THE RATIO OF RESPIRATION TO SYNTHESIS FOR HETEROGENEOUS POPULATIONS OF MICROORGANISMS

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CHAPTER I

INTRODUCTION

General

The activated sludge process of wastewater stabilization is based on the biochemical reactions involved in the life cycle of microorganisms. One of the fundamental characteristics of this life cycle is that of reproduction, which consists of synthesis of new cell tissue. Suspended and dissolved organic matter in wastewater is the available substrate which, when brought into contact with active microorganisms, is assimilated into functional protoplasm and storage products. To enable the microorganisms to use the substrate, energy is supplied by other biochemical reactions in addition to the synthesis reactions. These energy-supplying reactions are known collectively as respiration and are generally of the oxidation-reduction type.

Thus, the activated sludge process consists of the two related processes of respiration and synthesis. The overall process may be represented as:

organic matter + oxygen + organisms $\longrightarrow$ new organisms + waste products + energy.

It was the purpose of the research herein reported to determine relationships which might exist between the partition of an exogenous substrate between respiration and synthesis, and factors which might exert a controlling influence on such partition.

CHAPTER II

LITERATURE REVIEW

Many of the studies which have been reported in the literature have been concerned with the relationships between sludge growth and substrate removal rate, and with the prediction of sludge yields. Those investigators who have considered the partition of an exogenous substrate between respiration and synthesis have been concerned primarily with the variance of this partition throughout the growth cycle of single systems only.

Ruchhoft, et al. (1) conducted one of the first series of such investigations, and found the percent substrate channelled into synthesis and respiration to vary during growth. Ruchhoft, et al. (2) concluded from their studies that great differences in the ratio of the extent of oxidation to the extent of net adsorption and synthesis occurred under conditions of underdosing and overdosing. Placak and Ruchhoft (3) later found that after twenty-four hours of aeration, activated sludge had oxidized and synthesized varying proportions of several substrates; the variance depending apparently on the class of the substrate employed (e.g., carbohydrate, alcohol, amino acid, organic acid).

The technique which was employed to measure oxygen consumption (respiration) has, however, been found to give misleading results (4).

Clifton and Logan (5), working with a pure culture of Escherichia coli, found the portion of substrate assimilated by the cells to be dependent on the nature of the substrate and independent of its concentration. They suggested a close connection between dissimilation and assimilation through coupled reactions. It was proposed that the ratio of synthesis to oxidation must be constant for a given system for, in order for synthesis to occur, i.e., for work to be done by a spontaneous reaction, there must be a simultaneous reaction involving an increase in free energy.

Porges, et al. (6) employed skim milk as substrate, and found sixty-three per cent of the original COD to be channelled into synthesis, while the remainder was oxidized and used for energy required for growth processes.

Wuhrmann (7) studied the effect of ammonia-nitrogen supplementation of synthetic carbohydrate wastes on the partition of respiration and synthesis. His data indicated that the addition of ammonia salts invariably increased the amount of substrate oxidized, thereby increasing the oxygen requirements of the systems.

Sawyer (8) summarized data from several investigators which indicated large variations in the percent of substrate converted to new growth by activated sludge. Extremes reported were ten to sixty per cent conversion of

organic acids and forty-four to sixty-four per cent conversion of glucose to new growth.

McKinney (9) asserted that the rate of excess sludge production in the complete mixing activated sludge system will be dependent on the ratio of food to microorganisms. He proposed that a high food:microorganism ratio would result in a large excess sludge problem, while a low food: microorganism ratio would result in a minimum production of excess sludge. Only two years later the same investigator suggested that the relationship between oxidation and synthesis is fixed and independent of the chemical nature of the substrate employed. It was concluded that two-thirds of the ultimate oxygen demand of the substrate would be converted into cellular mass, the remaining one-third being oxidized to satisfy the energy requirements of the system (10). McKinney (11) contended that the synthesis of a unit mass of protoplasm requires so many transformations that the energy requirements are the same regardless of the substrate being metabolized, and proceeded to derive formulae for the prediction of sludge yield and substrate removal based on this contention. None of the above three proposals has been supported by data and must, therefore, still be considered as only theories.

Gaudy and Engelbrecht (12) conducted studies on the partition between respiration and synthesis, and concluded that the ratio of respiration to synthesis is not constant, but continually increases at a diminishing rate in both

respiring and growing systems as a result of synthesis and oxidative assimilation. The finding of greater respiration under growth conditions than under respiring conditions was attributed to the presence of more cells to actively respire the substrate.

Eckenfelder and O'Connor (13) reviewed the work of others and concluded that the amount of sludge growth varies with the nature of the substrate being metabolized. Data on which they reported from various mixed wastes indicated that twenty-five to fifty per cent of the BOD removed is oxidized and the remainder synthesized to new sludge, neglecting endogenous respiration.

Rao, Speece, and Engelbrecht (14) obtained data from an apparently old sludge, using glucose as substrate, which indicated an increase in the ratio of respiration:synthesis with an increase in the ratio of food:microorganisms. This deduction is in opposition to the proposal of McKinney (9).

Hetling, et al. (15), in their study on variations in synthesis, concluded that the true yield of microorganisms is not proportionate to the COD or changes in the free energy of oxidation of the substrate utilized.

Simpson (16) studied the partition between respiration and synthesis by plotting suspended solids concentration against oxygen uptake throughout the growth cycles of several systems and calculating the reciprocal of the slope, i.e., the ratio of respiration to synthesis (R/S),

for the linear portion of the plot. The systems studied included several pure cultures using glucose as substrate, settled sewage using glucose, alanine, or sodium acetate, and activated sludge using glucose; the initial substrate concentration remaining constant for all systems. It was found that with glucose and small initial concentrations of microorganisms the value of R/S did not vary significantly, i.e., 1.21 to 1.27. With other substrates the ratio R/S varied, reportedly due to the differences in the energy released during the oxidation of the several compounds. With higher inoculums the value of R/S was considerably less, indicating maximum synthesis in these systems. Due to the extremes in inocula of the systems employed and the limited number of systems studied, it is not practical to draw any significant conclusions from this investigation.

In recent years, more emphasis has been placed on the thermodynamic relationships of the activated sludge processes of respiration and synthesis (17)(18)(19). Servizi and Bogan (17)(18) indicated that biological synthesis and concomitant biochemical oxygen utilization are thermodynamically linked reactions, and theorized that both could be quantitively defined from the free energy of substrate oxidation. Their logic proceeded from a proportionality between yield of cell tissue and the quantity of adenosine triphosphate (ATP) formed from a unit of substrate, through a proportionality between ATP production and the free energy released from the substrate, and thus to a direct

relationship between yield of cell tissue and free energy. The data obtained by Servizi and Bogan did indicate a proportionality between yield and free energy of the substrates employed. However, as only one experiment was conducted on each substrate studied, it is obvious that the authors assumed yield to be constant for each substrate in all cases, an assumption which is not in accordance with the data of other investigators (20). Data obtained by Siegel and Clifton (21)(22) were recalculated by Servizi and Bogan for a comparison of their thermodynamic relationships. It was apparently the authors' belief that the recalculated data of Siegel and Clifton supported their proportionality between yield and free energy. Siegel and Clifton, however, interpreted their own data as indicating the proportion of substrate carbon converted to cell carbon to be unrelated to the free energy of substrate oxidation. The difference in interpretation of the same data by Servizi and Bogan and Siegel and Clifton appears to lie in the amount of variation one is willing to accept as "constant" (23). In their postulation of the concept of a constant yield from a given substrate, Servizi and Bogan relied upon the work of Bauchop and Elsden (24) for support of their initial hypothesis. While Bauchop and Elsden did find a direct relationship between yield and ATP production for pure cultures of S. faecelis, S. cerevisiae, and Ps. lindneri, it must be recognized that their investigation was conducted solely on anaerobic, i.e., energy-limiting,

systems. Thus, the application of the relationship found by Bauchop and Elsden to the postulation of Servizi and Bogan concerning aerobic, i.e., carbon-limiting, systems is not reasonable, as the two types of systems under consideration are not similar. In the case of limiting factors other than energy, the substrate may continue to be degraded either at a full or a diminished rate (25). In such cases the energy produced cannot be used for growth, and must be dissipated in some other fashion.

The work of McCarty (19) was also concerned with the thermodynamic relationships of respiration and synthesis. In the derivation of his theoretical equations, McCarty apparently fell into the same trap in which Servizi and Bogan were found: the assumption of a constant yield from a given substrate. Using COD' to indicate the theoretical oxygen equivalent of the organic substrate, McCarty used the free energy of formation (ΔF_f^o) of the substrate to determine the free energy (ΔF_r) per gram COD' of substrate converted for energy. Since ΔF_f^o is constant for any given substrate, ΔF_r would necessarily be constant. According to the relationship proposed, yield would then be constant for the particular substrate. In order to account for varying yields, however, the irregularity of the efficiencies of microorganisms in capturing energy was recognized. It was suggested that energy losses would result from inefficiencies in the transfer of energy both from substrate to ATP and from ATP to the cell for use. The

conclusion was drawn that variations in growth predictions were probable as a result of differences in substrates, microorganisms, and environmental conditions.

It has been argued that the classical laws of thermodynamics have been derived from studies of idealized closed systems, i.e., a material region in space ideally isolated in all respects from all other regions, and thus do not apply generally to living organisms (26). Free energy values are simply indicators of the maximum amount of energy available to the microorganism. The resulting growth depends first on the efficiency of the organism in obtaining the energy, and second, on the portion of the energy obtained which can be used for growth (27).

In an analysis of the data of Siegel and Clifton (22), Lamanna and Mallette (26) pointed out that E. coli was able to assimilate as much carbon from pyruvate as from lactate (monobasic compounds) and as much from fumarate as from succinate (dibasic compounds), in spite of the fact that pyruvate has a lesser free energy content than lactate and fumarate than succinate. Thus, the assimilation appeared to be more dependent on the chemical nature of the substrate than the free energy content. Clifton and Logan (5) had also recognized this relationship in their work with E. coli and concluded that the energy of substrate molecule would not provide a propitious criterion for the prediction of cell growth.

Investigations recently reported by Rao and Gaudy (20)

and Krishnan and Gaudy (28) indicated that high inocula would result in a significant amount of early carbohydrate synthesis which would remove dissolved COD with diminished autocatalytic growth when compared with systems of lower inocula. Such a relationship between synthesis and inoculum would result in a higher energy requirement (respiration) at low inocula than at higher inocula. It was suggested that the expectation of yield to be solely a function of the structure or free energy of the substrate would be a gross oversimplification due to the biological efficiencies of oxidative phosphorylation and ATP coupling for biosynthesis, factors which apparently vary in different bacteria and are largely unknown (20).

The effects of sodium chloride and sodium pentachlorophenol (SPCP) on the partition between respiration and synthesis have been studied independently and recently reported. Kincannon and Gaudy (29)(30) found that high concentrations of sodium chloride, under both long and short term exposures, caused a change in the metabolic process of the microorganisms which resulted in greater use of the substrate for respiration than for synthesis. It was reported that a continuous long term exposure resulted in a much more severe change in the ratio of respiration:synthesis than did shock loadings. It was suggested that uncoupling of oxidative phosphorylation and/or the effect of sodium chloride on a control mechanism of protein synthesis (e.g., salting out of key enzymes)

could be logical explanations for the observed results (30). It was found that the shock loading of systems with SPCP resulted in a linear increase of the ratio of respiration: synthesis as the dose of SPCP was increased, while systems acclimated to SPCP exhibited a non-linear decrease in the ratio as the level of SPCP was increased (31). In both of these independent studies the ratio of respiration to synthesis was determined at the point of maximum biological solids.

CHAPTER III

MATERIALS AND METHODS

Growth Medium

The medium composition employed in all experiments was such that carbon source was the growth-limiting nutrient. Both glucose and glycerol were used separately as single sources of carbon. It was anticipated that 2000 mg/l would be the highest COD concentration employed, therefore, a standard synthetic waste was designed for use at this COD concentration. All systems were then grown on the standard synthetic waste regardless of the COD concentration employed. This practice was maintained to eliminate unnatural deviations in growth which might have resulted from variations in osmotic pressue due to any changes imposed in the salt concentrations of the media. A potassium phosphate buffering system was used to maintain a pH of 7.0 in the systems. The composition of the medium is given in Table I for 2000 mg/l glucose COD concentration. Aeration was maintained at approximately 1000 ml/min/l of medium by means of compressed air introduced through carborundum diffusing stones. Agitation was accomplished through aeration.

TABLE I

COMPOSITION OF GROWTH MEDIUM FOR 2000 mg/l GLUCOSE COD

Constituent	Concentration
Glucose	1888 mg/l
Ammonium sulfate, $(NH_4)_2SO_4$	1000 mg/l
Magnesium sulfate, $MgSO_4 \cdot 7H_2O$	200 mg/l
Ferric chloride, $FeCl_3 \cdot 6H_2O$	1.0 mg/l
Manganous sulfate, $MnSO_4 \cdot H_2O$	20.0 mg/l
Calcium chloride, $CaCl_2$	15.0 mg/l
KH_2PO_4	1054 mg/l
K_2HPO_4	2140 mg/l
Tap water	200 ml/l

Young Cell Cultures

Growth in a batch activated sludge unit of 1.5 l aeration volume containing 2000 mg/l glucose COD medium was started from a sewage seed. The seed was obtained from the effluent of the primary clarifier of the Stillwater municipal sewage treatment plant. The plant influent has an average 5-day BOD of approximately 275 mg/l, consists largely of domestic wastes, and includes the sewage from a large university. The unit was operated for three days in the following manner: Prior to daily feeding, 500 ml of

mixed liquor were wasted and the remaining mixed liquor was allowed to settle for one hour, after which 500 ml of supernatant were wasted. The volume was then restored with the standard 2000 mg/l glucose COD medium previously described. Subsequent transfers, including those for the carryover of seed from one experiment to the following, were small percentages of the total mixed liquor volume (e.g., five per cent, eight per cent). Such small transfers were carried out for five days prior to commencing with the experiments.

Old Cell Cultures

A batch activated sludge unit of 6.0 l aeration volume containing 2000 mg/l COD medium was started from sewage seed obtained from the above described source. The daily operating procedure was similar to that delineated above, in that one-third of the total volume was wasted prior to settling, and one-third following settling. After ten days of operation the feed concentration was doubled in an attempt to increase the accumulating biological mass. The feeding procedure was followed throughout the course of the experiments, the settled sludge at the conclusion of each experiment being returned to the 6.0 l batch unit.

Experimental Procedure

Using the transfer procedures already described, seed for the young cell experiments was allowed to grow for eight days before experiments were commenced. Seed for the old cell experiments was allowed to grow until a well-flocculating sludge was developed. This required

twenty-three days in the case of the glucose system, and thirty-seven days in the case of the glycerol system.

For each experiment, seed which had been transferred twenty-four hours earlier was removed from the appropriate batch unit and centrifuged under conditions of reduced temperature (0 to 3°C) at a relative centrifugal force of 25,400xG for fifteen minutes. The supernatant was discarded and the seed was resuspended in 0.02M phosphate buffer solution to form the desired inocula concentrations. Media was prepared in batch tubes of 1.5 l aeration capacity, inoculated with the resuspended seed, and aerated. Samples were withdrawn immediately for the determinations discussed below, and at appropriate time intervals thereafter until the point of maximum COD removal was attained. The high solids concentrations employed in many of the experiments resulted in a high rate of substrate removal which necessitated rapid sampling. To maintain uniformity while attempting to accomplish this end, all samples, excepting those at the ends of experiments number fifty through fifty-three were centrifuged at a temperature of 0 to 3°C for ten minutes at a relative centrifugal force of 34,800xG. Due to a breakdown of the refrigerated centrifuge near the ends of experiments number fifty through fifty-three, it was necessary to centrifuge some of the samples in these experiments at room temperature for five minutes.

Analytical Techniques

I. Oxygen Uptake

Oxygen uptake was determined on a Warburg respirometer using 25 ml mixed liquor samples in 125 ml flasks, with 1.0 ml KOH (twenty per cent) in the center well for carbon dioxide absorption. A shaker rate of 110 osc/min was employed at a constant temperature of 25°C. The respirometers were calibrated in accordance with the recommended mercury calibration technique (32).

II. Biological Solids

Optical density determinations were employed in the measurement of biological solids in experiments number one through nine, as the inocula were relatively small. The optical densities of mixed liquor samples were determined with a Bausch and Lomb "Spectronic 20" spectrophotometer at a wave length of 540 mμ using 19 mm diameter cuvettes. Biological solids concentrations of several samples were determined by both the optical density technique and the membrane filter technique. Optical density was then plotted against solids concentration and a line of best fit for the data was protracted. Using this calibration curve, the solids concentrations corresponding to the various optical densities determined were obtained (33).

In all other cases, biological solids were measured using membrane filters (Millipore Filter Corp., HA, 0.45μ or equivalent). The centrifuged samples were filtered through dried and desiccated tared filters. The filters

were then placed in their respective aluminum dishes, oven-dried at 103°C for two hours, desiccated, and weighed.

III. Substrate Removal

Substrate removal was observed through the chemical oxygen demand (COD) determination as delineated in Standard Methods (34). This determination was made on the supernatant of the centrifuged samples.

Calculation Procedures

I. Ratio Respiration:Synthesis

Biological solids concentration (mg/l) and oxygen uptake data (mg/l) were plotted against time for each experiment. Values of accumulated oxygen uptake were then plotted against corresponding values of accumulated biological solids concentration. The slope of the linear portion of the curve thus obtained was calculated as the ratio of respiration to synthesis (R/S).

II. Statistical Methods

Curves of best fit for data were obtained through the use of least square regression techniques. Inasmuch as the least square line approximating the set of points (X_1, Y_1), (X_2, Y_2), . . ., (X_n, Y_n) has the equation

$$Y = a_0 + a_1X$$

the intercept a_0 and the coefficient a_1 were determined by solving simultaneously the equations

$$\Sigma Y = a_0 N + a_1 \Sigma X$$

$$\Sigma XY = a_0 \Sigma X + a_1 \Sigma X^2,$$

the normal equations for the least square line. Similarly, the least square parabola approximating the set of points (X_1, Y_1), (X_2, Y_2) . . ., (X_n, Y_n) has the equation

$$Y = a_0 + a_1 X + a_2 X^2$$

where the intercept a_0 and the coefficients a_1 and a_2 were determined by solving simultaneously the equations

$$\Sigma Y = a_0 N + a_1 \Sigma X + a_2 \Sigma X^2$$

$$\Sigma XY = a_0 \Sigma X + a_1 \Sigma X^2 + a_2 \Sigma X^3$$

$$\Sigma X^2 Y = a_0 \Sigma X^2 + a_1 \Sigma X^3 + a_2 \Sigma X^4,$$

the normal equations for the least square parabola.

Tests were conducted to determine the congruency of the computed equations with the observed data. The technique employed was that suggested by Mack (35). As it is desirable to reject an incorrect hypothesis, the null hypothesis tested in each case was that the state of no relation is more likely than the existence of a linear or quadratic relation between Y and X. The test applied was the F test, which requires acceptance of the null hypothesis where $F < F_c$.

In the case of linear relationships s_1^2, s_2^2, and F were defined as

$$s_1^2 = \Sigma (Y_i - \bar{Y})^2, \quad s_2^2 = \left\{\Sigma (Y - Y_i)^2\right\}/N-2, \quad F = s_1^2/s_2^2$$

where $Y_i = a_0 + a_1 X$, Y = observed values, and N is the

number of observations. Degrees of freedom for values of s_1^2 were 1, and for values of s_2^2 were (N - 2). F values were calculated and compared with critical F values (F_c) at the confidence level of ninety per cent ($\alpha = 0.10$) for the appropriate degrees of freedom as found in prepared tables (36). The hypothesis that the least square line fits the observed data better than the state of no relation at all was accepted where $F>F_c$.

In the case of quadratic relationships S_1^2, S_2^2, and F were defined as

$$S_1^2 = \left\{\sum(Y_i-\bar{Y})^2\right\}/2,\ S_2^2 = \left\{\sum(Y-Y_i)^2\right\}/N-3,\ F = S_1^2\ S_2^2$$

where $Y_i = a_0 + a_1X + a_2X^2$, Y = observed values, and N is the number of observations. Degrees of freedom for values of S_1^2 were 2, and for S_2^2 were (N-3). F values were calculated and compared with F_c values as above. The hypothesis that the least square quadratic curve fits the observed data better than the state of no relation at all was accepted where $F>F_c$.

Tests were also conducted to examine for the improvement of a quadratic over a linear relationship. F values were calculated as $(2S_1^2 - s_1^2)/S_2^2$ and compared with F_c values at the ninety per cent confidence level for degrees of freedom of 1 for the numerator and (N-3) for the denominator. The hypothesis that the quadratic is an improvement over the linear relationship was accepted where $F>F_c$.

III. Material Balances

Material balances were made for each experiment according to the method of Gaudy and Engelbrecht (12) where all measurements were converted to weight of substrate. COD and oxygen uptake data were converted to equivalent weights of substrate based on the theoretical amount of oxygen required for complete oxidation of 1 mole of substrate. Substrate mass was assumed to be converted directly to cell mass; therefore, the substrate utilized for cell synthesis was measured as increase in dry weight of cells. The balance thus obtained could be expressed as

$$\text{Weight of substrate removed} = \text{weight of cells produced} + \text{weight of substrate oxidized}$$

Percent recovery was then calculated by dividing the sum of the increase in cell weight and the weight of substrate oxidized by the weight of substrate removed.

CHAPTER IV

RESULTS

A total of seventy-four experiments were conducted in this investigation of the partition of an exogenous substrate between respiration and synthesis. A summary of these experiments is presented in Table II, where it can be seen that the experiments have been arranged in four groups identified by their respective Roman numerals. Groups I and II consist of experiments conducted on glucose systems employing young cells. Group III experiments were also conducted on glucose systems, but employed old cells, while the experiments in Group IV were conducted on old cells using glycerol as the sole carbon source. The ratio of respiration:synthesis (R/S) was calculated for each experiment in the manner previously described. The ratio of food: microorganisms (F/M) was calculated for each experiment by dividing the initial COD (mg/l) of the system by the initial solids concentration (mg/l). The percent recovery was computed for each experiment and listed in the table. The average value of percent recovery for the experiments was found to be ninety-two per cent.

The biochemical response of a typical batch system is

TABLE II

SUMMARY OF EXPERIMENTS

Group No.	Date 1967	Exp. No.	Initial Solids mg/l	Initial COD mg/l	F/M	R/S	% Recovery
I	9-23	1	0	289		0.77	85
		2	0	552		1.09	93
		3	0	1025		0.92	90
		4	0	1525		0.99	91
	9-26	5	175	505	2.89	0.84	96
		6	175	740	4.23	0.31	121
		7	175	1135	6.49	0.55	109
		8	180	1570	8.73	0.61	96
		9	180	2090	11.61	0.47	91
	10- 5	10	217	218	1.05	0.66	95
		11	218	482	2.21	0.33	106
		12	220	935	4.25	0.43	86
		13	200	1430	7.15	0.45	91
		14	220	1980	9.00	0.44	87
	10- 7	15	322	275	0.85	0.50	90
		16	312	573	1.84	0.75	86
		17	318	1070	3.36	0.72	92
		18	308	1535	4.98	0.72	93
		19	303	2075	6.86	0.84	92
	10-10	20	1345	315	0.23	0.29	119
		21	1302	567	0.44	0.29	78
		22	1390	1070	0.77	0.29	86
		23	1460	1550	1.06	0.38	80
		24	1420	2035	1.43	1.11	77
	10-19	25	762	178	0.23	0.57	130
		26	840	482	0.57	0.64	90
		27	588	954	1.62	0.42	88
		28	623	1425	2.29	0.37	96
		29	750	1940	2.59	0.46	93
II	10-19	30	175	975	5.56	0.50	91
		31	428	965	2.25	0.57	94
		32	711	975	1.37	0.32	89
		33	875	956	1.09	0.31	76
		34	1342	1010	0.75	0.35	88
	10-26	35	157	500	3.18	0.50	83
		36	423	468	1.11	0.34	92
		37	749	500	0.67	0.38	95
II	10-26	38	934	488	0.52	0.29	85
		39	1436	487	0.34	0.74	90
	11-21	40	160	1755	10.98	0.55	80
		41	404	1700	4.21	0.36	81
		42	650	1700	2.62	0.39	84
		43	832	1715	2.06	0.30	82
		44	1205	1710	1.42	0.23	72
III	12- 5	45	220	533	2.42	0.44	85
		46	645	521	0.81	0.19	83
		47	1133	546	0.48	0.09	76
		48	1564	580	0.37	0.06	58
		49	2108	617	0.29	0.07	67
	12- 7	50	192	1540	8.02	0.63	90
		51	620	1540	2.48	0.41	91
		52	1115	1530	1.37	0.35	91
		53	1505	1570	1.04	0.30	82
		54	2050	1560	0.76	0.31	72
	12-14	55	284	957	3.37	0.54	89
		56	856	925	1.08	0.30	89
		57	1489	937	0.63	0.20	89
		58	2008	915	0.46	0.16	80
		59	2492	960	0.39	0.16	67
IV	1-22-68	60	148	948	6.41	0.76	98
		61	370	944	2.55	0.70	101
		62	650	935	1.44	0.60	96
		63	962	935	0.97	0.50	100
		64	1280	902	0.70	0.47	101
	1-24	65	170	1340	7.89	0.96	119
		66	404	1270	3.14	0.84	150
		67	760	1250	1.64	0.66	102
		68	1008	1315	1.31	0.61	102
		69	1312	1330	1.01	0.50	101
	1-26	70	187	461	2.46	1.00	117
		71	345	464	1.35	0.75	134
		72	576	484	0.84	0.63	82
		73	840	488	0.58	0.61	103
		74	1028	520	0.51	0.52	94

presented in Figure 1. Biological solids concentration (growth), oxygen uptake (respiration), and COD remaining are shown plotted against elapsed time for the system. The data presented is that obtained from experiment number 64, an old cell system employing glycerol at an initial COD of approximately 900 mg/l, and a F/M ratio of 0.70. The solids and COD concentrations found in the corresponding Warburg flask at the end of the experiment are also indicated. Figure 2 is a plot of oxygen uptake (respiration) versus accumulated solids (synthesis). Corresponding values of oxygen uptake and solids concentration were obtained from Figure 1 and plotted in Figure 2. The ratio R/S was obtained as the slope of the line thus derived. These figures are included to indicate typical results obtained from the experiments conducted.

Upon the conclusion of several of the experiments, samples were simultaneously drawn from the batch tubes and their corresponding Warburg flasks in order to compare the growth and substrate removal occurring in each. The purpose of this comparison was to certify growth in the Warburg flask as sufficiently similar to that in the corresponding batch tube to allow the assumption that oxygen uptake measured via the Warburg respirometer was equivalent to the oxygen consumption which occurred in the batch tube. The results of these comparisons are presented in Table III, where the percent difference is based on the batch tubes as the reference and was determined by

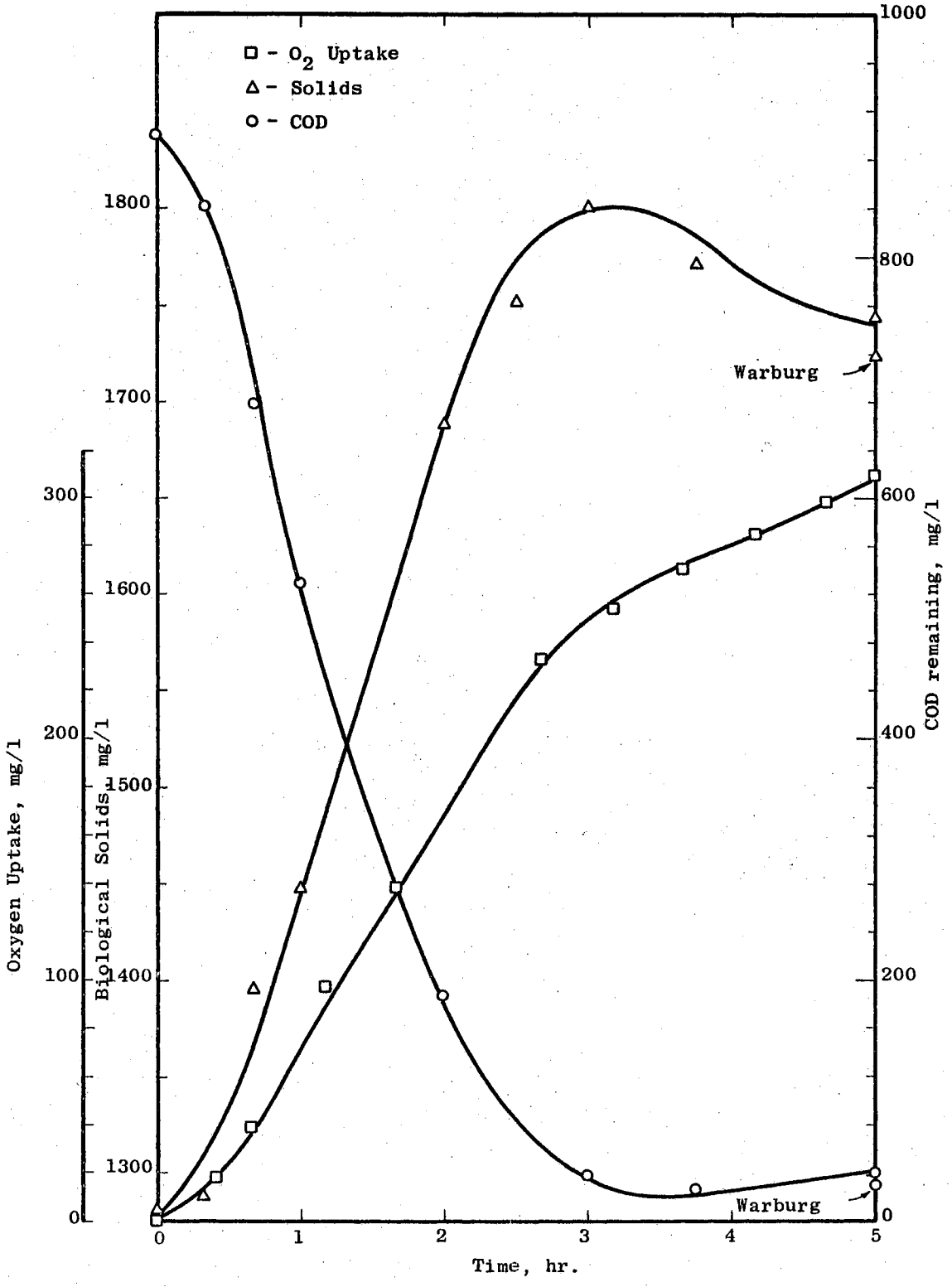

Figure 1 - Typical biochemical response of systems studied.

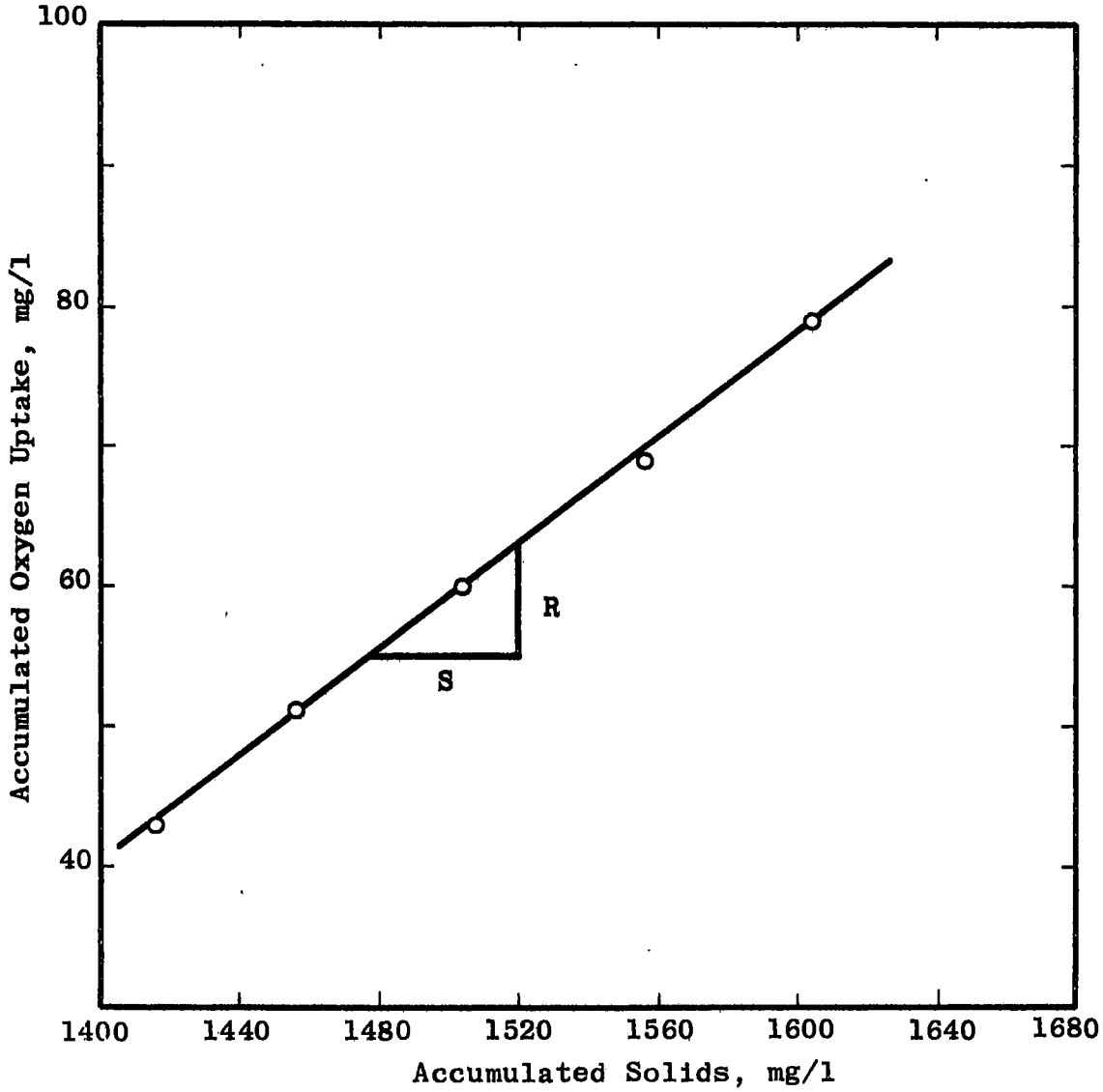

Figure 2 - Respiration versus synthesis for a typical system studied.

TABLE III

CORRELATION BETWEEN WARBURG FLASKS AND BATCH TUBES

Exp. #	COD Remaining mg/l		%	Accumulated Solids mg/l		%	%
	Tube	Flask	Difference	Tube	Flask	Difference	Recovery
40	55	55	0	964	876	+ 9	80
41	90	50	+44	1110	1090	+ 2	81
42	65	75	-15	1384	1328	+ 4	84
43	80	90	-12	1520	1440	+ 5	82
44	80	80	0	1780	1720	+ 3	72
50	60	35	+42	995	892	+10	90
51	55	65	-18	1450	1350	+ 7	91
52	30	30	0	1780	1845	- 4	91
53	55	30	+27	2270	2260	0	82
54	50	60	-20	2785	2780	0	72
55	150	165	-10	761	768	- 1	89
56	53	47	+11	1363	1335	+ 2	89
57	43	35	+19	1976	1988	- 1	89
58	55	50	+ 9	2468	2488	- 1	80
59	46	62	-35	2872	2944	- 3	67
60	526	417	+21	348	416	-20	98
61	16	18	-12	883	844	+ 4	101
62	26	38	-46	1145	1128	+ 1	96
63	26	30	-15	1455	1425	+ 2	100
64	43	29	+33	1740	1724	+ 1	101
65	668	486	+27	640	495	+23	119
66	50	65	-30	1140	1084	+ 5	150
67	20	45	-125	1484	1460	+ 2	102
68	30	30	0	1742	1676	+ 4	102
69	35	45	-29	2004	2012	0	101
70	78	31	+60	412	420	- 2	117
71	33	36	- 9	595	572	+ 4	134
72	33	34	- 3	818	800	+ 2	82
73	44	38	+14	1095	1088	+ 1	103
74	30	36	-20	1284	1248	+ 3	94

$$\% \text{ difference} = \frac{\text{tube} - \text{flask}}{\text{tube}} \times 100$$

The comparisons of COD remaining yielded an average percent difference of twenty-four per cent, while that of accumulated solids was four per cent. For these experiments, the average percent recovery was found to be ninety-five per cent.

Glucose Systems:Young Cells, Group I

The R/S ratios obtained from the experiments of Group I (experiments number 1 through 29) were plotted in various forms against the parameters of initial solids concentration, initial COD, and F/M in an attempt to obtain a characteristic correlation. In Figures 3 and 4, R/S is plotted against initial solids and F/M ratio with respect to experiments having approximately the same initial COD values. This was accomplished by arranging the experiments of Group I into four subgroups on the basis of their initial COD concentrations. Initial COD concentrations of approximately 200, 500, 1000, 1500, and 2000 mg/l were employed. No two experiments within any one subgroup were conducted with seed from the same batch unit.

Although there is no correlation between R/S and initial solids concentration in Figure 3, where experiments of differing inocula but similar initial COD concentrations are considered, a general trend of decreasing R/S with increasing initial solids concentration can be detected. This lack of correlation is indicative of changes which

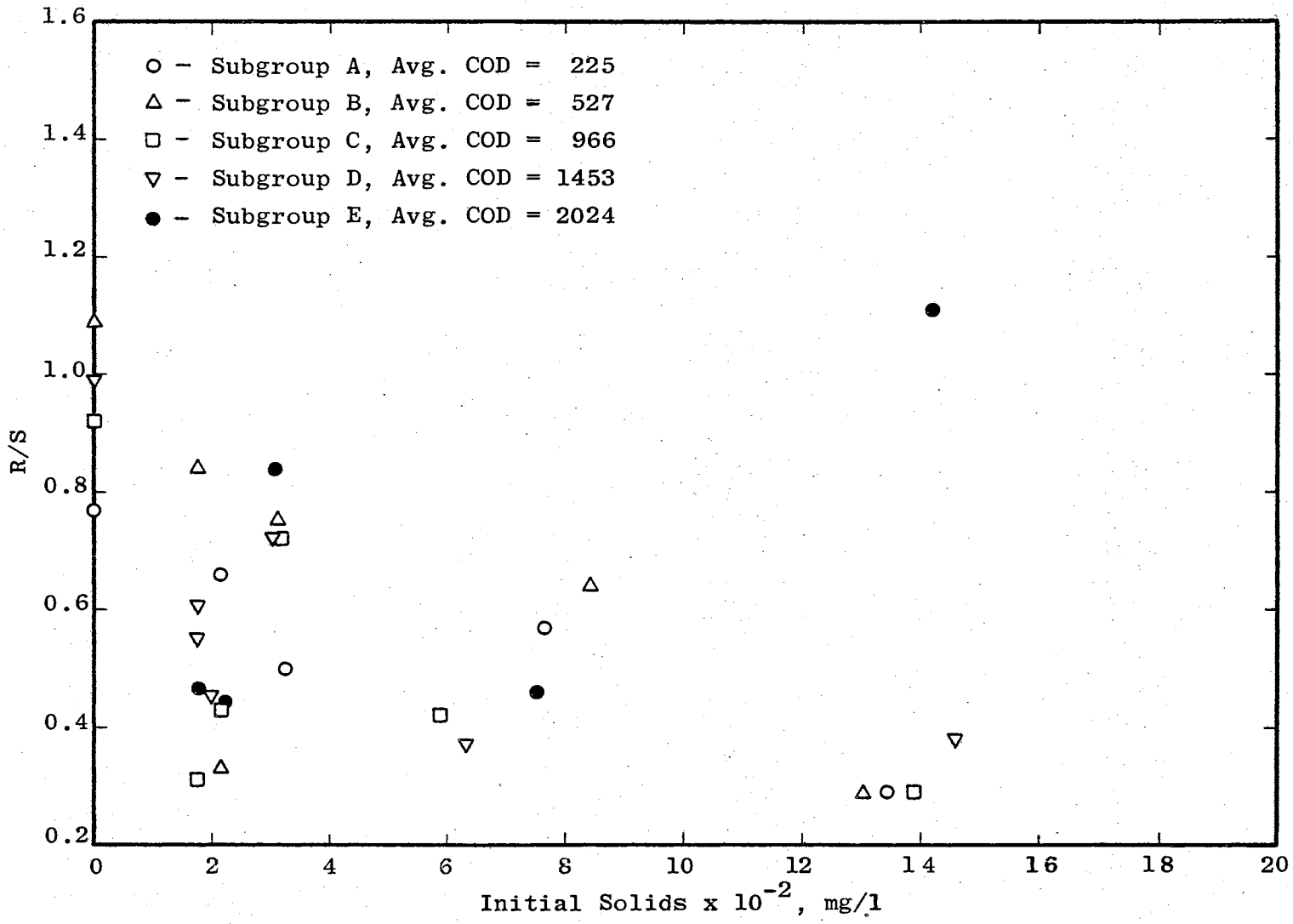

Figure 3 - Effect of initial solids concentration on R/S for young glucose cultures, Group I.

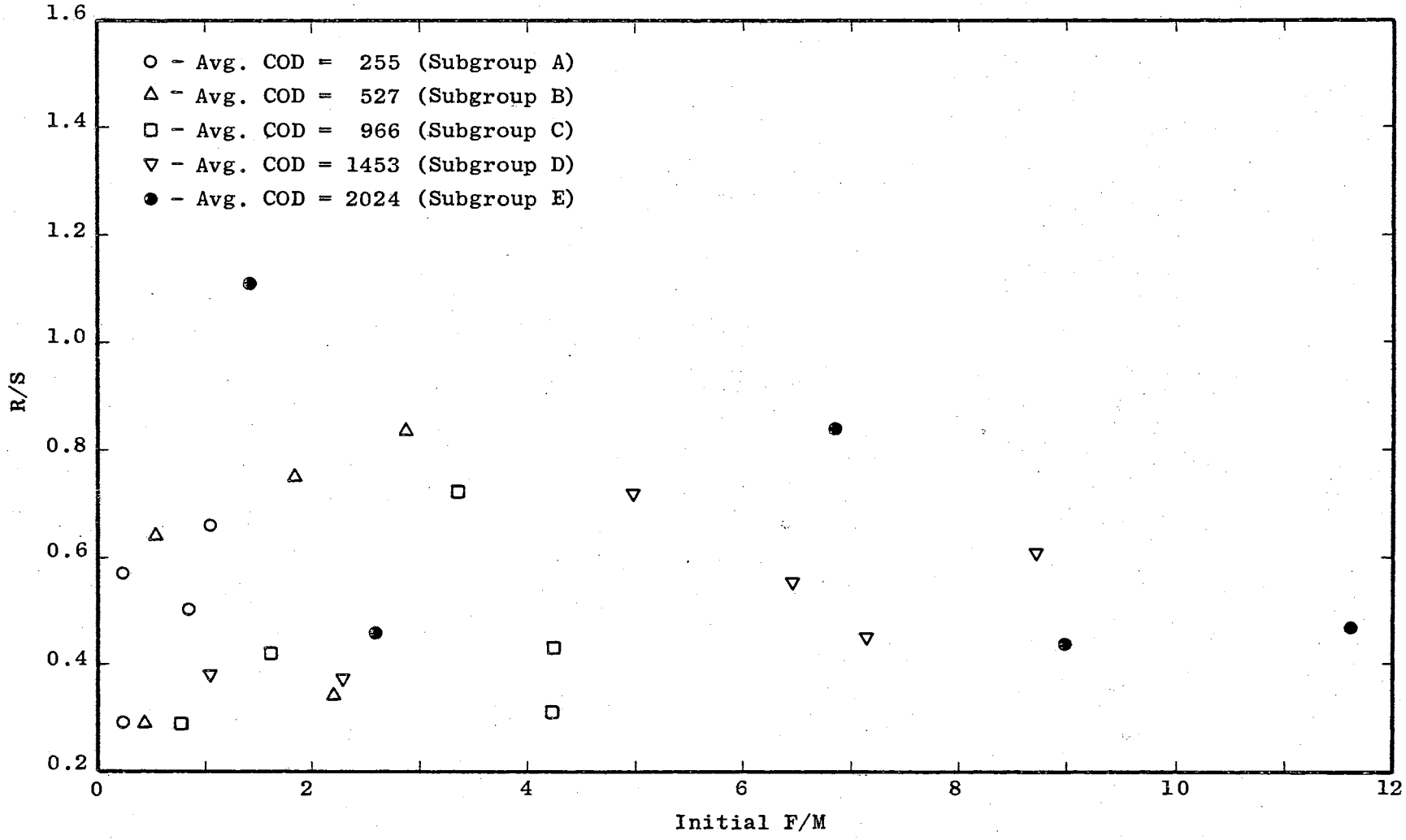

Figure 4 - Effect of F/M on R/S for young glucose cultures, Group I.

occurred in the seed cultures of young cells as a result of the small transfers employed in their growth. A plot of R/S for these same subgroups versus initial F/M (Figure 4) also yielded a lack of correlation. No general trend of R/S with F/M was indicated.

The data presented in Figures 5 and 6 were obtained from the same experiments as those of Figures 3 and 4; however, the data were plotted with respect to groups of experiments of differing initial COD concentrations but identical inocula. From the plot of R/S versus initial COD concentration for the young cell systems (Figure 5) it is apparent that there is no correlation between R/S and initial COD even where identical inocula are employed. When values of R/S for the systems employing identical inocula were plotted against initial F/M (Figure 6), it was again indicated that there was no correlation between the ratios R/S and F/M for young cell systems.

A comparison of Figures 4 and 6 indicates that for young cell systems, whether the effect of differing inocula or initial COD concentrations is considered, there is no relationship between R/S and F/M.

Glucose Systems:Young Cells, Group II

The experiments of Group II involved systems similar to those of Group I. These experiments, however, were conducted in such a manner that several of the experiments contained varying amounts of inocula from the same batch unit, the initial COD of the experiments being approximately equal.

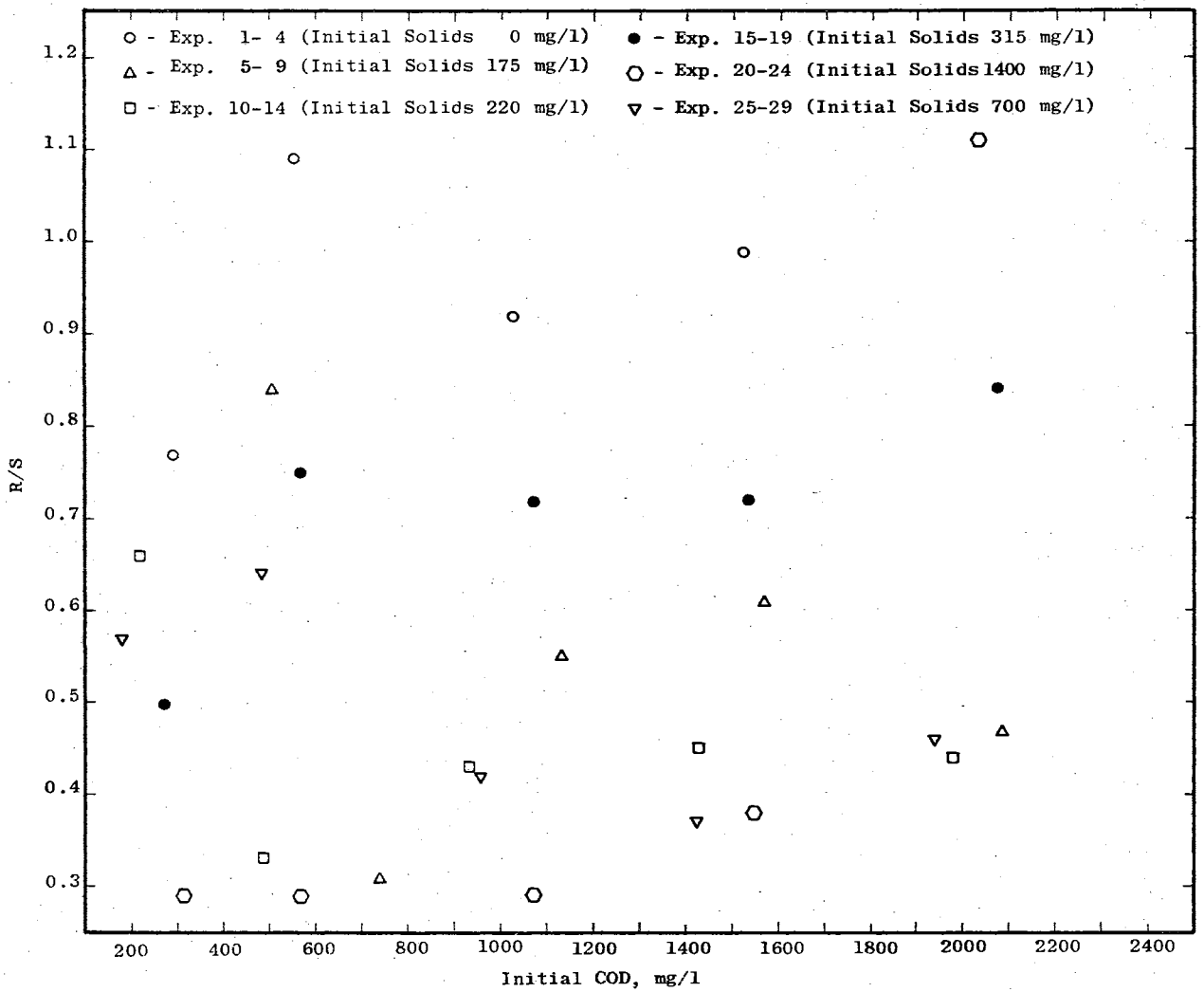

Figure 5 - Effect of initial COD on R/S for young glucose cultures, Group I.

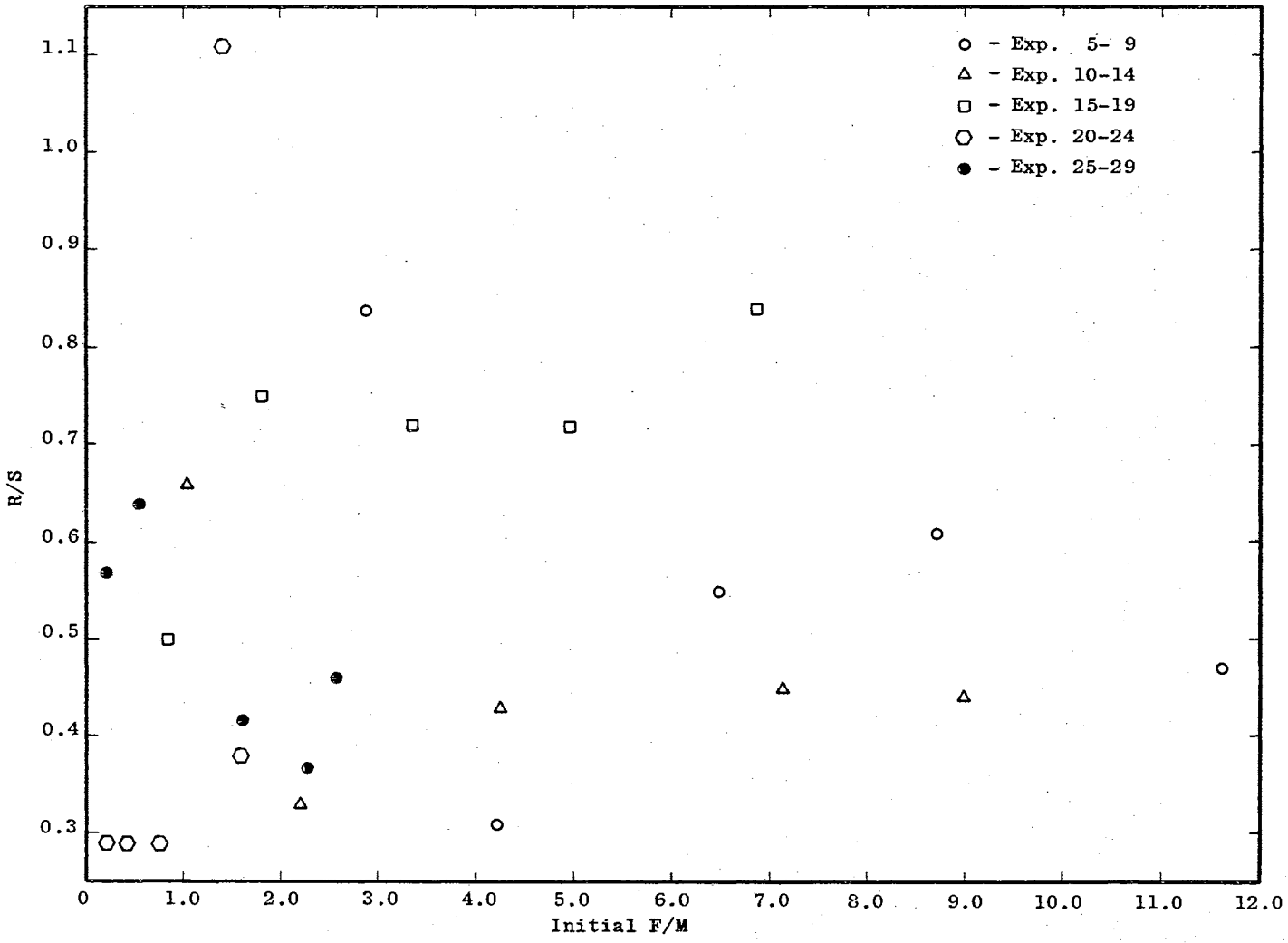

Figure 6 - Effect of F/M on R/S for young glucose cultures, Group I.

The R/S values obtained from the Group II experiments are presented in Figures 7 and 8 plotted against F/M and initial solids concentration, respectively. Figure 7 again indicates the state of no relation between the ratios R/S and F/M for young cells metabolizing glucose, although a trend of a slight increase in R/S with increasing F/M ratio is evident. As the data presented in Figure 8 suggested the possibility of a linear relationship between R/S and initial solids concentration, a statistical analysis was performed. This analysis yielded the linear relationship of

$$R/S = 0.522 - 0.213X$$

where X is initial solids concentration multiplied by 10^{-3}. This relationship was found to be better than the state of no relation at all or a quadratic relationship, at a level of significance of ninety per cent. Thus, an increase in initial solids concentration resulted in a decrease in the ratio R/S, regardless of initial COD concentration.

Glucose Systems:Old Cells, Group III

The values of R/S obtained from the Group III experiments were also plotted against initial solids concentration and F/M and are presented in Figures 9 and 10, respectively. A statistical analysis of the data presented in Figure 9 revealed the following relationships for the experimental parameters:

Initial COD 500 mg/l: $R/S = 0.514 - 0.495X + 0.125X^2$
Initial COD 950 mg/l: $R/S = 0.640 - 0.458X + 0.107X^2$
Initial COD 1550 mg/l: $R/S = 0.748 - 0.613X + 0.200X^2$

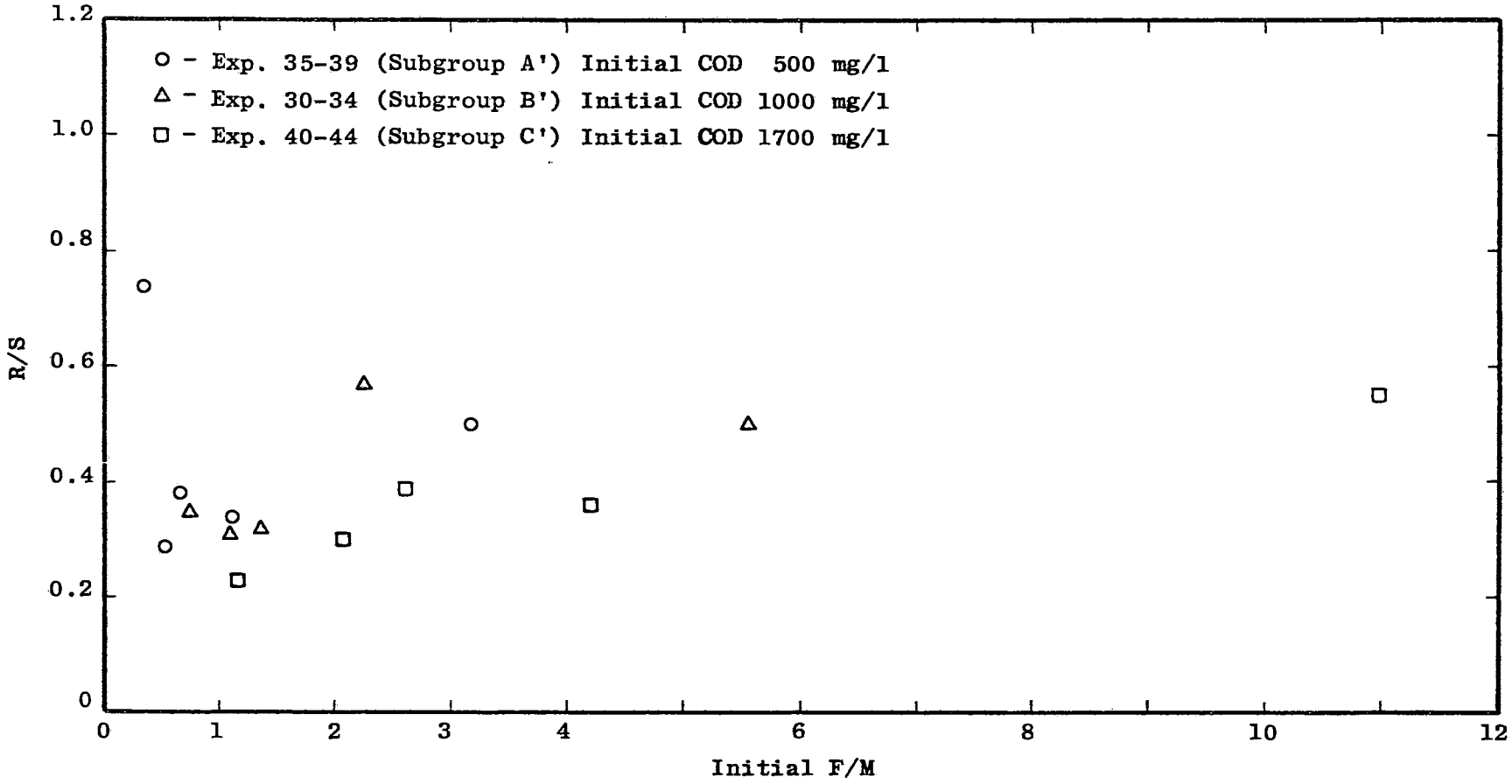

Figure 7 - Effect of F/M on R/S for young glucose cultures, Group II.

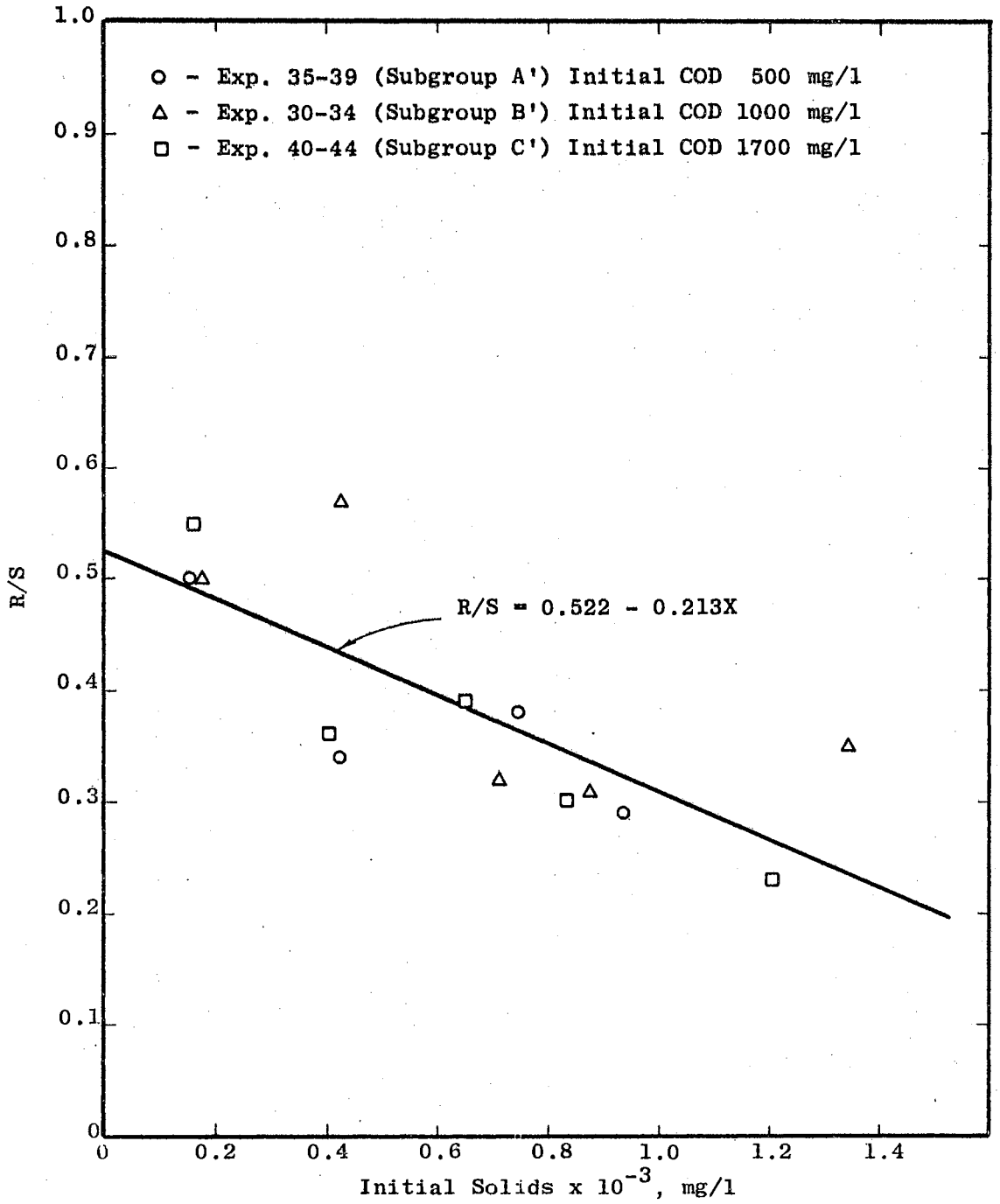

Figure 8 - Effect of initial solids on R/S for young glucose cultures, Group II.

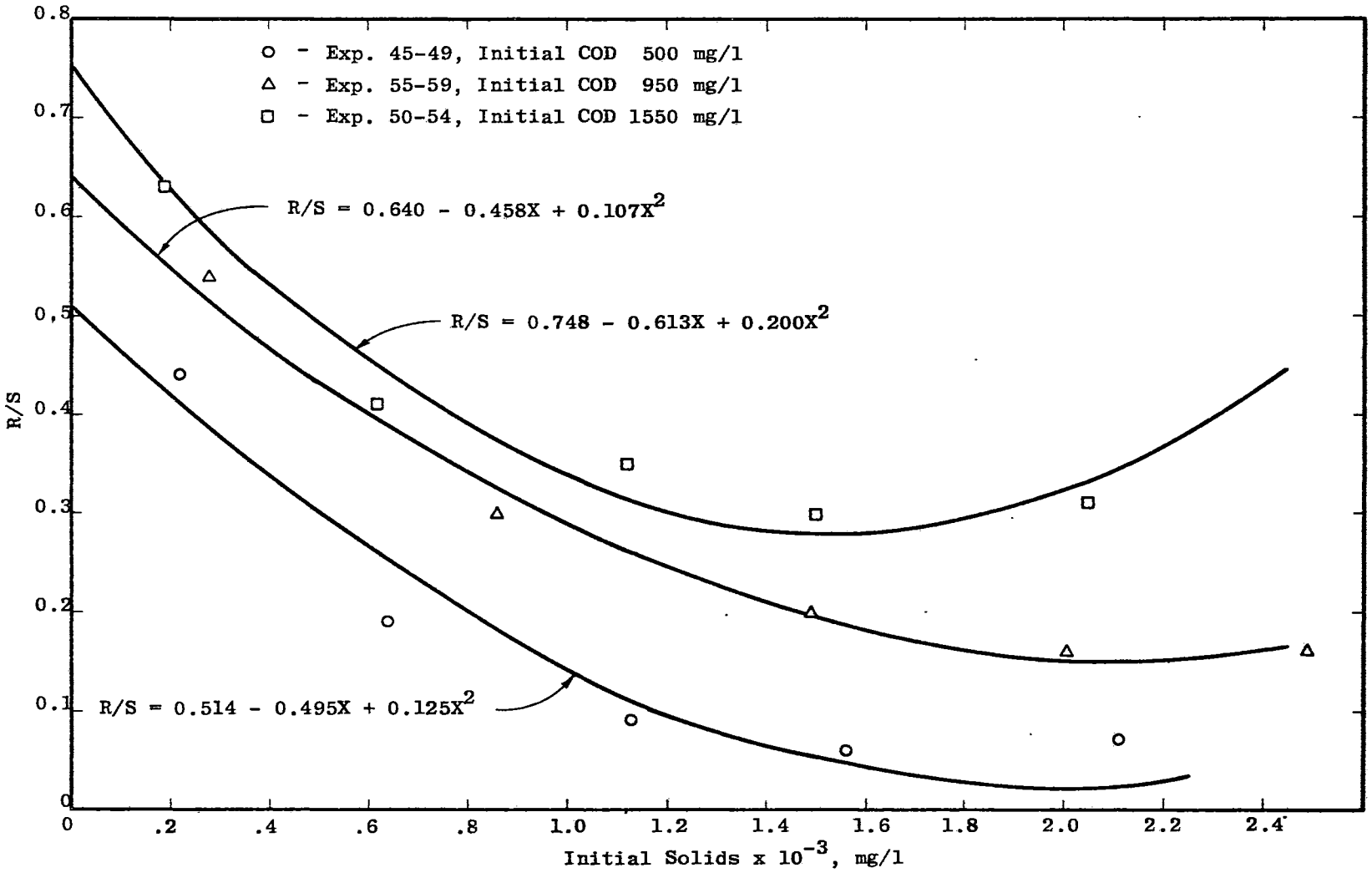

Figure 9 - Effect of initial solids on R/S for old glucose cultures, Group III.

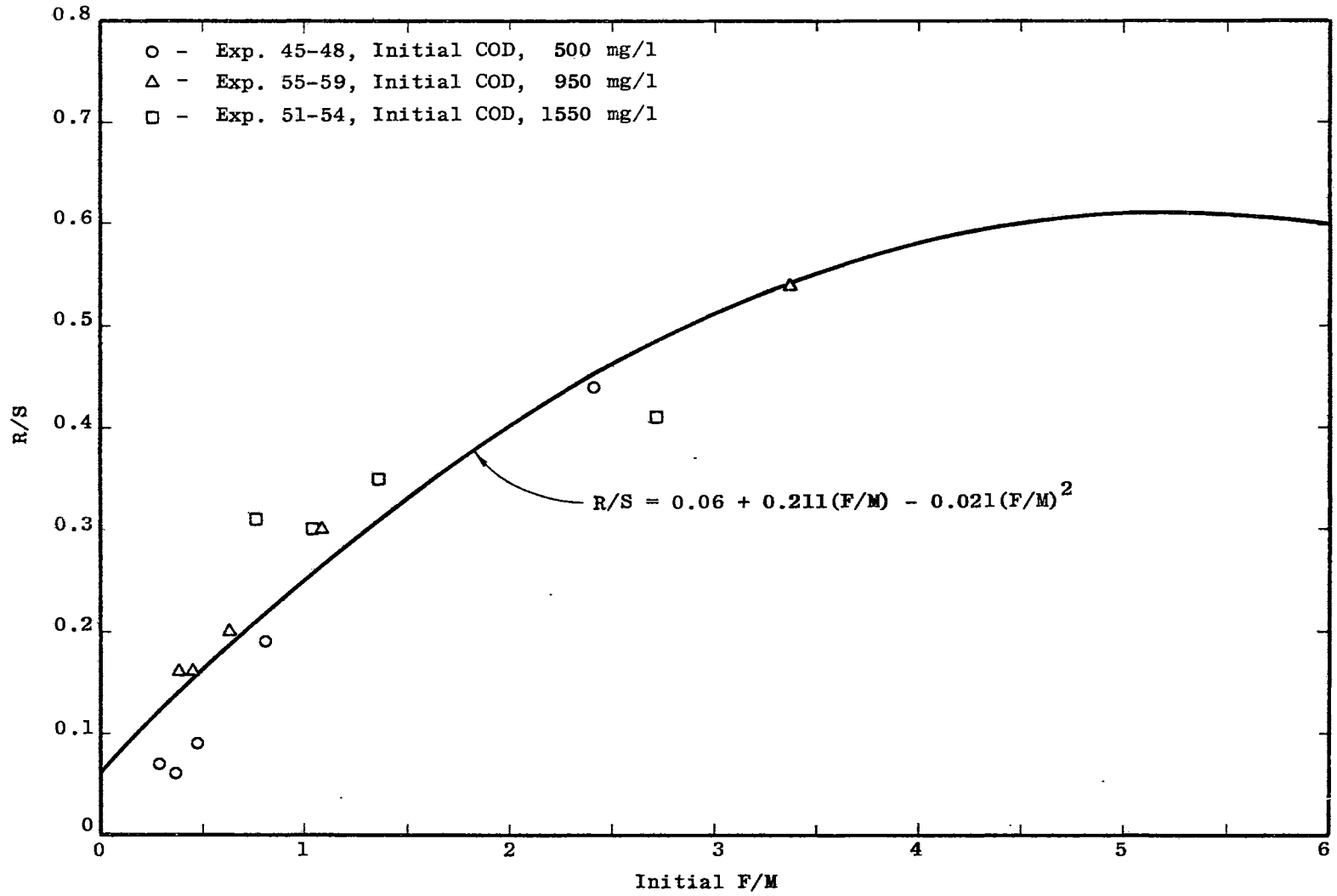

Figure 10 - Effect of F/M on R/S for old glucose cultures, Group III.

where X is initial solids concentration multiplied by 10^{-3}. These relationships were tested at the ninety per cent significance level, and found to be an improvement over a linear relationship and the state of no relation at all. When the values of R/S for the experiments of this group were plotted against the corresponding initial F/M ratios (Figure 10), the appearance of the data suggested the possibility of the existence of a single relationship for all systems studied in this group. Statistical analyses indicated the relationship of

$$R/S = 0.06 + 0.211(F/M) = 0.021(F/M)^2$$

to be preferred for the data as presented in Figure 10.

Glycerol Systems:Old Cells, Group IV

Figures 11 and 12 are presentations of the data acquired through the experiments of Group IV. A statistical analysis of a plot of R/S versus initial solids concentration (Figure 11) indicated that the following relationships are to be preferred:

Initial COD 900 mg/l:R/S = 7.89 - 0.268X
Initial COD 500 mg/l:R/S = 9.89 - 0.483X
Initial COD 1300 mg/l:R/S = $10.62 - 0.611X + 0.014X^2$

The values of R/S for these systems were plotted against initial F/M (Figure 12). The appearance of this data, as plotted in Figure 12, inferred the insignificance of a statistical analysis; therefore, none is presented. The data, however, was not so scattered as to inhibit the protraction of reasonable curves, representative of the trends of the data.

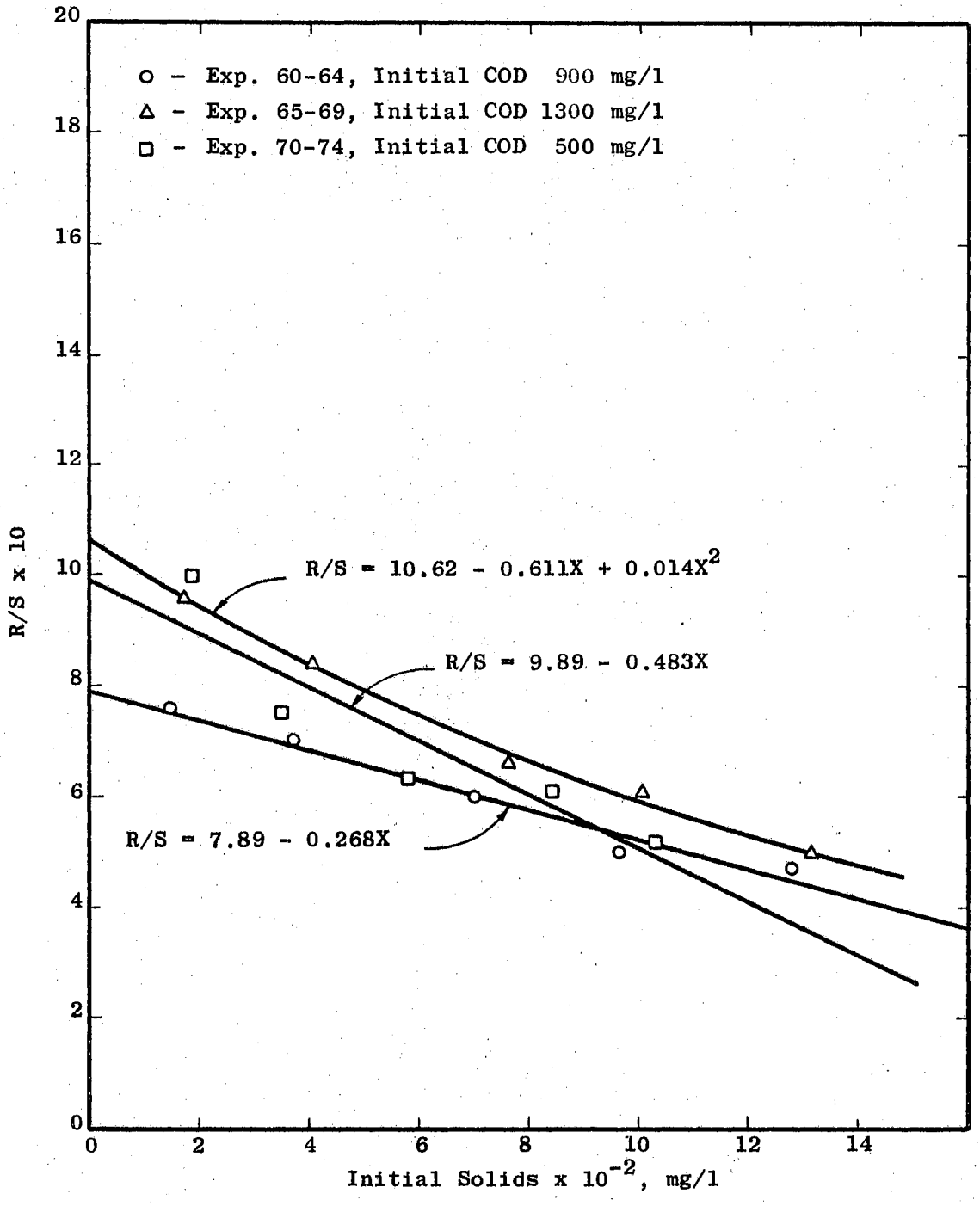

Figure 11 - Effect of initial solids on R/S for old glycerol cultures, Group IV.

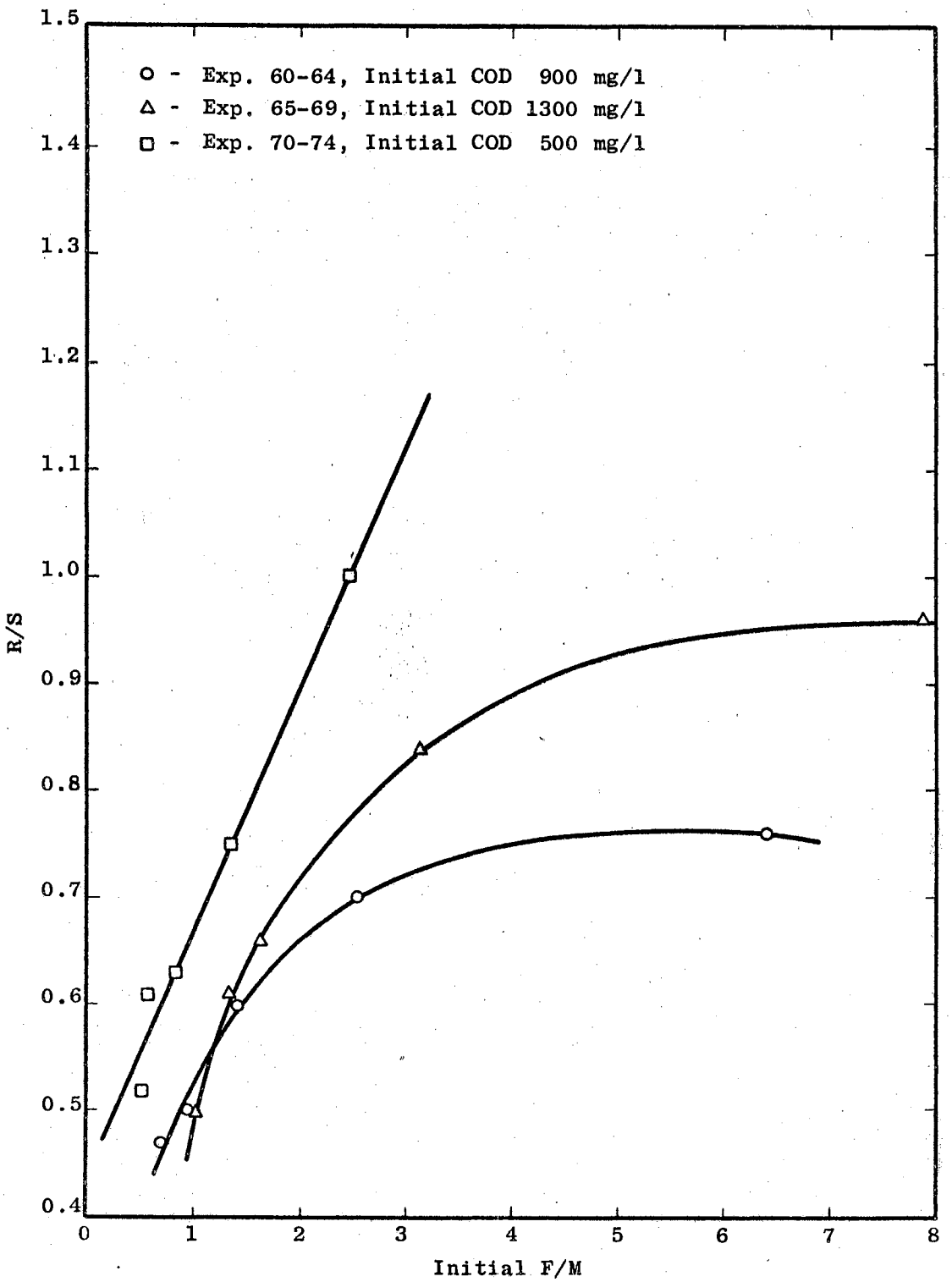

Figure 12 - Effect of F/M on R/S for old glycerol cultures.

CHAPTER V

DISCUSSION

This investigation on the partition between respiration and synthesis was conducted on a heterogeneous population of microorganisms in order that it might be applied generally to the situation of wastewater treatment. As many investigators have employed the soluble organic compound glucose as a substrate, glucose was the primary substrate studied in this investigation in order that the data thus obtained could be compared and correlated with that of others. It was also considered that many industrial wastes contain soluble organic compounds, as do the effluents of industrial and municipal wastewater treatment plants; therefore, an investigation on this type of substrate was in order. Fifteen experiments were conducted in which glycerol, a soluble organic compound of a nature differing from that of glucose, was the sole source of carbon. These experiments were conducted to determine if the use of a different type of substeate would yield relationships different from those determined with glucose. It has been determined that there exists a difference in the physio-chemical condition of bacterial cells of different ages (37); therefore,

experiments were conducted on both young and old cell cultures.

The results of the Group I (young cells metabolizing glucose) experiments as presented in Figure 3 indicated that systems having similar initial COD concentrations but unsimilar inocula manifest a completely random behavior when the partition between respiration and synthesis is considered with respect to the initial solids concentration. Figure 4 also indicates a random type of behavior when the F/M ratio is considered. When the R/S values of the experiments having similar inocula but unsimilar initial COD concentrations were plotted against initial COD concentration and initial F/M (Figures 5 and 6, respectively) it was again found that the partition between respiration and synthesis is not dependent on factors other than the microorganisms themselves.

When the results of the Group II (young cell cultures employing glucose) experiments were plotted, there was no significant trend in the ratio R/S as the ratio F/M was increased for the subgroups of experiments, i.e., systems with varying inocula from the same batch unit but having similar COD concentration. Regardless of the COD concentration employed, however, it was found that the ratio of R/S could be decreased as a constant function of the initial solids concentrations of the systems (Figure 8).

By comparing Figures 3 and 8, the effect of unsimilar inocula can be observed. In Figure 3 the experiments

plotted within each subgroup employed inocula from different batch units. Although a general trend of decreasing R/S with increasing initial solids concentration may be discerned, the trends of the individual subgroups do not support such an observation. When experiments using similar inocula, i.e., subgroups A', B', C', are considered in the same respect (Figure 8); however, it can be seen that the trends of the individual subgroups are in support of the overall trend, the initial COD concentrations exhibiting no effect. Hence, it may be concluded that it is the microorganisms themselves which, in the systems studied, have the most significant influence on the partition of the substrate between respiration and synthesis.

It is evident from Figure 9 that the initial COD concentrations of the systems studied did affect the partition between respiration and synthesis with old cells. There is a genuine trend of decreasing R/S with increasing initial solids concentration. This trend is supported and indicated by the three parabolic functions computed for the three series of experiments with initial COD concentrations of 500, 950, and 1550 mg/l. The effect of initial COD concentration is to increase the value of R/S, i.e., more of the substrate is channelled into respiration where more substrate is available at a particular level of initial solids concentration. This effect of initial COD is further illustrated in Figure 10 where it is evident that an increase in the ratio of F/M increases the ratio R/S. The

minimal amount of scatter of the data obtained from the old cell systems is attributed to the thirty-three per cent transfers employed in feeding the cultures which apparently prevented gross changes in the system from occurring. The observed trend of decreasing R/S with increasing solids concentration can best be explained when it is considered in the light of previous studies. Krishnan and Gaudy (28) and Rao and Gaudy (20) observed the early synthesis of low energy-requiring compounds for the buildup of metabolic pools required for synthesis. Gaudy and Gaudy (23) reported the product of this synthesis to be predominantly carbohydrate, especially where the substrate is a carbohydrate. It was reported that where the initial solids concentration is low, considerable cell replication was required before the oxygen uptake, i.e., energy expended, could be measured. However, as the initial solids concentration is increased, the number of cells functioning to synthesize low energy-requiring compounds is increased, resulting in increased removal of substrate during the period of low energy requirement. A comparison of the systems would thus yield less oxygen uptake required for synthesis in the case of high initial solids than in the case of low initial solids. In the systems herein considered, it is apparent that the same type of situation was encountered. As the initial solids concentration was increased (i.e., F/M decreased) a lower value of R/S was observed, thus indicating that with large inocula more of the substrate was removed for purposes

requiring small quantities of energy than was removed for similar purposes in systems of smaller inocula. This relationship between R/S and F/M is consistent with McKinney's (9) anticipation of a large excess sludge problem with higher F/M ratios, while a low F/M ratio would result in a minimum of excess sludge.

For the systems studied, it was found that, as presented in Figure 10, R/S was related to F/M by the parabolic function

$$R/S = 0.06 + 0.211(F/M) - 0.021(F/M)^2$$

This unique relationship between substrate partition and biological environment is unprecedented in the field of wastewater treatment, and may prove to have significant applications. As such a function apparently becomes constant for a sludge as it ages, it is obvious that, knowing the COD and biological solids concentrations of a system, one could easily predict the partition of the substrate into respiration and synthesis. Oxygen supply and sludge handling facilities could then be regulated to accommodate such partition. An analysis of the data of Rao (38) in accordance with the techniques herein employed was conducted to indicate the applicability of this parabolic relationship to other cultures. When the values of R/S were plotted against initial solids concentration (Figure 13), a trend of decreasing R/S with increasing initial solids concentration according to the parabolic function $R/S = 0.61 - 0.38X + 0.08X^2$ was evidenced. This relationship is similar

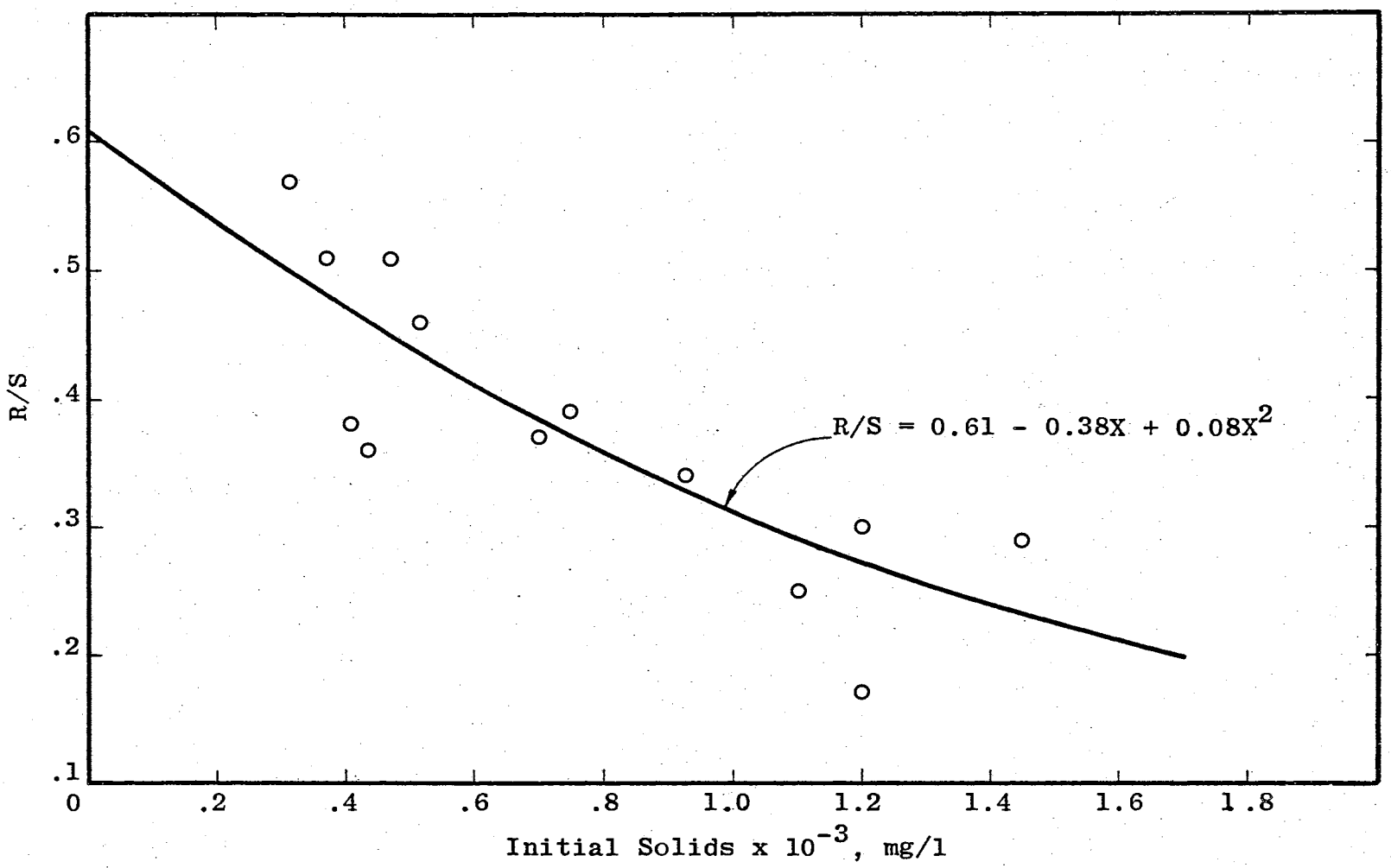

Figure 13 - Effect of initial solids on R/S for old glucose cultures studied by Rao.

to that observed for the systems studied in Group III of this investigation (Figure 9). In Figure 14, values of R/S were plotted against initial F/M for the recalculated data of Rao. The curve describing the relationship between the same parameters for the Group III (old cells metabolizing glucose) experiments of this investigation is also presented for comparison. The function describing the curve of best fit for the data of Rao:

$$R/S = -0.04 + 0.211(F/M) - 0.021(F/M)^2$$

compares favorably with the equation of the curve for the experimental data of this investigation:

$$R/S = 0.06 + 0.211(F/M) - 0.021(F/M)^2$$

the only difference between the two functions being the slight displacement of the vertical intercepts from one another. It should be noted that Rao employed an old sludge and an initial glucose COD concentration of approximately 2000 mg/l in all systems studied. The parabolic relationship between the ratios R/S and F/M can be discerned as valid for systems such as these herein reported. As a result of the above discussed glucose systems, the effect of sludge age may be postulated as a stabilization of the biochemical nature of the sludge. In order that the effects of the nature of the substrate employed on the trends in the ratio of R/S established with glucose might be observed, the tri-hydroxy alcohol glycerol was also utilized as substrate. From Figure 11 it can be seen that

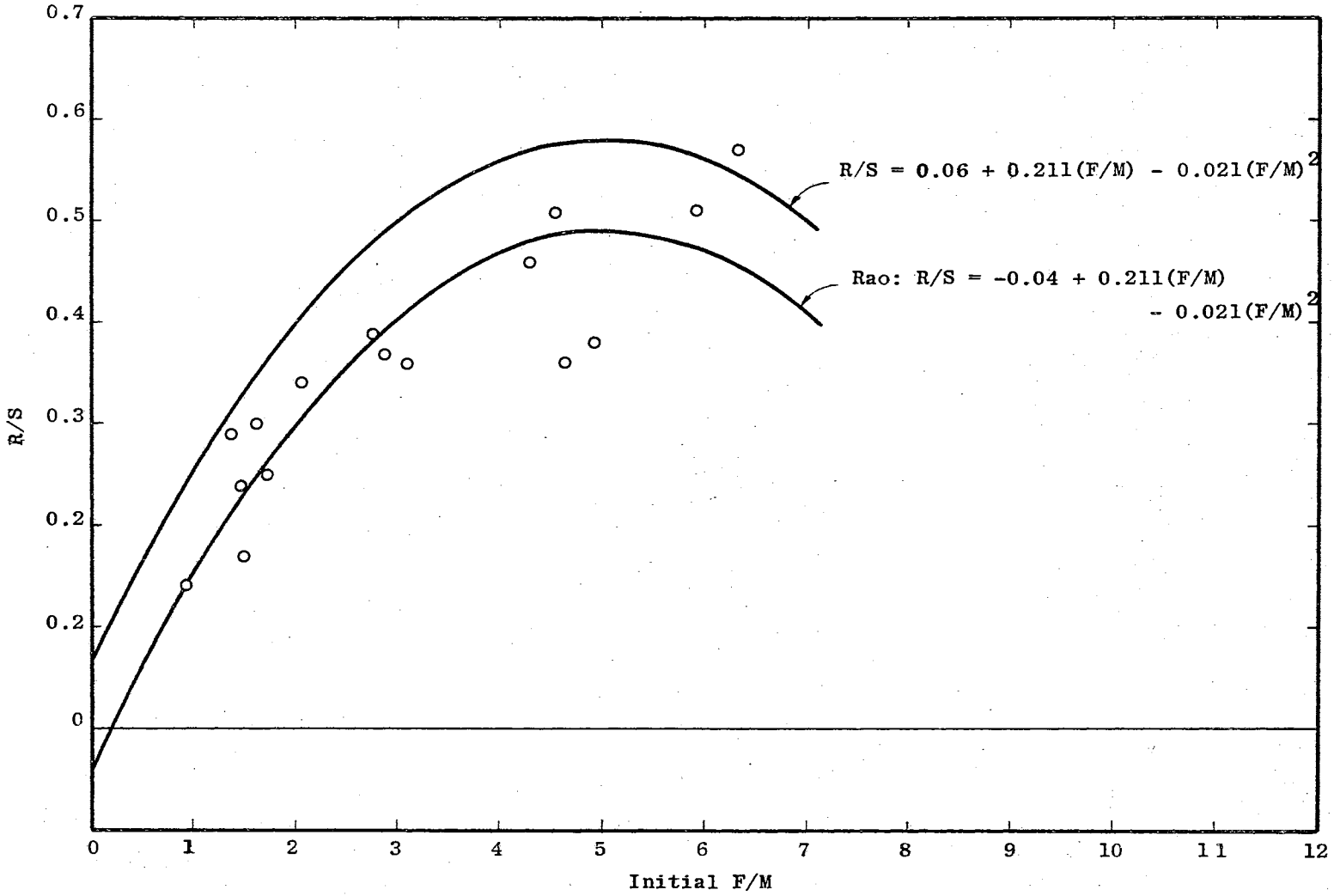

Figure 14 - Effect of F/M on R/S for old glucose cultures studied by Rao.

there is a decreasing trend approaching linearity for the plot of R/S vs. initial solids concentration for each of the initial COD levels studied. Statistically, the initial COD concentration has no definite effect on the partition between respiration and synthesis, although a scatter plot might indicate otherwise. Assuming the absence of a COD effect, the three sets of points were considered together and the effect of initial solids concentration on the ratio R/S was determined to adhere to the relationship $R/S = 9.7 - 0.62X + 0.02X^2$, where X represents initial solids concentration multiplied by 10^{-2}. The general trends exhibited in Figures 11 and 12 are consistent with those established by the glucose systems; however, the mathematical functions of the systems employing different substrates are not in agreement. A comparison of Figures 9 and 11 indicates that, for old cells, the ratio R/S at a particular initial solids concentration is higher where glycerol is metabolized than where the substrate is glucose, i.e., with glycerol more of the substrate is channelled into respiration than with glucose. It may, therefore, be generalized that the nature of the substrate employed does exert an influence on the manner in which the substrate is utilized, i.e., the partition between respiration and synthesis.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The partition of an exogenous substrate between respiration and synthesis has been studied, employing the ratio of respiration to synthesis (R/S) to indicate such partition. This partition of a substrate by microorganisms, and thus the parameter R/S, is considered to be a function of microbial systems, the importance of which has been generally neglected. A genuine understanding of the ratio of R/S and the factors related to its control could be applied advantageously in the prediction of excess sludge production and oxygen requirements in the wastewater treatment field, in economical operation in such processes as enzyme production, and in equipment selection and design for any microbial process.

From the investigation herein reported, it is concluded that:

1. Young cell systems exhibit a random partition of substrate between respiration and synthesis. The partition is apparently a function of the microbial population involved.

2. Cell age has the effect of stabilizing the biochemical nature of a microbial population. With older cells, the magnitude of the partition between respiration and synthesis in relation to other variables can be defined through laboratory studies.

3. The variables of initial COD and biological solids concentrations exert an influence on the manner of substrate utilization, i.e., the channelling of substrate into respiration and synthesis, by microorganisms, and can be employed as controlling factors in systems where their relationship can be identified. With systems employing old cells metabolizing glucose at initial COD concentrations of 500 to 2000 mg/l and initial F/M ratios of approximately 0 to 6.5, the partition of the substrate may be predicted by the equation:

$$R/S = a + 0.211(F/M) - 0.021(F/M)^2$$

where a, the value of R/S at a F/M ratio of 0.0, may vary with the physiological condition of the sludge.

4. The nature of the substrate employed will affect the magnitude of the partition, but not the general trends of decreasing R/S with increasing initial solids concentration and increasing R/S with increasing F/M.

CHAPTER VII

SUGGESTIONS FOR FUTURE WORK

As a result of the investigation herein reported, the following suggestions are presented for future study of the partition of exogenous substrates between respiration and synthesis in microbial systems:

1. This study has indicated that the nature of the substrate employed will exert an influence on the ratio of R/S. Further work on the nature of such an influence with various types of substrates could be of considerable value.

2. Investigations on substrate partition employing whole wastes as substrates could serve as a further indication of the applicability of the concepts herein presented to the field of wastewater treatment.

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