.5	Brown	"	-	480	27.0	Chlorella, Ankistrodesmus, Oscillatoria
" " III	30.0	"	0.88	-	651	12.8	" " "
" " IV	30.0	"	3.76	-	714	23.1	" " "
" " V	30.5	Straw	1.08	-	924	15.6	" " "
" " VI	30.04	"	0.94	-	486	34.2	" " "
7.00 A.M.					7.00 A.M.		7.00 A.M.
Inlet to Pond I	23.0	Black	Nil	230	-	-	--
Outlet from Pond I	31.6	"	"	70	-	-	--
" " II	31.0	Red	"	75	186	24.1	Chlorella, Pandornia, Ankistrodesmus
" " III	29.7	"	"	85	744	13.3	" " "
" " IV	29.5	"	2.86	-	717	22.2	" " "
" " V	20.0	"	"	40	975	12.6	" " "
" " VI	30.0	Y.Green	0.46	55	521	14.7	" " "
11.00 P.M.					11.00 P.M.		11.00 P.M.
Inlet to Pond I	27.5	-	Nil	-	-	-	--
Outlet from Pond I	30.7	-	"	85	-	-	--
" " II	29.0	-	"	75	993	29.0	Chlorella, Ankistrodesmus
" " III	28.6	-	"	-	672	31.0	" "
" " IV	29.4	-	"	80	510	21.2	" "
" " V	29.1	-	1.18	90	693	11.3	" "
" " VI	28.5	-	2.24	75	642	19.0	" "

TABLE NO. 3(Contd.)

	Temp. C	Colour	D.O. mg./1	5day BOD mg./1	Total orga-nisms per ml. x10^3	Thiopedia rosea % of the total	Other algal organisms(remaining % of the Total)
3.00 A.M.					3.00 A.M. (30.4.62)		3.00 A.M.
Inlet to Pond I	23.5	-	Nil	180	-	-	--
Outlet from Pond I	29.0	-	"	90	-	-	--
" " " II	27.5	-	"	-	687	14.6	Chlorella, Ankistrodesmus
" " "III	27.5	-	"	60	297	27.3	" "
" " " IV	28.0	-	"	51	306	21.5	" "
" " " V	27.5	-	"	35	327	24.0	" "
" " "VI	27.5	-	0.46	40	340	36.2	" "
7.00 A.M.					7.00 A.M.		7.00 A.M.
Inlet to Pond I	-	-	Nil	-	-	-	--
Outlet from Pond I	30.0	-	"	-	-	-	--
" " " II	28.8	Brown	"	-	519	48.5	Chlorella, Ankistrodesmus
" " " III	27.6	"	"	-	504	60.0	" "
" " " IV	27.2	Green	"	-	459	47.0	" "
" " " V	27.0	Brown	"	-	564	46.3	" "
" " " VI	27.0	"	0.96	-	702	44.5	" "