"SECTION - B"

STUDIES ON CATALASE ACTIVITY AND ITS RELATION TO CERTAIN IMPORTANT WASTE WATER PARAMETERS:

INTRODUCTION

Microorganisms are basically responsible for stabilising decomposing organic matter of waste waters in the three principal methods of waste treatment known today. It is also well-known that the removal of dissolved organic matter is achieved partly by complete bacterial oxidation and mostly by cellular synthesis. Various methods have been used to evaluate the latter in these treatment systems.

Monod (1942) found the growth of aerobic bacteria to be directly proportional to the total quantity of substrate used up. He expressed the growth yield in terms of dry bacterial weight per unit of substrate used. Another method is the determination of volatile suspended matter in anaerobic fermentation systems (Stewart 1958, Andrews et al 1964, Lawrence and McCarty 1967, and Tourien et al 1967). But this method of estimation is applicable only to systems fed essentially with soluble substrates (Agardy et al 1963). A third method is to measure the elementary cellular constituents like C, N and P (Agardy et al 1963, Lawrence and McCarty 1967). In all these cases no attempt is made to

distinguish between living and dead cells.

Micro-organisms can be expressed also in terms of the number of living cells per unit volume but such determinations are not possible in anaerobic fermentation systems. Holm (1957), Herbert (1959,1961) have shown that the average size of the cells and the formation of intracellular storage products may vary considerably during the assimilatory and endogenous phases, so that expressing growth in terms of mass or number is not always equivalent. But Herbert (1961) found that the DNA content of bacterial cells was fairly constant under a wide range of growth conditions. In support of the above Agardy et al (1963) used DNA as an active parameter in digesters and Hattingh et al (1967) found a good correlation between DNA content of the cells and volatile suspended solids in anaerobic fermenters receiving soluble substrates.

In the light of the above facts it is surprising to note that the design of the treatment units like the activated sludge process is still being based largely on pilot plant studies because of the limitations of the BOD_5 tests, which neither furnishes the rate of oxygen demand nor the demands of a large concentration of biologically active sludge. So, for a proper design and effective operation of aerobic biological waste treatment

systems, it is necessary to know the actual number of micro-organisms or their enzymatic activity at any time.

Again, experience in aquatic microbiology shows that the method for evaluating the potential activity of specific metabolic groups of the natural microflora is based on a bio-assay technique, in which the increase of the metabolic activity after the addition of selected specific substrates like glucose or acetate to samples of waste water is measured. These methods are often preferable to the usual methods of counting these bacteria in neutral media. The maximum velocity-uptake is an useful indicator of the heterogenous activity and a relative measure of bacterial population (Wright and Hobbie 1965).

Sridhar and Pillai (1965, 1966, 1969-a, 1969-b and 1969-c) have done a good amount of work in the field of waste water treatment using catalase activity as a parameter for estimating organic impurity in sewage, waste water and effluents qualitatively.

Data relating to enzyme activities in waste water treatment systems are very meagre. So, for a rational design of any waste treatment unit, it is necessary to evaluate the relationship existing between organic loadings, removal and the quantity along with the

"unit activity" of the organisms present in the system (Agardy 1964). The development of a well-tested analytical enzymatic procedure is necessary at the present time. So, an attempt has been made to determine the catalase activity quantitatively and to correlate it with three important waste water parameters - BOD_5, COD and turbidity.

Methods

Sewage samples were drawn from different situations like upper channel, lower channel and continuous flow settling tank (CFST) effluents of the Wadi Sewage Disposal Works at Baroda. They were tested for turbidity at 420 m/u, BOD_5 at 20°C, COD and catalase activity:

1) Catalase activity:- It was determined by Euler and Josephson's method (1927). 250 /u moles of H_2O_2 (25ml of 0.01 M H_2O_2 pH 7.0) were added to 25 ml of the sample and incubated for one hour at room temperature (26° to 28°C). 250 /u moles of H_2O_2 added to 25 ml of distilled water was run as blank. After one hour the reaction was stopped by the addition of 5 ml of 2N H_2SO_4 and the left-over H_2O_2 was titrated against 0.1M $KMnO_4$.

   The enzyme activity is expressed in /u moles of H_2O_2 per 100 ml per hour.

2) COD:- It was determined by dichromate reflux method (Standard methods 13th Ed. p.495).

3) Turbidity:- It was measured at 420 mμ using distilled water as blank in Klett-Summerson Colorimeter (Sridhar and Pillai).

4) BOD_5 at 20°C:- It was estimated by Winkler's azide modification (Standard methods 13th Ed.).

Results

The results of the tests carried out are shown in the Table -7. Upper channel samples showed more activity than CFST effluents while lower channel samples showed more activity than CFST effluents. Upper channel sample represented the raw sewage pumped from the city and the lower channel sample was a mixture of CFST effluents and the raw sewage pumped from the city. Also, the upper channel samples showed the highest turbidity, BOD_5 and COD while the CFST effluents showed the lowest turbidity, BOD_5 and COD, when compared with the samples studied. Thus the enzyme activity in the samples seem to run almost parallel to turbidity, COD and BOD_5.

So, an attempt was made to find out relation between respective values of catalase activity and turbidity,

Fig. 27

RELATION BETWEEN CATALASE ACTIVITY AND TURBIDITY WITH BARODA RAW SEWAGE

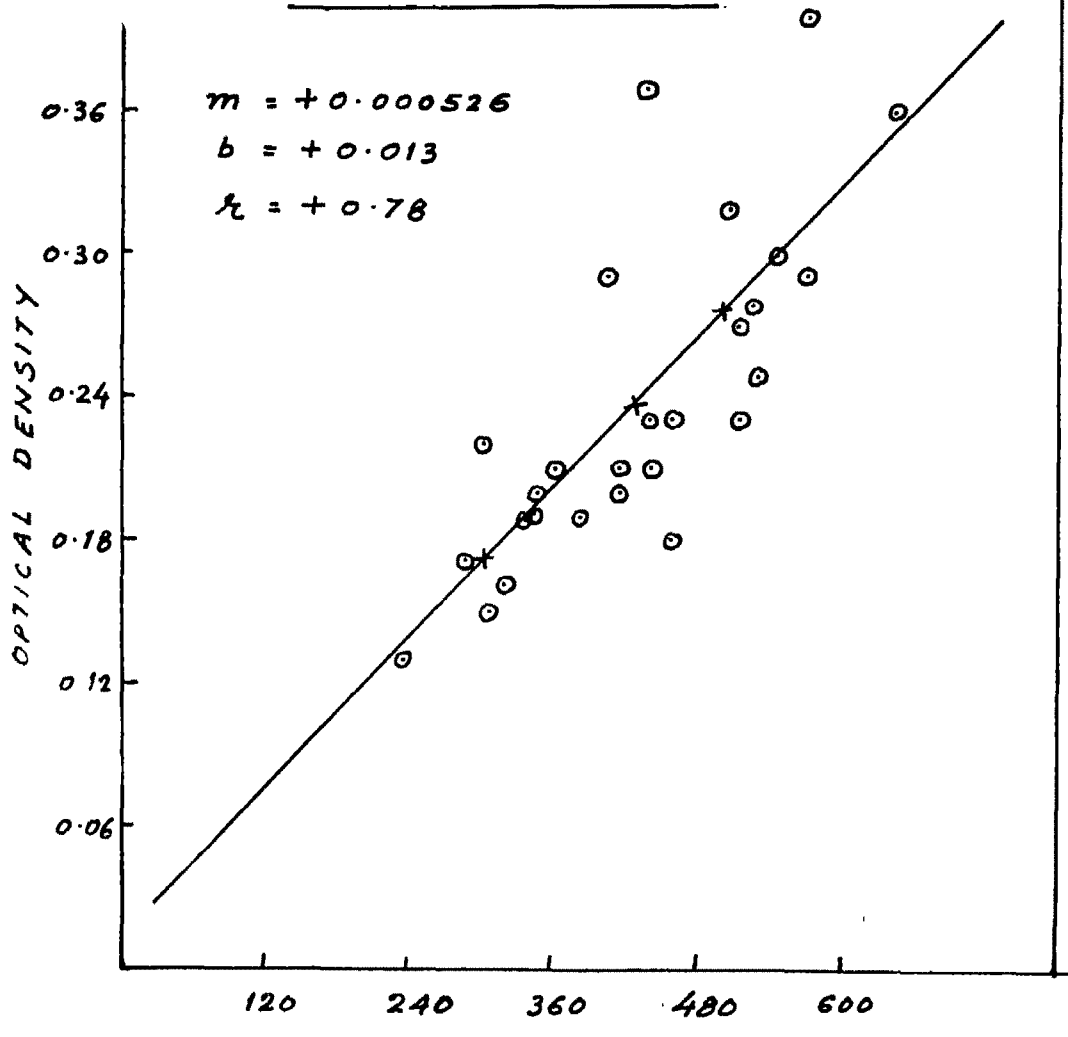
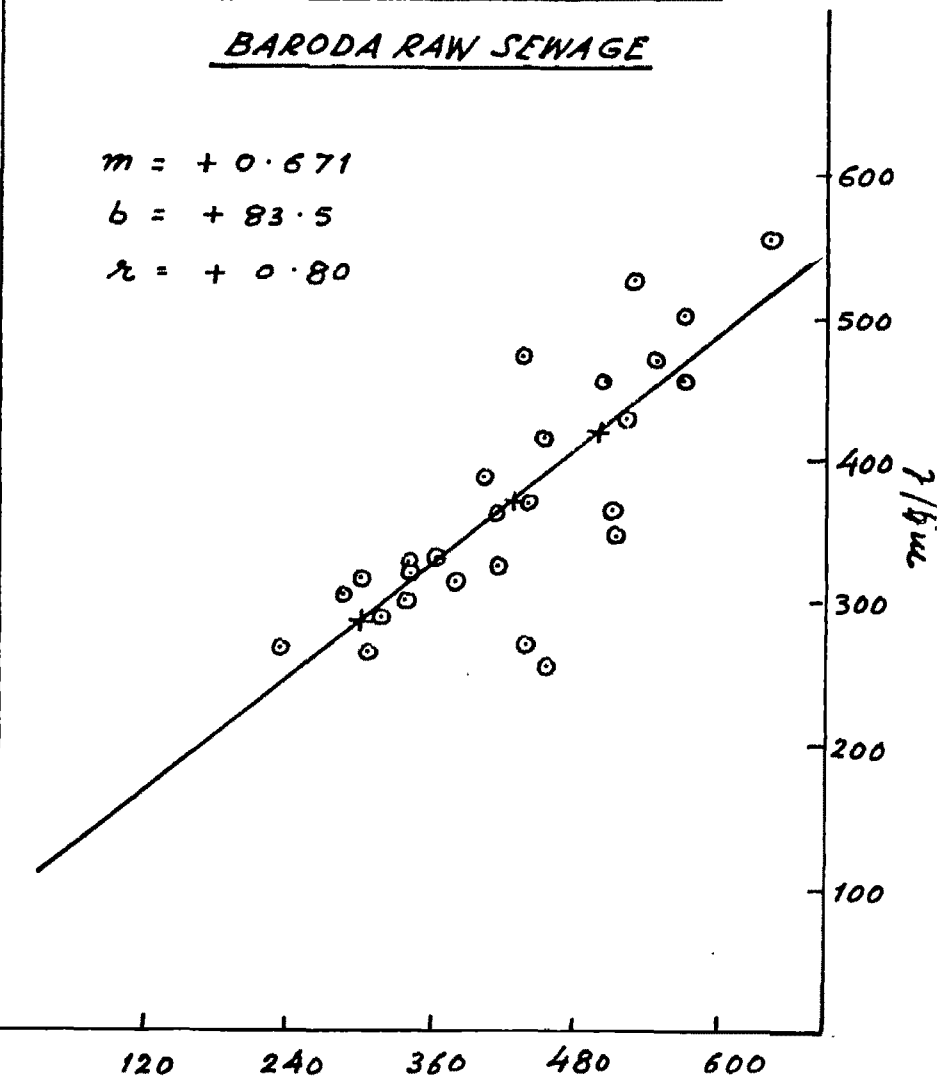


Fig. 28

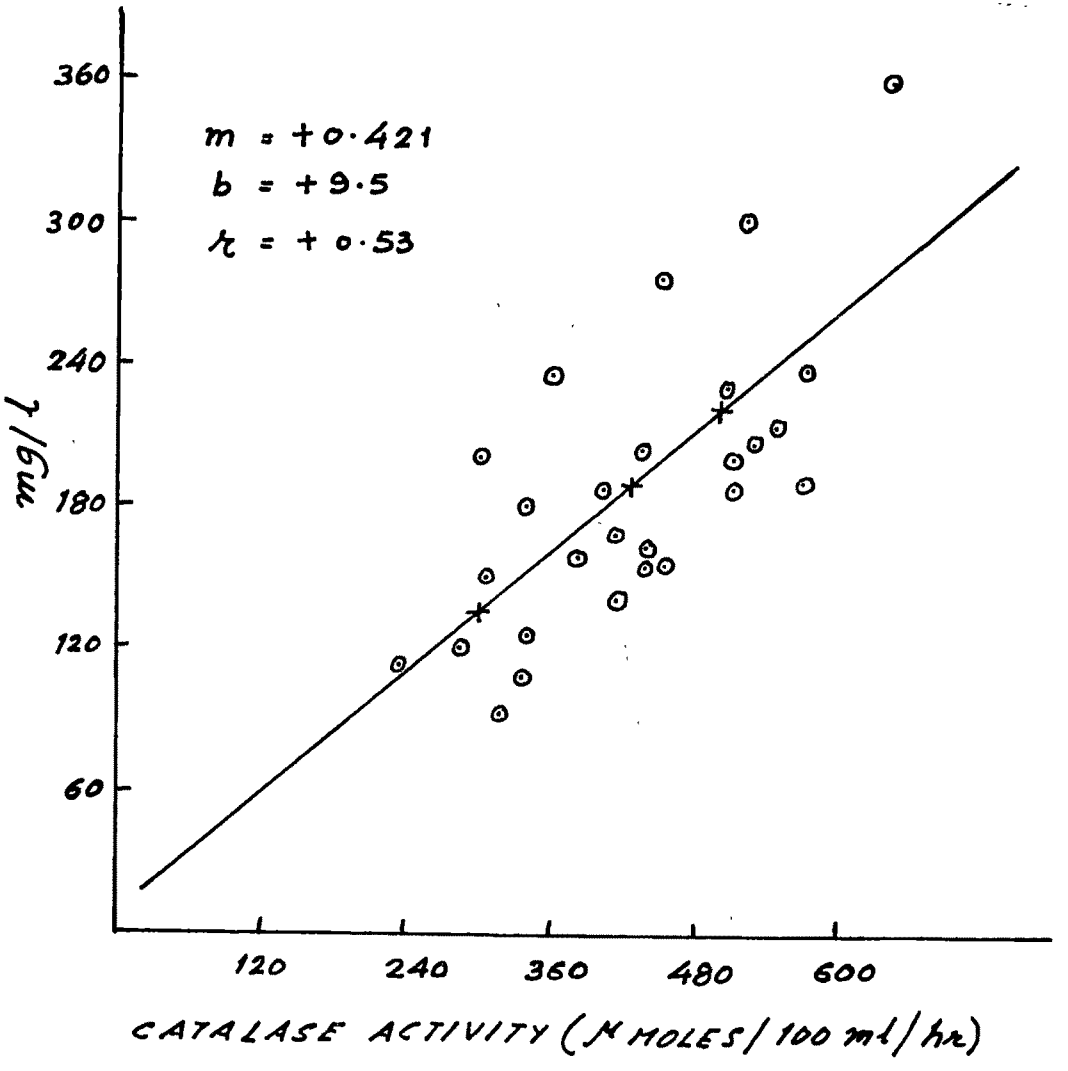
RELATION BETWEEN CATALASE ACTIVITY AND C·O·D. WITH BARODA RAW SEWAGE



CATALASE ACTIVITY (M MOLES / 100 ml / hr)

Fig. 29

RELATION BETWEEN CATALASE ACTIVITY AND B.O.D. WITH BARODA RAW SEWAGE



catalase activity and COD, and catalase activity and BOD_5 by the regression analysis. Values of m and b were calculated according to the least square method for each pair of values. (Standard methods 13th Ed.). Also, Pearson's correlation coefficient "r" was calculated for each pair (Fischer, 1970).

The values of m, b and r thus obtained are shown below:

Parameters :	'm'	'b'	'r'
Turbidity	+0.000526	+ 0.013	+ 0.78
COD	+0.671	+83.5	+ 0.80
BOD_5 at 20°C	+0.421	+ 9.5	+ 0.53

From the above table it will be seen that the correlation coefficient, r, is greater than 0.5 in all the three cases. The correlation coefficient for COD and turbidity are far greater than that for BOD_5.

With the help of 'm' and 'b' values regression lines were drawn and all the values were plotted on the graph for each pair of values. The regression lines are shown in Figs. 27,28,29.

Discussion

All organic reactions taking place in biological treatment systems are enzyme-catalyzed and the enzymes

are both oxidising and reducing. The oxidising enzyme, catalase, is known to be present in all aerobic living organisms (Sumner 1947, Thorell 1951) and has the capacity to reduce hydrogen peroxide. Weetall et al (1965) found a reasonable correlation between catalase activity and bacterial content of solids. The determination of catalase activity is a rapid and sensitive test and can be used in studying organic pollution or saprobicity in lakes and streams. So, it can be used for evaluating accurately and quickly the biochemical conditions existing in any waste water treatment system for checking their efficiency.