

INVESTIGATIONS INTO THE MECHANISMS OF INTERACTION BETWEEN TO MICROBIAL POPULATIONS IN ACTIVATED SLUDGE

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INTRODUCTION

The A/O process for waste water treatment places strong selective pressures on the microbial community of the activated sludge. These pressures result in domination of the sludge by two microbial populations. One of these populations actively accumulates and stores phosphate from the wastewater. The other population has a marked tendency to form large flocs. This second population gives the sludge excellent settling characteristics; the former insures that the effluent water will be low in phosphate.

In studying the characteristics of isolates from each population, it was found that the phosphate accumulators were unable to grow on glucose as the sole source of carbon and energy, but that the clumping organism could. This information, coupled with the knowledge that glucose is a major component of the influent wastewater, led to the hypothesis that a "cross-feeding" phenomenon occurs in the system, with the "clumper" supplying a glucose metabolite which could be used for growth by the phosphate accumulator. Support for this hypothesis has come from observations of growth of the phosphate accumulators in mixed culture with the clumper when glucose was supplied as the only carbon source.

The aim of this study was to supply a quantitative data base which could be applied to the study of the interactions between these two populations. This includes an evaluation of the abilities of isolates to utilize carbohydrates supplied in the culture medium and an assessment of the ability of a phosphate accumulating isolate to grow on metabolites of glucose produced during clumper growth on minimal media. Also, an attempt was made to identify the products of glucose metabolism in a floc-forming isolate. Finally, some preliminary experiments were conducted with the aim of characterizing the nature of the interactions involved in floc-formation.

METHODS

I. Glucose Utilization by Clumper (CH-1) and Isolate RS-1

CH-1 or RS-1 were inoculated into 50 ml of minimal medium-containing either glucose or a mixed carbon source. Cells were harvested at appropriate intervals by filtration through pre-dried, tared 0.2 μ Gelman membrane filters. Total particulate dry weight was determined by drying the filters for two to three hrs. The filtrate was assayed for glucose using the diphenylamine reagent. Particulate protein was determined using the Bio-Rad Protein Reagent.

The minimal medium used throughout the work described here consisted of the following per liter: NH_4Cl (56 mg), NaHCO_3 (337.5 mg), K_2SO_4 (30.2 mg), K_2HPO_4 (27.9 mg), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (99.3 mg), CaCl_2 (34.4 mg) and FeCl_3 (unknown, but high). This basal salts medium was supplemented either with glucose (143 mg/l) or a mixed organics preparation. The mixed organics consisted of per liter: sucrose (20.2 mg), glucose (31.8 mg), glutamic acid (20.3 mg), Na-citrate- $2\text{H}_2\text{O}$ (36.2 mg), Na-acetate (16.8 mg) and triacetin (18.1 mg).

II. Growth of RS-1 on CH-1 Filtrate

This experiment was conducted to test the hypothesis that CH-1, when grown on glucose only, is capable of producing a metabolite which can be used by RS-1 for growth. The incorporation of ^3H -thymidine by RS-1 was used as the measure of growth. The experiment was conducted as follows:

Five 500 ml DeLong flasks containing 50 ml of minimal media with glucose only as the carbon source were inoculated with .1 ml of an overnight culture of CH-1. The flasks were harvested by filtration (0.45 μ Gelman membrane filters) at six hr intervals. The filtrate was saved and the total particulate dry weight was determined. The glucose concentration of the filtrate was determined.

After all of the flasks had been harvested, triplicate 10 ml aliquots of the culture filtrate were transferred into sterile 25 ml Erlenmeyer flasks with filter sterilization through 0.2 μ filters. Triplicate controls of sterile glucose-only minimal media were also prepared (the equivalent of time zero samples except that they were not filtered). 1.0 μCi of ^3H -thymidine was added to each flask before inoculation with 0.3 ml of stationary phase RS-1 culture (grown on minimal media with mixed organics). The flasks were incubated for 30 hrs, then harvested by filtration through 0.2 μ Gelman membrane filters. The particulate radioactivity was then determined.

III. Determination of Volatile Fatty Acid (VFA) Production

VFA production was assayed for by acidifying a 50 ml sample of culture filtrate to pH2 with concentrated HCl. The acidified samples were extracted with 4x5ml volumes of diethyl ether. The ether fractions were pooled and dried by addition of a small amount of MgSO_4 . The ether was analyzed for VFA's by gas chromatography using a Resoflex column with thermal conductivity detection.

IV. Dissociation of CH-1 Floccs

The degree of dissociation of CH-1 floccs which occurred when they were treated with Tween 80, EDTA or DMSO was measured in an attempt to characterize the nature of the surface interactions. The settling characteristics of a preparation were used as a measure of clump dissociation. Settling was measured by transferring 1 ml sample to a 1.5 ml cuvette and suspending it by inverting several times. The initial OD was read at 680 nm in a Beckman DBG T Spectrophotometer. The sample was allowed to settle in the cuvette for 2 hrs and the final OD was read. Settling was expressed as the Settling Index (SI).

$$SI = \frac{OD_{\text{initial}} - OD_{\text{final}}}{OD_{\text{initial}}}$$

More efficient settling (indicating the presence of clumps) is represented by high values for SI. Low values indicate that less settling has occurred and that the clumps have dissociated somewhat.

RESULTS AND DISCUSSION

I. Glucose Utilization and Growth of RS-1

When RS-1 was inoculated into minimal medium containing glucose as the sole source of carbon and energy, no growth occurred. Also, no glucose was removed from solution over the 30 hr period of the experiment. Although these

results are by far the "noisiest" of all of the experiments, comparison of the initial (0 hrs) and final (30 hrs) values for both particulate dry weight and glucose concentration support the conclusion that RS-1 neither grows nor removes glucose from solution when glucose is supplied as the sole carbon source.

The profile of growth and glucose utilization when RS-1 is grown on minimal media with a mixed source of carbon and energy is shown in Figure 1B. Yield coefficients have been calculated considering both the total organics as substrate and excluding the sucrose and glucose from this calculation. The results are shown in Table 1.

The yield coefficient (Y) is calculated as follows):

$$Y = \frac{x_{\text{final}} - x_{\text{initial}}}{s} \quad \text{where: } \begin{array}{l} x = \text{particulate dry weight (mg)} \\ s = \text{substrate concentration (mg)} \end{array}$$

Table 1:

Organism	Y_{glucose}	$Y_{\text{mixed total}} (s=143 \text{ mg})$	$Y_{\text{mixed-gluc+suc}} (s=91.4 \text{ mg})$
RS-1	0	0.11	0.17
CH-1	0.26	0.32	---

The profile of glucose utilization shown in Figure 1B is surprising, based on the inability of RS-1 to either grow or remove glucose from the culture media shown above. Under these conditions, diphenylamine reactive material is definitely removed as RS-1 grows. It should be noted that glucose is not the only diphenylamine reactive compound in the mixed organics media -- sucrose will react as well. All other substrates present were tested for color production with the diphenylamine reagent, but none was detected. Also, the total diphenylamine reactive material, expressed as glucose equivalents, seems to be more than twice as high as it should be. The extinction coefficient of the sucrose-diphenylamine reaction product (which was not determined) may be much higher than that of the glucose-diphenylamine reaction product. If so the high value for glucose equivalents can be accounted for easily.

These results suggest that, whereas RS-1 cannot use glucose when it is present alone, the presence of other organic compounds may allow its uptake. It is not clear that RS-1 derives energy from glucose under these conditions, however. Perhaps an examination of the yield of RS-1 biomass when grown on the mixed organics media with and without glucose/sucrose will answer this question. If they extract additional energy from the carbohydrates, the biomass yield would be greater in their presence. If they do not, the yield should be unaffected by the presence of the compounds. Radiolabeled glucose could be used to determine if the sugar is being incorporated and/or respired.

II. Glucose Utilization and Growth of CH-1

Figure 2A shows the profiles of particulate dry weight increase, particulate protein increase and removal of glucose from solution when CH-1 is grown on minimal media with glucose as the sole source of carbon and energy. The lag in particulate protein increase relative to particulate dry weight increase may indicate unbalanced growth, if it is shown to be reproducible. Glucose removal seems to be more closely correlated with increase in particulate protein than with the dry weight increase. Again, this may indicate that initial growth is unbalanced, possibly resulting from cell wall

synthesis or storage of large amounts of carbohydrate polymer before the cells are ready to use the glucose in other aspects of metabolism.

The growth of CH-1 on a mixed organics carbon source is represented in Figure 2B. The increase in particulate protein more closely parallels the increase in particulate dry weight. This is more a result of a shorter lag period for protein increase than any change in the dry weight profile. The yield of CH-1 on the mixed organics substrate is significantly higher than on glucose only (see Table 1).

III. VFA Production by CH-1 Grown on Glucose

We could not demonstrate the production of acetate (or any other VFA's) by CH-1 when grown on glucose since the partition coefficient was very low ($K_d = \frac{[\text{acetate}]_{\text{ether}}}{[\text{acetate}]_{\text{aqueous}}} = 0.43$). Therefore, under the best of conditions, the detection limit is approximately 25 mM. The maximum amount of acetate which could be produced by complete conversion of glucose to acetate (through glycolysis: $1 \text{ C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{ C}_2\text{H}_3\text{O}_2\text{H}$) is 1.6 mM, well below the detection limit of the method. More sensitive methods for the detection of acetate should be utilized.

Figure 3B shows the growth of CH-1 as particulate dry weight increase and the removal of glucose from the culture filtrate. Figure 3A shows the incorporation of ^3H -thymidine into RS-1 grown on the culture filtrate. Filtrate was harvested at 0, 6, 12, 18, 24 and 30 hrs. The error bars show one standard deviation of the values from triplicate samples. Although the scatter about the mean values is relatively large, there is an indication that more growth occurred on filtrates from cultures in which CH-1 actually grew than on the control media.

The results of the dissociation experiments are shown in Figures 4 and 5. Tween 80 is the only compound which dissociates the flocs to any extent. Its maximum effect is at a low concentration. These results suggest that some of the forces involved are hydrophobic. DMSO, which should also disrupt hydrophobic forces, has no effect. EDTA, which should interfere with divalent cation dependent processes, also was ineffective in dissociating clumps.

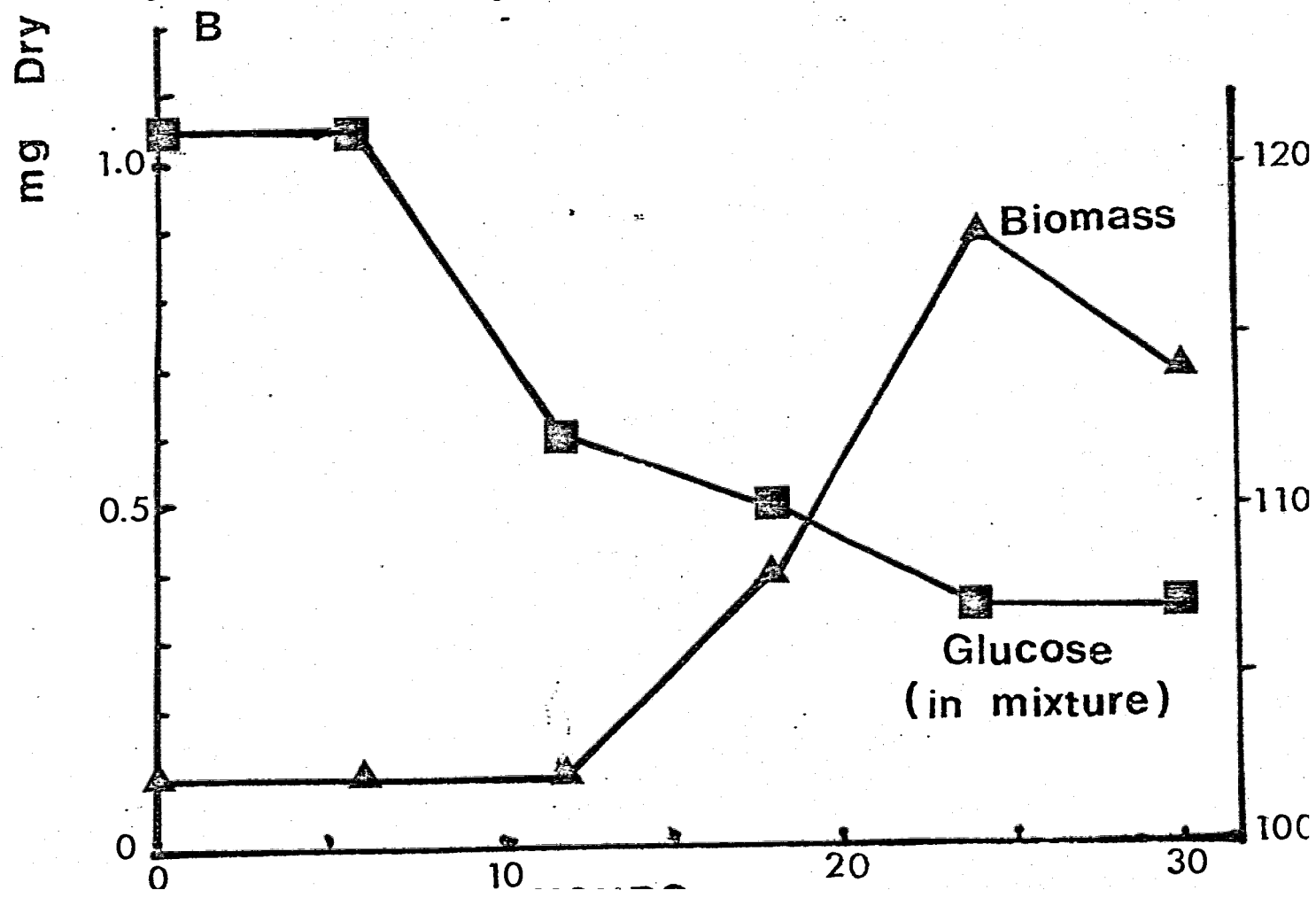
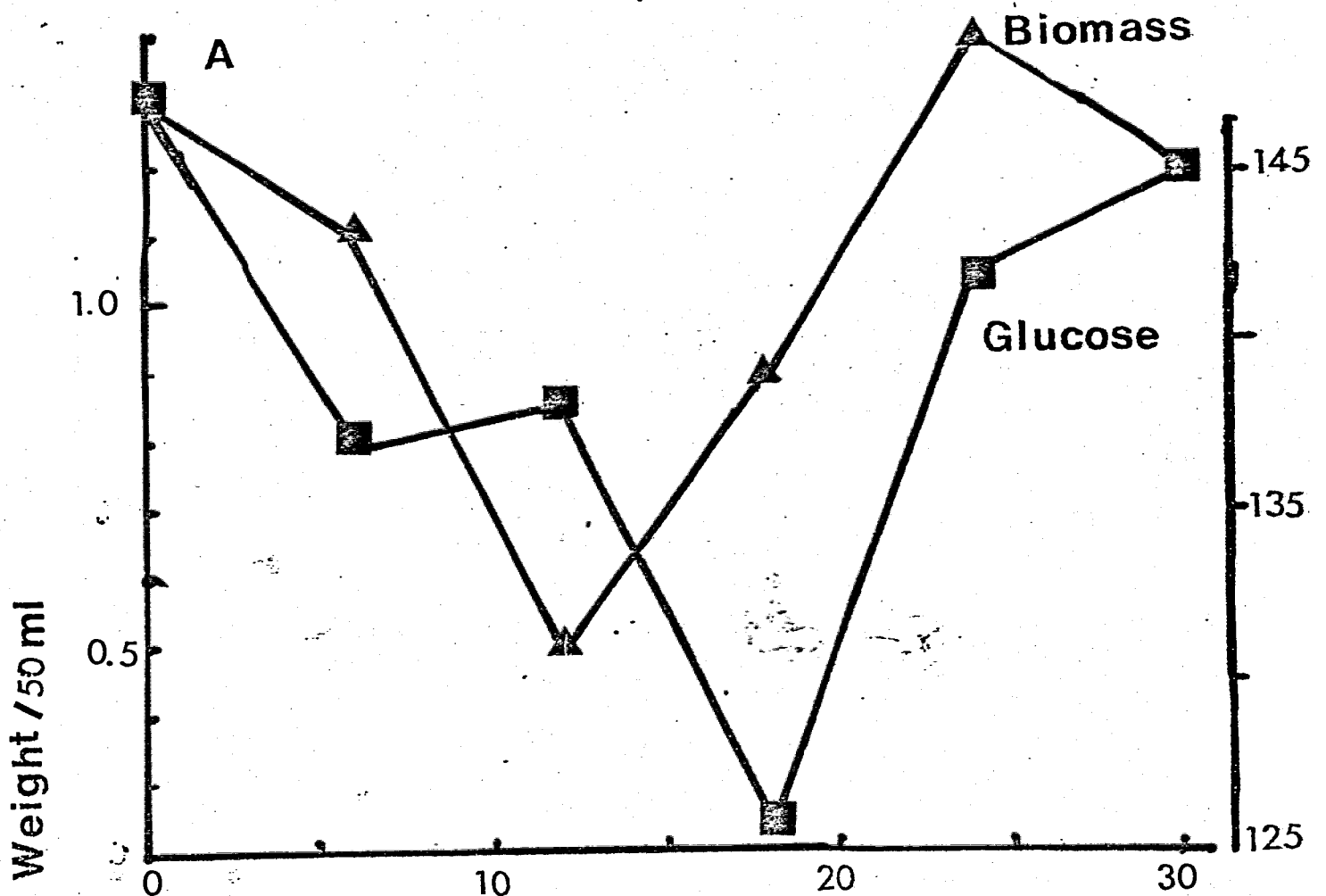
CH-1 was not tested for the ability to grow on Tween 80. It is conceivable that the observed effect of Tween 80 could have been due to stimulation of growth by the compound.

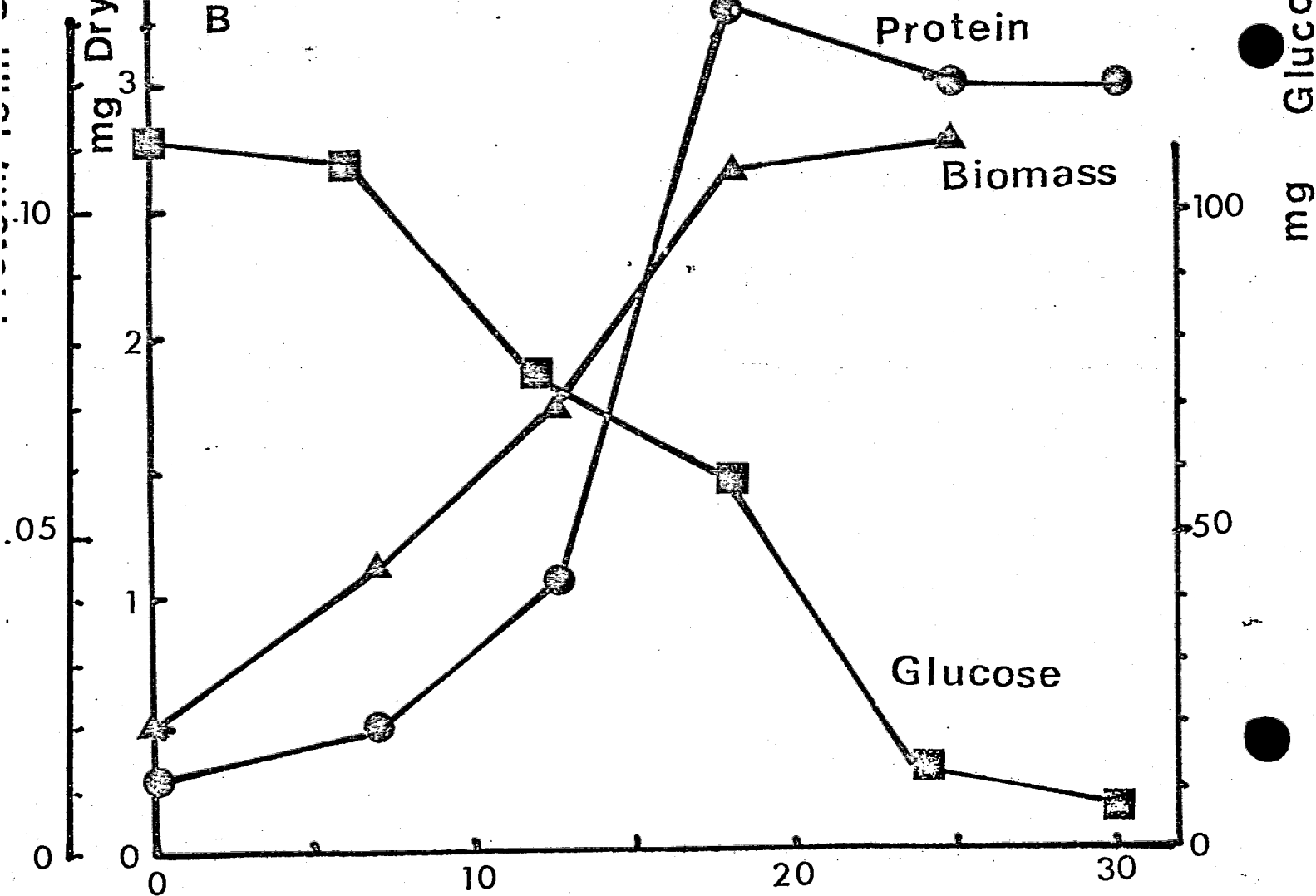
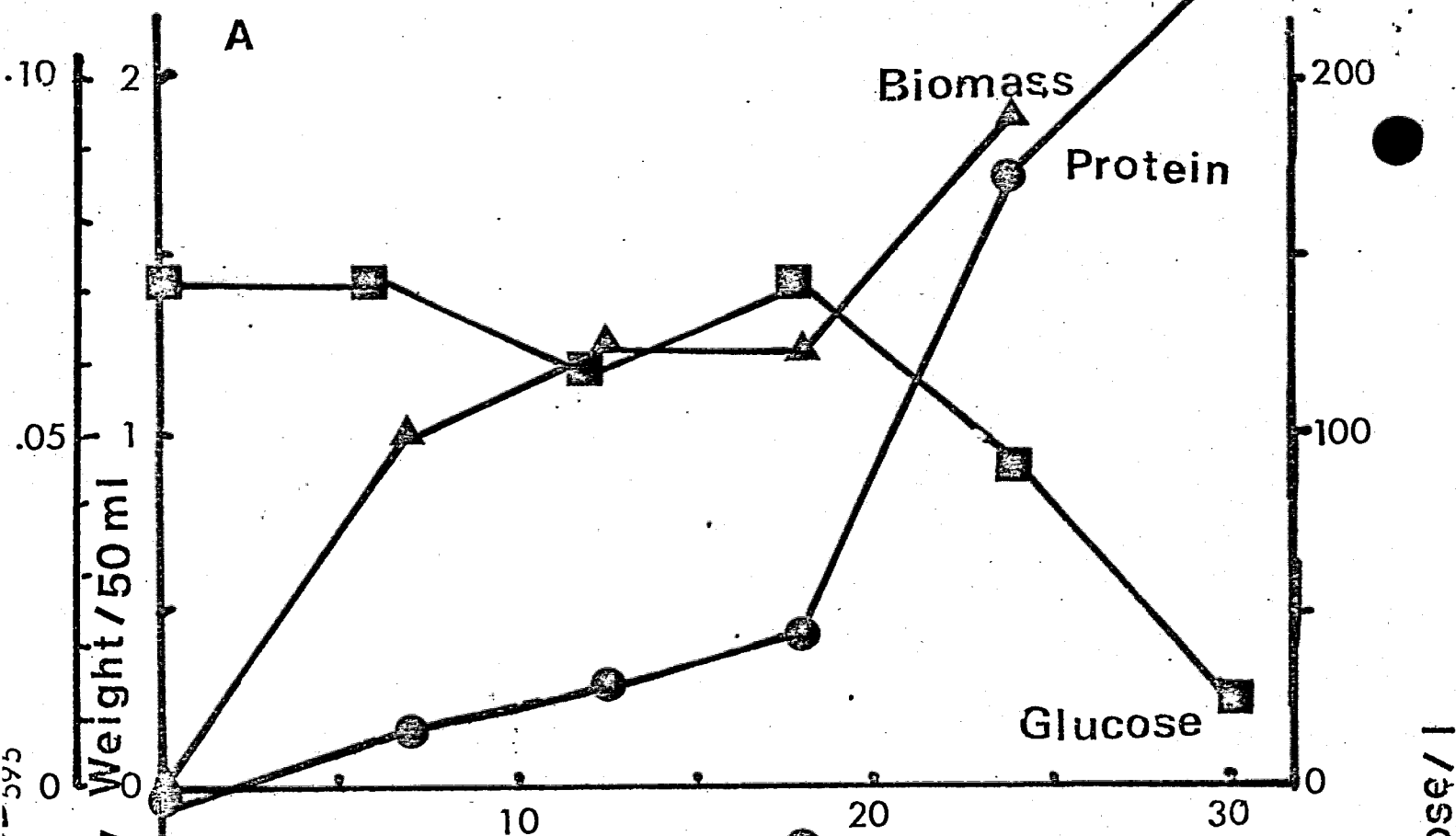
IV. Nature of the Cell Surface of CH-1

Preparations of CH-1 were stained with Alcian Blue for the positive visualization of any carbohydrate capsular material. Cells stained intensely with the Alcian Blue, but it made no difference whether the cells were from clumped or unclumped cultures. The capsule appeared to be of uniform thickness.

In an attempt to isolate capsular material, the cells were boiled for about 15 min in phosphate buffer. The cells were removed by centrifugation and the supernatant was saved. This supernatant liquid contained a high MW (greater than 5000 daltons) material which was anthrone-positive. This material could be precipitated by adding ethanol to a final concentration of 70%. On this basis it was concluded that the material was carbohydrate.

figure 1





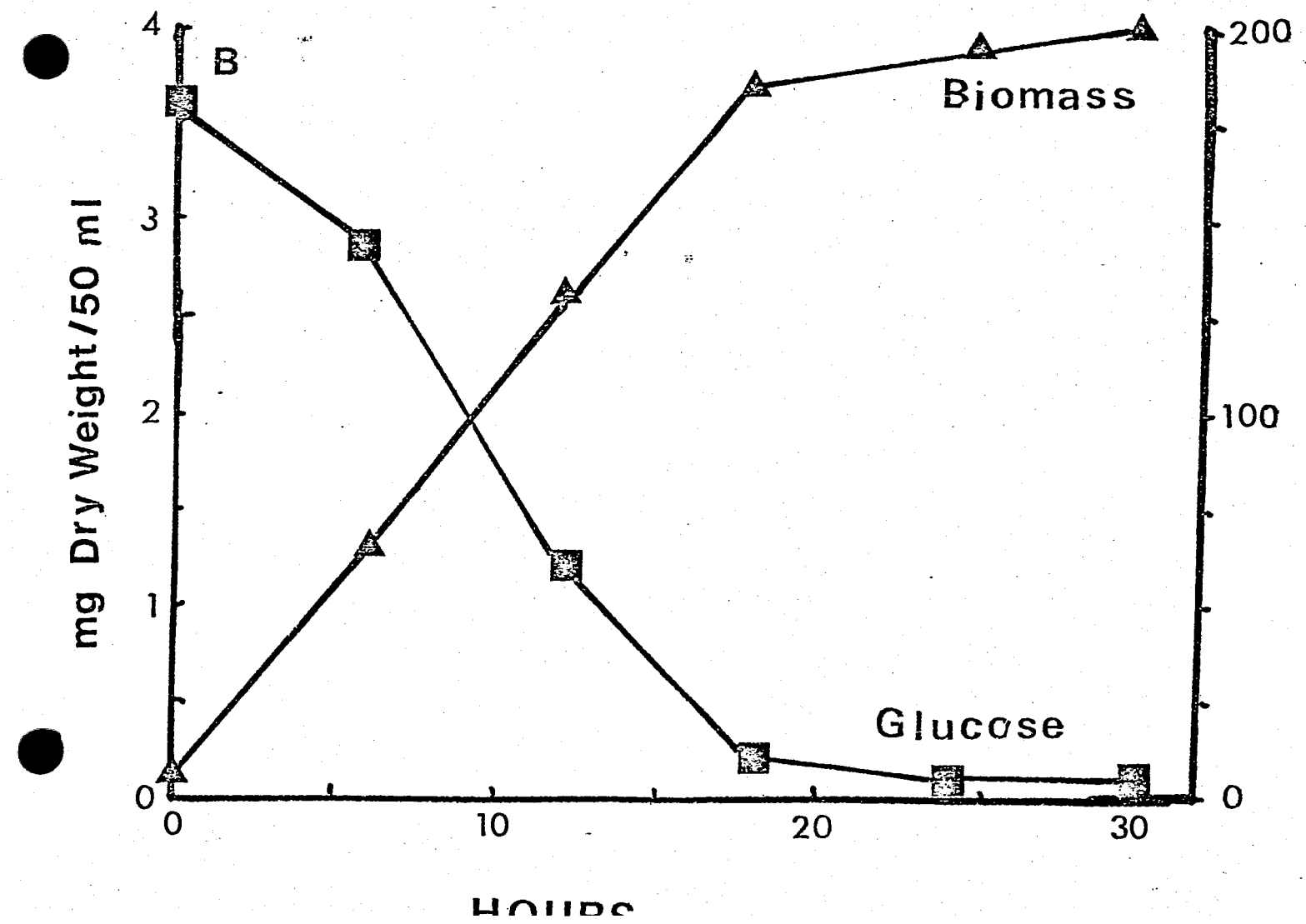
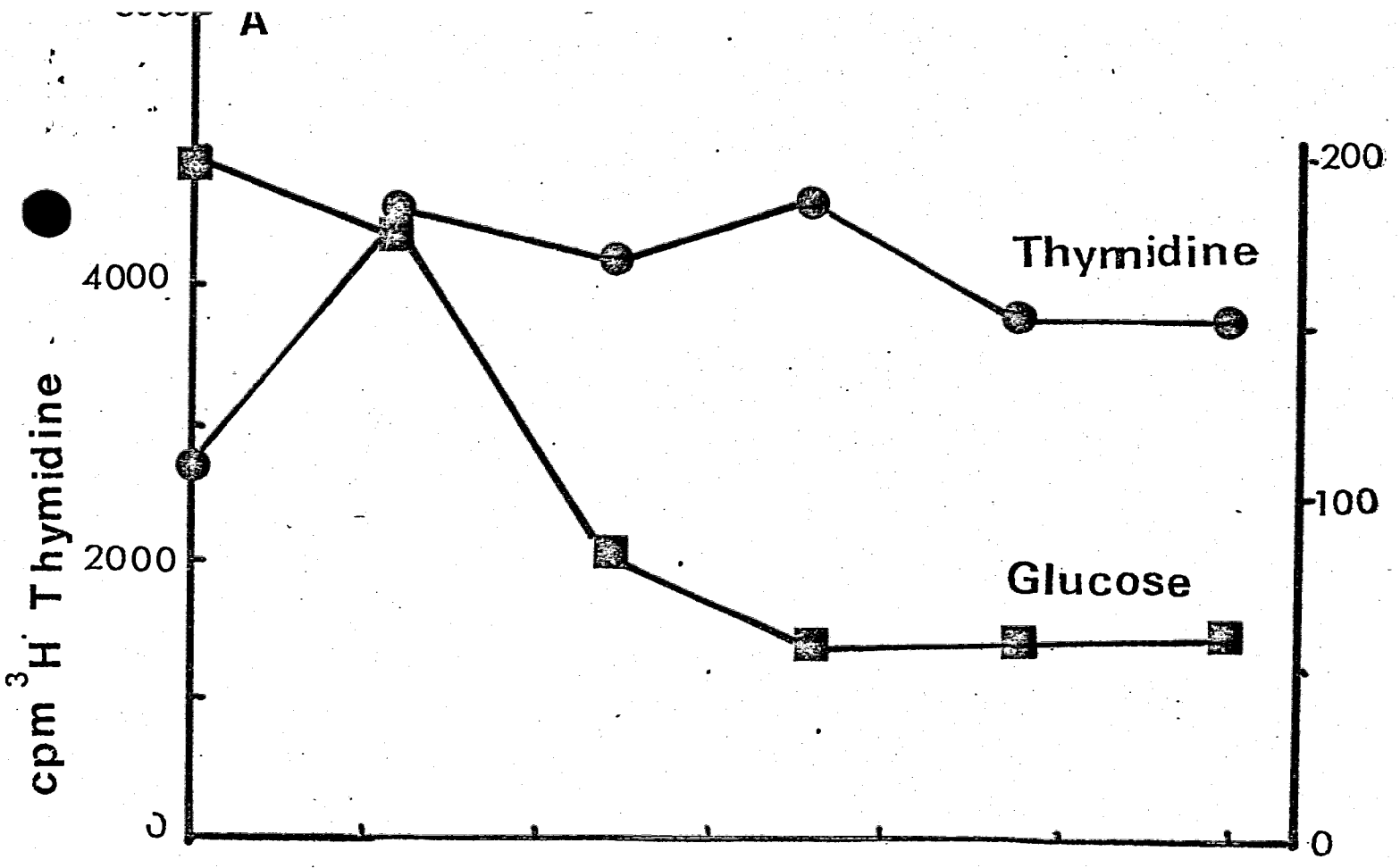


Figure 4

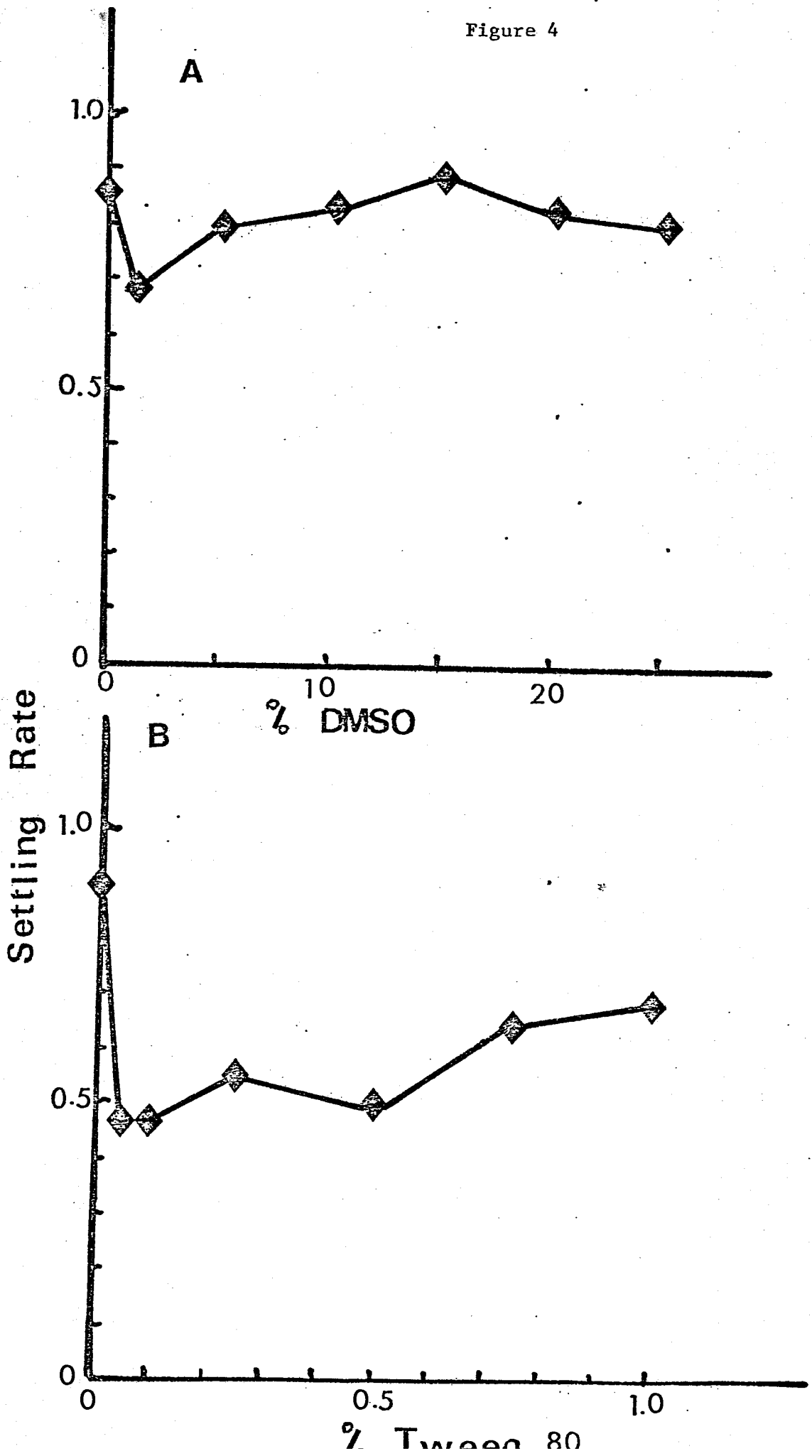
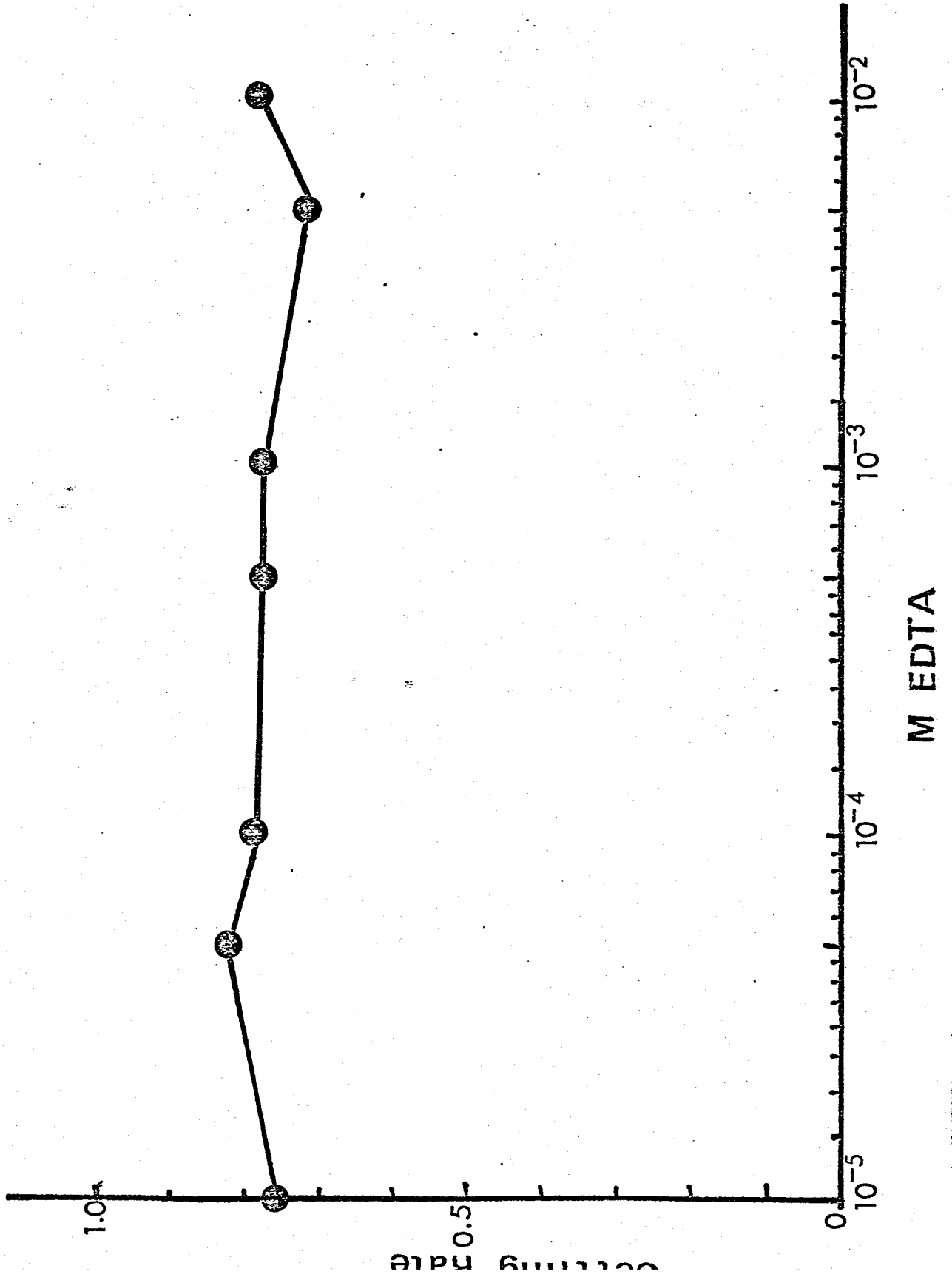


Figure 5



ABSTRACTS GENERAL SCIENTIFIC MEETINGS

AUGUST 16 - 19, 1983

- Ackerman, E.B. and H.L. Kornberg. Mutants of Escherichia coli affected in "inducer exclusion".
- Boyer, J.N. and R.S. Wolfe. An aerobic chitin degradation as a carbon and hydrogen source for sulfate reduction and methanogenesis in salt marsh bacteria.
- Halvorson, H.O., A. Keynan and T. Tiernan. Studies on manganese oxidizing, spore-forming bacteria.
- Muehlstein, L. and R.W. Castenholz. Sheath pigment formation in a blue-green alga, Lyngbya aestuarii, as an adaptation to high light.
- Skladany, G.J., B.A. Wrenn and R.R. Hall. The design and construction of a bench top reactor to model an anaerobic/oxic waste-water treatment system.
- Sutherland, D.F. and H.L. Kornberg. Factors affecting growth inhibition of enteric bacteria by methyl- α -D-glucoside.
- Vincent, W.S. and R.R. Hall. Microbial selection in an artificial ecosystem.