dium' and those of the Stemonitales, an 'aphanoplasmodium', it was of interest to find out the nature of the plasmodium in *Diachea* which forms a connecting link between these two orders. In so far as the rapid fruiting of the cultures permitted only a limited observation of the plasmodia, no definite statement can be made regarding the type of plasmodium in this genus. From chance observations of delicate fans and slender veins it may be suggested that the plasmodium of *D. splendens* is perhaps an intermediate type between the phanero- and aphanoplasmodia, such as that found in *Arcyria cinerea*.[4,5]

Morphological variations produced in culture, such as formation of sessile sporangia, imperfect development of the capillitium and formation of monstrous spores can perhaps be attributed to excessive moisture in the culture flasks, as it has been frequently observed that excessive moisture at the time of fruiting usually produces such irregularities.

The stipitate or sessile nature of the sporangia is sometimes used as a criterion for roughly separating the species *D. splendens* and *D. subsessilis*, the latter species tending to produce nearly sessile sporangia whereas the former is said to be prominently stipitate.[6,7] It is borne out from the present account that stipe length is a variable feature and *D. splendens* produces stipitate as well as sessile sporangia. The distinguishing feature is the ornamentation of the spore wall, as has also been pointed out by Hagelstein.[8] This feature has evolved into a well-developed, delicate reticulation in *D. subsessilis*, whereas in *D. splendens* it shows a primitive reticulation crudely formed by ridges which in turn have developed from a union of coarse warts and spines that were originally perhaps scattered, and to which state the species seems to revert under certain conditions. Such a state is perhaps common in nature, along with an intergrading series towards the reticulate form, as the spore ornamentation in this species is variously described as very coarsely warted,[6] marked with raised bands and tubercles,[9] marked with stout scattered protuberances often confluent to form ridges,[8] etc.

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CATALASE IN ACTIVATED SLUDGE

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INTRODUCTION

SINCE the early years of the scientific study of sewage and sludges, attention has been devoted mostly to the practical aspects of their treatment and utilization. Comparatively very little work has been done on the fundamental aspects of sewage purification. One of the aspects of this biological process, on which the evidence is very meagre, is the enzymes in sewage and sludges. The available information on this aspect relates mostly to the occurrence of some hydrolytic and oxidative enzymes in the slime on the material in the sewage filter,[1,2] in sewage and effluents,[3-7] in the iron bacterium M7[8] and other bacteria,[9-11] in activated sludge,[12-16] in anaerobic sludge[17] and in the sludge from synthetic media.[18] No attempt has, however, been made to extract and isolate the enzymes from even the rapidly purifying system of activated sludge and to study their relation to the aerobic process of purification.

Activated sludge contains a selective group of aerobic micro-organisms which efficiently purify sewage. Such a system may be expected to contain all the enzymes which have been reported to be present in aerobic micro-organisms and possibly other enzymes. The earlier work on the enzymes in activated sludge was probably limited by the lack of adequate methods and techniques, which are now available. Thus it was reported that activated sludge did not contain catalase,[19] a specific hemin containing enzyme, which is essential for the decomposition of hydrogen peroxide, a harmful product formed during metabolic oxidation. In view of the present state of knowledge of the enzymes in sewage and sludges, a systematic study of this aspect has been undertaken. The evidence collected on the catalase in activated sludge is presented in this article.

Extraction of Catalase from Activated Sludge

The samples of activated sludge employed in the experiments were originally obtained from the plant at this Institute and were maintained in the laboratory by aeration. The efficiency of the sludge samples was daily assessed with reference to the quality of the effluents they yielded in 6 hours on aeration with raw sewage. The turbidity of the effluents ranged from 7 to 12 units (Klett-Summerson colorimeter with No. 42 filter) and the 3-minute and 4-hour permanganate values ranged from 1 to 3 mg./l. and 5 to 9 mg./l. respectively.

The sludge samples were allowed to settle for 15 minutes and then they were taken out and washed twice with tap water and once with distilled water. The water was removed by decantation and finally by centrifugation for 5 minutes. Weighed aliquots were homogenized for 10 minutes in a mortar with acid-washed sand, using various homogenizing media (Table I) at the rate of 80 ml. for 20 g. of sludge, and centrifuged at 1475 × g. for 15 minutes. The supernatant liquids were assayed for catalase activity by the method of Euler *et al.*[20] All the operations were carried out at 0° C. The protein content was determined by the method of Lowry *et al.*[21]

Assay of the Enzyme

The reaction mixture for catalase assay consisted of 2·5 ml. of 0·2 M sodium phosphate buffer pH 7·0, 2·5 ml. of 0·2 N (250 μ moles) hydrogen peroxide (Merck) and 1 ml. of enzyme preparation. The reaction mixture was incubated at room temperature (26° to 28° C.) for 15 minutes, and the reaction was arrested by adding 5 ml. of 2 N sulphuric acid and the residual hydrogen peroxide was titrated against 0·1 N potassium permanganate. The specific activity was expressed as μ moles of hydrogen peroxide decomposed per mg. protein under the assay conditions.

Table I

Effect of different homogenizing media on the extraction of catalase from activated sludge

Homogenizing media	Catalase activity (μ moles H_2O_2 decomposed)
Distilled water ..	5*
Ethanol, 30% ..	10*
Distilled water ..	115
Sodium phosphate buffer, M/15 pH 7·0	115
Ethanol, 30% ..	185
Acetone, 30% ..	175

* These results were obtained when the sludge samples were merely shaken with distilled water and 30% ethanol (without homogenization).

Factors Influencing the Catalase Activity in the Sludge

Effect of Different Homogenizing Media on the Extraction of Enzyme.—The sludge samples were shaken separately with water and with 30% ethanol at 20% level for 10 minutes and the supernatant liquids were tested for catalase activity. The activity in the liquid was negligible. When, however, the sludge samples were homogenized with (*a*) water, (*b*) sodium phosphate buffer, and varying concentrations of (*c*) ethanol, and (*d*) acetone, the enzyme activity was observed, and it was maximum with 30% ethanol (Table I). These observations show that activated sludge contains catalase and that its activity is apparently inside the cells of the associated organisms.

Effect of Hydrogen Ion Concentration.—The catalase activity in the sludge samples was followed, using appropriate buffers, at different pH values, 2·7 to 11·0 (Fig. 1). The buffers used were: citrate-phosphate (0·2 M) for pH 2 to 5; sodium phosphate (0·2 M) for pH 6 to 8; and sodium bicarbonate-sodium carbonate (0·2 M) for pH 9 to 11. The enzyme activity was appreciable in the range of pH 5 to 9, the optimum pH being 7·0. It may be pointed out in this connection that one of the conditions for the efficient operation of the activated sludge process is pH around 7·0.[1,22]

Effect of Substrate Concentration.—The influence of varying concentration of the sub-

strate (hydrogen peroxide) on the activity of the enzyme was studied and 500 μM of the substrate was found optimal. Above this concentration, the activity of the enzyme decreased to a certain extent and then remained constant (Fig. 2).

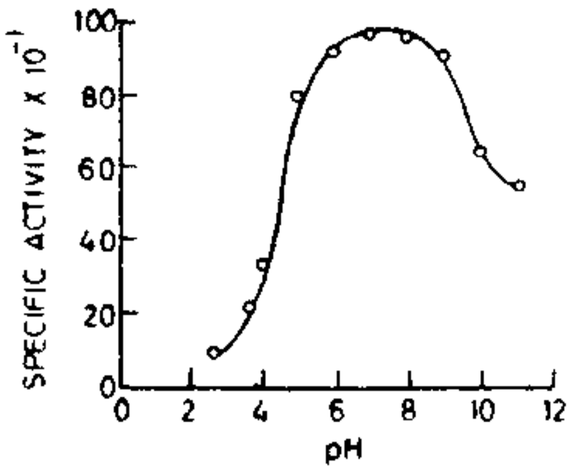

FIG. 1

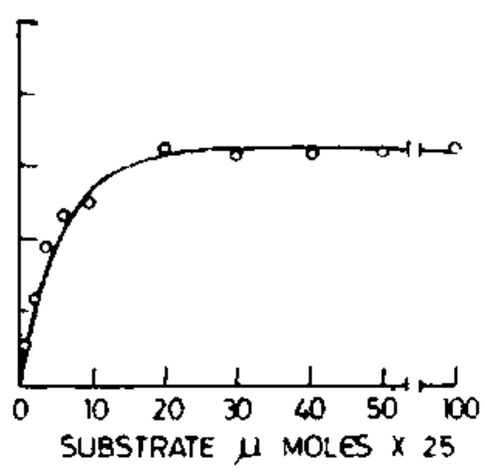

FIG. 2

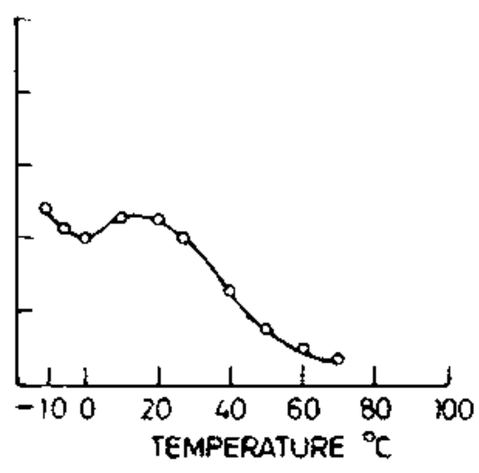

FIG 4

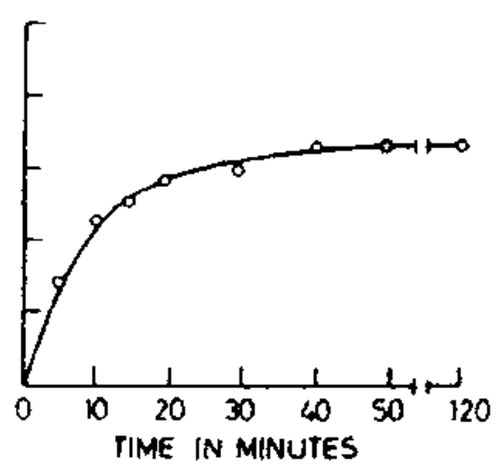

FIG. 5

Effect of Enzyme Concentration.—The activity of the enzyme at varying concentration, from 0·1 to 5 ml, was studied. There was increased activity of the enzyme with increasing concentration, like other enzymes (Fig. 3).

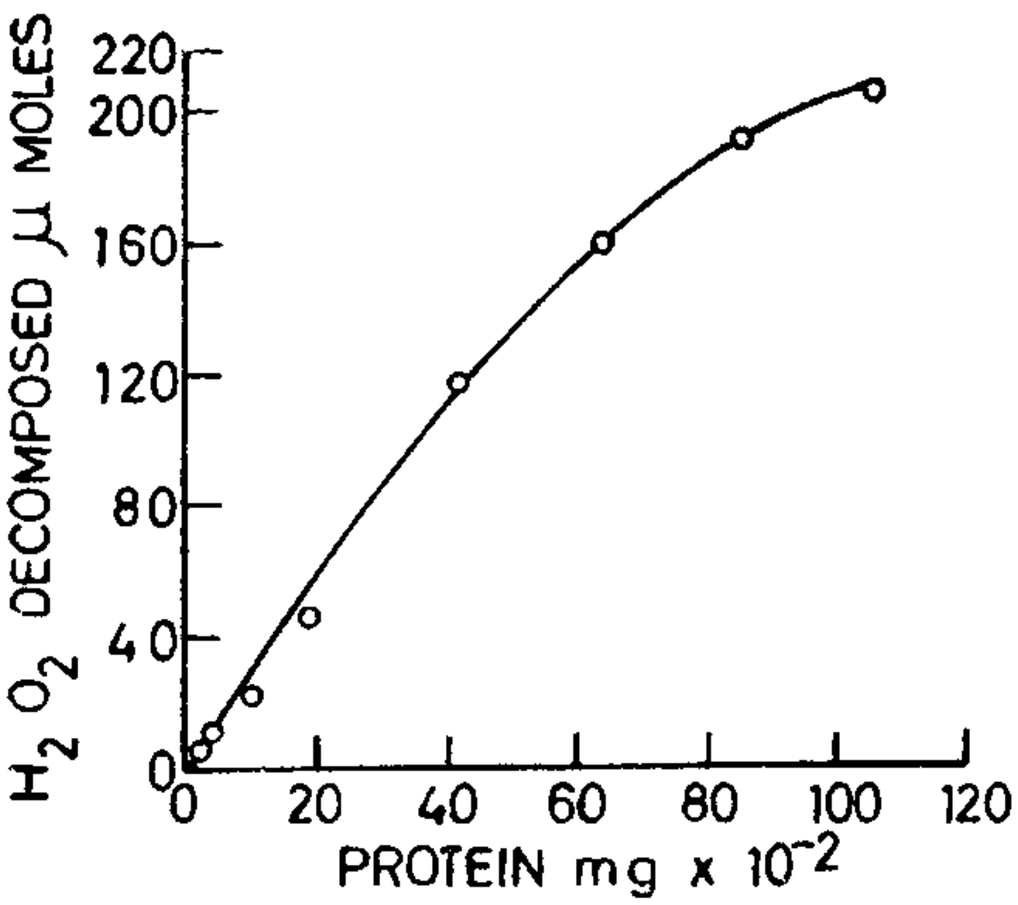

FIG. 3

Effect of Temperature.—The catalase activity at different temperatures, from — 10° C. to 90° C., was followed (Fig. 4). The maximum activity was found between — 10° C. and 20° C. and then as the temperature increased, the activity decreased. The enzyme was completely inactivated at 90° C.

In this connection it may be useful to refer to some of the reports on the effect of temperature on the activity of catalase from different sources. Stoland and Walling[23] reported that catalase seemed to differ from other enzymes of the animal body, as it did not show optimum activity at body temperature. Margulis *et al.*[24] observed that the maximum activity of catalase was between 0° and 10° C. and that inactivation of the enzyme began at 40° C. Liotti,[25] working with blood and tissues of several plant and animal species, recorded that the catalase activity at pH 7·0 increased with decreasing temperature.

Effect of Drying Activated Sludge on Its Catalase Activity.—The excess activated sludge from the plant at the Institute is generally dried in the sun in a specially constructed bed with cement floor for using it as a manure and as a feed supplement for chicks. The sun-dried sludge material did not show any catalase activity.

Time Course Reaction.—The reaction mixtures were incubated at room temperature for varying period up to 2 hours. At different intervals the catalase activity was measured. After incubation for 40 minutes the reaction rate remained the same (Fig. 5). This may perhaps be due to the inhibition of the enzyme activity by hydrogen peroxide on prolonged exposure.

Effect of Inhibitors.—The effect of the following inhibitors on the enzyme activity was studied : sodium azide, mercuric chloride, copper sulphate and cobalt nitrate at varying concentrations, 0 to 500 μ moles (Table II). Sodium

TABLE II

Effect of different inhibitors on the catalase activity

Inhibitor concentration (μ moles)	Specific activity of catalase (per cent.)			
	$HgCl_2$	NaN_3	$CuSO_4$	$Co(NO_3)_2$
0	100·0	100·0	100·0	100·0
1	100·0	—	—	—
2	94·1	—	—	—
10	76·4	82·3	—	—
20	58·7	29·3	—	—
50	—	—	—	100·0
100	29·3	11·7	100·0	94·1
200	Nil	Nil	88·2	88·0
300	—	—	82·3	76·4
400	—	—	76·4	76·4
500	—	—	58·7	58·7

azide and mercuric chloride at a concentration of 200 μ moles completely inactivated the enzyme. At this concentration copper sulphate and cobalt nitrate had little effect on the enzyme activity. As the concentrations of copper sulphate and cobalt nitrate were increased, the enzyme activity decreased, although this decrease was not much, even with 500 μ moles.

Quality of Sludge and Catalase Activity.—A large number of samples of activated sludge and sludge samples from septic tank were examined for catalase activity. The efficiency of these sludges, as reflected in the quality of effluents given by the sludges on aeration with raw sewage for 6 hours, was studied (Table III). The sample of activated sludge which purified sewage to a greater extent showed more catalase activity. The sludge from septic tank did not show any catalase activity.

TABLE III

Catalase activity and quality of effluents yielded by different samples of activated sludge

Activated sludge sample	Catalase activity in sludge	Quality of effluent		
		T	3-min. P.V.	4-hr. P.V.
1	384	8	2	9
2	277	18	3	12
3	255	22	5	14
4	252	35	9	18

T = Values for turbidity; 3-min. and 4-hr. P.V. = 3-minute and 4-hour permanganate values.

Summary and Conclusions

The demonstration of catalase activity in healthy activated sludge produced from domestic sewage is of considerable interest, particularly in the investigation of the biological mechanism of purification of sewage. The relatively high activity of the enzyme in the sludge samples which purified sewage more rapidly, the correlation of the enzyme activity with the quality of the sludge and the absence of the enzyme activity in the sludge samples from septic tank lend some valuable evidence on the biochemical nature of the aerobic purification process.

The enzyme from activated sludge, like that from other sources, showed activity at a wide range of pH. The optimum pH for the sludge catalase was found to be at 7·0, and it is around this pH that the activated sludge process operates efficiently. Again, the effect of temperature on the sludge catalase is also similar to that on the catalase from other sources, but the significance of maximal activity at lower temperatures is not clear. Samples of sludge dried in the sun did not show any catalase activity.

It has been shown that the catalase activity in the sludge material is located inside the cells of the organisms and that it is not extracellular. Isolation of the dominant organisms from activated sludge and their examination for catalase activity with special reference to their relative influence on the purification process would throw further light on the mechanism involved.

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