RESIDUAL SOLUBLE ORGANICS FROM THE ACTIVATED SLUDGE PROCESS

RESIDUAL SOLUBLE ORGANICS FROM THE ACTIVATED SLUDGE PROCESS

BY

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A THESIS

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE

MASTER OF ENGINEERING

McMASTER UNIVERSITY

NOVEMBER, 1971

MASTER OF ENGINEERING (1971) McMASTER UNIVERSITY
(Chemical Engineering) Hamilton, Ontario

TITLE: Residual Soluble Organics from the Activated Sludge Process

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NUMBER OF PAGES: ix; 81

SCOPE AND CONTENTS:

A completely-mixed batch biochemical reactor was used to study the degradation of soluble organic carbon using a mixed microbial culture. Carbon sources, representative of actual wastes were chosen from among those whose metabolism has been described in the literature, and included:

a) Phenol - an aromatic hydrocarbon

b) Glutamic acid - an aminoacid

c) Glucose - a monosaccharide, and

d) Starch - a polysaccharide.

Experiments were performed to determine if a mathematical relationship could be developed between the level of residual organic carbon and operating parameters of the activated sludge process. An organic residue which could not be removed from the liquid medium even after an extended period of contact of the waste with activated sludge was monitored during the batch tests. Also, measurements of specific substrate degradation, and the total oxygen demand were analysed during the tests.

ACKNOWLEDGEMENTS

I wish to sincerely thank Dr. K. L. Murphy for his encouragement and valuable advice during this investigation. My thanks are extended to Mrs. A. Latoszek for professional assistance with the biological examinations and for providing the pictures of the bacteriological cultures. The financial assistance provided by the Department of Chemical Engineering, McMaster University, and the Federal Department of Energy, Mines and Resources is gratefully acknowledged.

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CHAPTER 1

INTRODUCTION

Discharged waste water at one point of a stream necessarily becomes part of the source water for further usage. Biological purification of pollutants in waste water is dependent upon a complex balance of pollutants, organisms and environmental conditions. The ability to renovate waste water so that it is acceptable for varied use depends in part upon a better understanding of the aerobic bio-oxidation process. Some organics remain in the effluent following any degree of biological treatment, and are found with laboratory units using a simple, single substrate and a pure culture. Further, conventional secondary treatment is not satisfactory with respect to the removal of nitrogen, and phosphorus, and must be coupled with advanced waste treatment processes to achieve an even higher quality of effluent.

If sewage effluent is to be reused for municipal water supply, it is necessary to determine what the remaining organic carbon and nitrogen compounds in these effluents are in order that the health aspects of these residuals can be estimated. The degree of treatment required must be modified, taking into account the specific uses as well as the nature of the constituents, so that the desired environmental protection can be accomplished.

This study was directed towards the attainment of a greater insight into the biological treatment process. The emphasis was placed upon examining the extent to which the incomplete oxidation of the substrate could produce residual organic matter in secondary effluents. Simple degradable substrates were fed to activated sludge microorganisms in batch reactors and the residual organics monitored. Attempts were made to correlate the concentration of residual matter to operating conditions and the type of substrate.

CHAPTER 2

LITERATURE REVIEW

Soluble organic matter remaining in effluents may be either biologically degradable or relatively non-degradable. This point was extended and described by Chudoba (1967), who proposed the ratio BOD:COD as a degree of biodegradability. He demonstrated this ratio was 0.5 for sewage and in the effluent it decreased below 0.2. He used eleven simple substrates as feed for both batch and continuous studies, and by means of the BOD_5:COD ratio of the effluents estimated the amount of residual biodegradable material. This ratio was about 0.5-0.7 in the influent and 0.2-0.03 in the effluent. It was interesting that the BOD_5 tended to an absolute limit (approximately 5 ppm), while the residual COD tended to a relative limit (approximately 1% of the initial value). After long term aeration (30-150 days), the biodegradable portion of the effluent (measured as BOD_5) approached zero, but the COD value decreased by only 50%, and the residual value remained significant (6-51 ppm as COD). These experiments have indicated that a mathematical correlation between the residual COD and the initial concentration of substrate yielded a "first order" response:

$$COD_r = a \times COD_i + b \qquad (1)$$

where: COD_r is the residual COD in mg/l,

COD_i is the initial COD in mg/l, and

a and b are constants.

Parameter b was reported almost zero. The effect of the activated sludge concentration was not considered, and a variation of "a" from 5×10^{-3} - 1×10^{-2} was reported.

A further attempt to find a mathematical model between residual and initial COD was made by Eckhoff and Jenkins (1966) using a continuous system. They developed the following relationship:

$$COD_r = a \times COD_i + b\ G \qquad (2)$$

in which

$$G = Dc + k \qquad (3)$$

where:

k is the endogenous metabolism coefficient,

D_c is the cell dilution rate, and

a and b are constants

The second term of equation (2) was negligible. However, this model can be used only for continuous study, as the sludge age can not be defined satisfactorily for batch study.

Experiments carried out by Chudoba (1967) indicated that a certain range of sludge loading existed in which it was possible to obtain minimum values of residual COD. He found this range to be 0.5-1.2 g COD/g MLSS-day. Outside this range, the percentage of residual COD increased. However, only four runs of each substrate were used to describe this phenomenon over a fairly large range of substrate (1-8 g/l) coupled with a relatively small variation in MLSS (approximately 1000 mg/l).

Further, the analytical methods used in this study, the BOD and the COD, have inherent weaknesses which must be considered. Neither is of fundamental significance. COD does not measure carbon, but the dichromate oxidation capacity, and the test itself oxidizes different organics from between 9 - 100% of the TOD (Heffler, 1970).

Other workers have reported that a great percentage of the residual COD is attributable to metabolic activity of the organisms in the sludge. Washington and Symons (1962) indicated an extracellular material containing polysaccharides accumulated in the activated sludge process. If metabolic activity is measured as simple substrate removal (eg. glucose), the substrate concentration reaches zero very rapidly and in one case utilizing only about 18% of the theoretical oxygen demand (McWhorter and Heukelekian, 1964). The usual explanation is that cells easily and quickly ingest simple substrates and later use the carbon for synthesis. During this endogenous phase, the cells continue to use oxygen and to produce extracellular material. When the supply of external food is exhausted, cells begin to die and cell protoplasm is progressively degradated, by so-called endogenous respiration, giving products which serve as food sources for the living cells which remain.

Some of the main degradation pathways for aerobic systems are illustrated in Figure 1 (transferred from Wilson, 1967).

FIGURE # 1

MAIN METABOLIC PATHWAYS (AFTER WILSON, 1967)

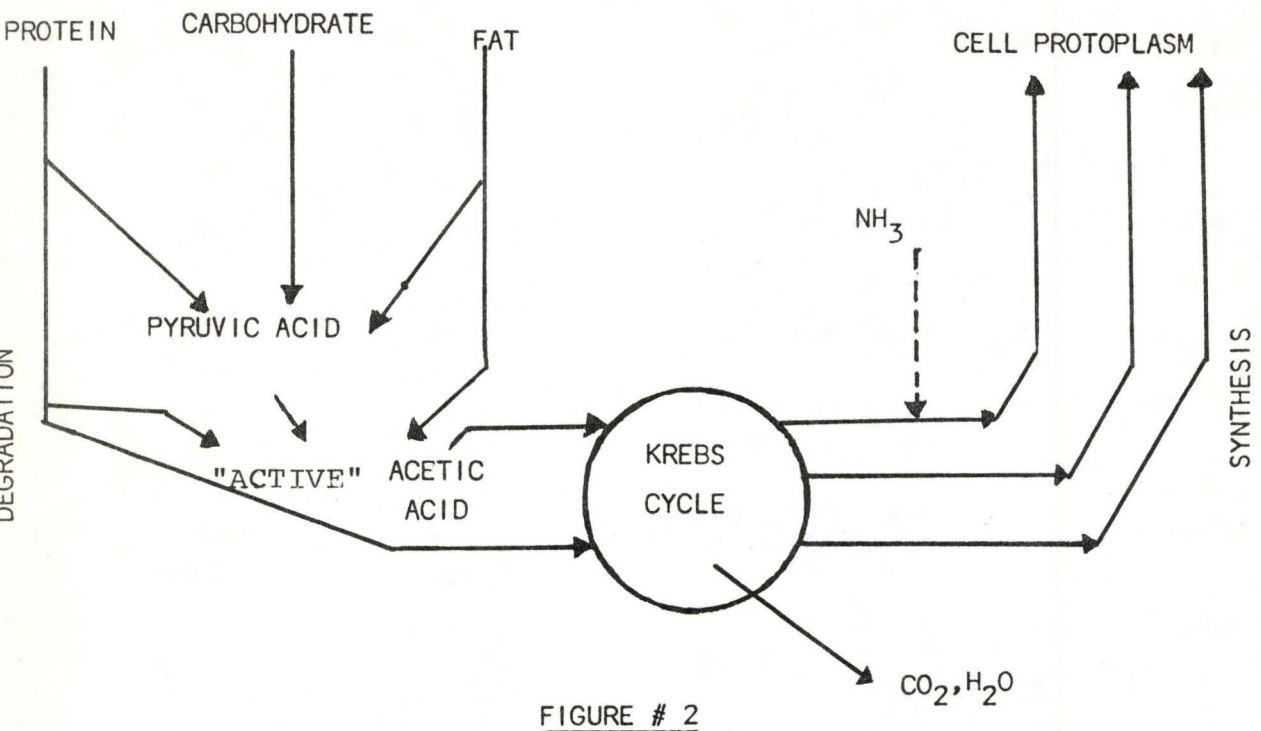
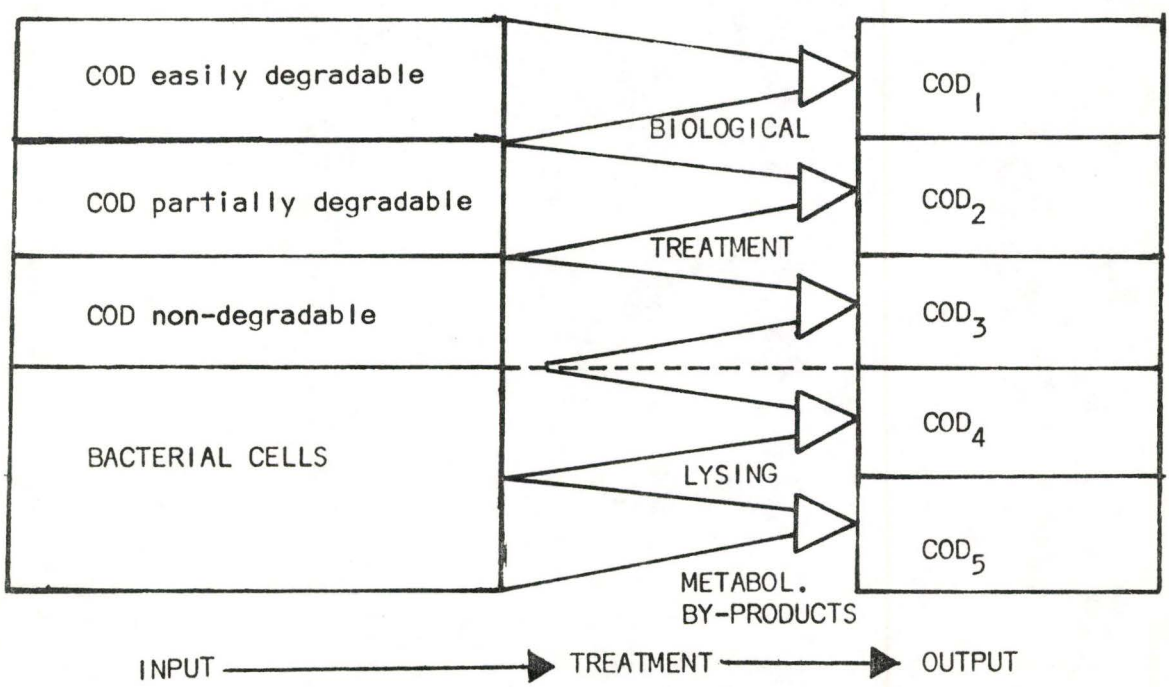


FIGURE # 2

SCHEME of the CHANGE in COD of SEWAGE in the BIOLOGICAL TREATMENT UNIT.

(AFTER CHUDOBA, 1967)



Another mechanism for the removal of starch has been postulated by Banerji et al. (1968). This hypothesis explains removal of the starch from solution by adsorption onto the cell surface followed by degradation and ingestion of the degradation products. The postulated model for this removal could be the Freundlich isotherm expression, which seems to fit as well as any method of correlation.

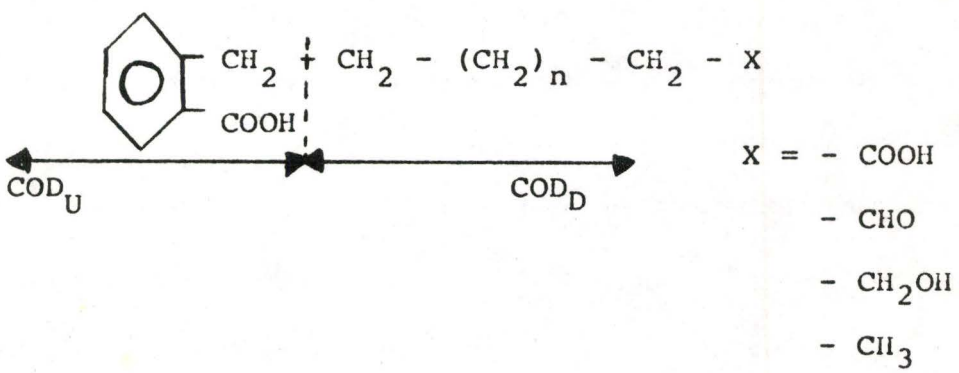
Generally all work in the field leads to the conclusion that four sources for residual organics can be postulated:

(1) Metabolic waste-products (by-products) of the organisms;

(2) Materials which enter with the influent and are at least partially degradable, but are not completely removed during treatment. The COD of this material can be divided into degradable (COD_D) and "non-degradable" (COD_U) portions.

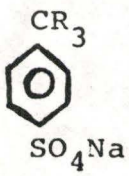
$$COD = COD_D + COD_U$$

For example:

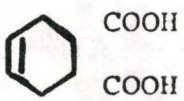


(3) Relatively non-degradable material entering the raw sewage. Material falling into this class is hardly degradated or adsorbed,

and passes the biological purification almost unchanged. For example:



ABS
(Alkyl benzene sulfonates)



THPA
(Tetrahydrophthalic acid)

(4) Cellular material which enters solution when the cells lyse. All possible sources of residual COD are illustrated in Figure 2 (after Chudoba, 1967).

Pirt (1959) studied the metabolism of glucose in a batch system. He observed that the removal of glucose occurred at a slow rate followed by removal at an accelerated rate with a distinct break point. A small amount of formic acid was produced towards the end of the period of utilization of glucose, but it was metabolized after the glucose was exhausted. After the break point in the removal curve of the glucose, acetic acid was produced. The possibility that acetic acid was metabolised later in the batch culture after exhaustion of the glucose was not investigated.

The last postulated source of residual organics is material from dead cells. Painter et al. (1961) detected muramic acid in solution in the culture medium which could only have arisen from bacterial cell walls. This suggests that dead cells do contribute to the residual organics in solution during the biological process. The same results were reported by Helfgott

et al. (1970). They made an extensive classification of effluent constituents and reported a certain quantity of organics in the effluent which could not be removed by activated carbon sorption. This class of residual material was classified as cationic organics. These materials remained cationic even at high values of pH (above 8). The authors hypothesized that the residual materials are intermediate breakdown products of protein that originate from the cell walls of the micro-organisms that thrive in biologically treated sewage. Bacterial cell walls contain in addition to polysaccharides the lipids a large quantity of amino acid polymers reported as "unusual proteins." The antibacterial effect of this group of organics is not excluded; cationic detergents are used because of their antibacterial ability.

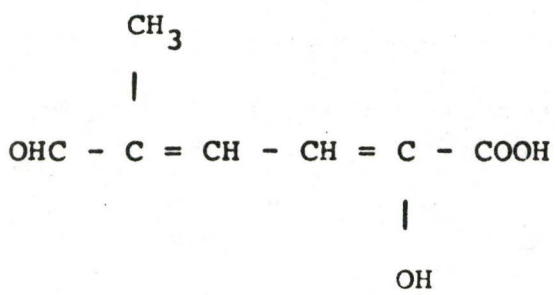
Hashimoto (1970) analysed the oxidation product p-cresol from a strain of yeast which had been isolated from the activated sludge previously acclimated to phenol. The author assumed the existence of only a few metabolites, and found only one. The metabolite p-cresol was extracted twice with ether and re-crystallized three times from chloroform-methanol mixtures. Analysis of the metabolite was carried out by means of paper chromatography, UV spectroscopy, IR spectroscopy and nuclear

magnetic spectra. During the fermentation, UV spectra were carried out frequently. At the start of the experiment, the absorption maximum was at 276 mμ due to p-cresol, which subsequently shifted to 264 mμ when oxygen uptake completely stopped. The result of elemental analysis yielded:

C - 53.7%

H - 5.3%

The titration analysis yielded one carboxyl group belonging to each molecule. The absorption maximum by UV spectroscopy was at 263 mμ in methanol. IR spectra suggested the presence of - OH, - COOH, - C = C - and - CH_3 groups. The most probable structure of this metabolate, $C_7H_8O_4$, seemed to be:



An extensive description of material in secondary effluents was done by Bunch et al. (1961). The sample was filtered and concentrated by evaporation. They gave an organic balance as:

ether extractable	< 10%
proteins	< 10%
carbohydrates	< 5%
xannins and lignins	< 5%
ABS	≐10%
unidentified	≐65%

The "unidentified" fraction was low molecular weight compounds which passed a semi-permeable membrane.

Hunter et al. (1965) found that the soluble organic matter in sewage was composed largely of ether extractable materials of which organic acids were the primary constituents (56%). Other components were amino acids and sugars, detergents, volatile acids, phenols and uric acid. This implies that sewage and effluents contain a complex mixture of organics rather than a few refractory materials. On the other hand, most of the constituents of effluents are yet unknown. Obviously the complete explanation of this problem depends upon the development of suitable analytical techniques which are still some distance in the future. The major problem is the very small concentration of organics found in effluents. The most practical and available methods appear to be conventional tests supplementing group analysis.

Kupchik et al.(1962) identified some of the lower fatty acids in secondary effluents, but this included only 7.1% of the total organic acid content. The lower aliphatic acids appear as intermediate products regardless of the original substrate.

Basically, present analytical methods can not determine whether the residual organics in effluent represent only a few constituents or are a complex mixture of materials. It can not be determined whether these residuals pass through the conventional treatment plant unaffected or are the product of the treatment

(in the case of raw sewage). Painter et al. (1961) stated that the residuals are small concentrations of a large number of compounds. Some fractions are highly volatile and can be found even in distilled water made of secondary effluent (Helfgott et al., 1970).

CHAPTER 3

EXPERIMENTAL METHODS AND EQUIPMENT

3.1 Description of Apparatus

The major component of the apparatus was a conical reaction vessel. Dispersed bubble aeration was used to mix the constant volume reactor and to supply the system with oxygen. Compressed air, supplied at 20 psi was introduced into the reactor through a fritted glass disc which was fitted to the bottom of the reactor. The system is illustrated in Figure 3. Four reactors, containing 2-6 litres, were used. The tops of reactors were capped to reduce evaporation and minimize contamination of the contents of reactors.

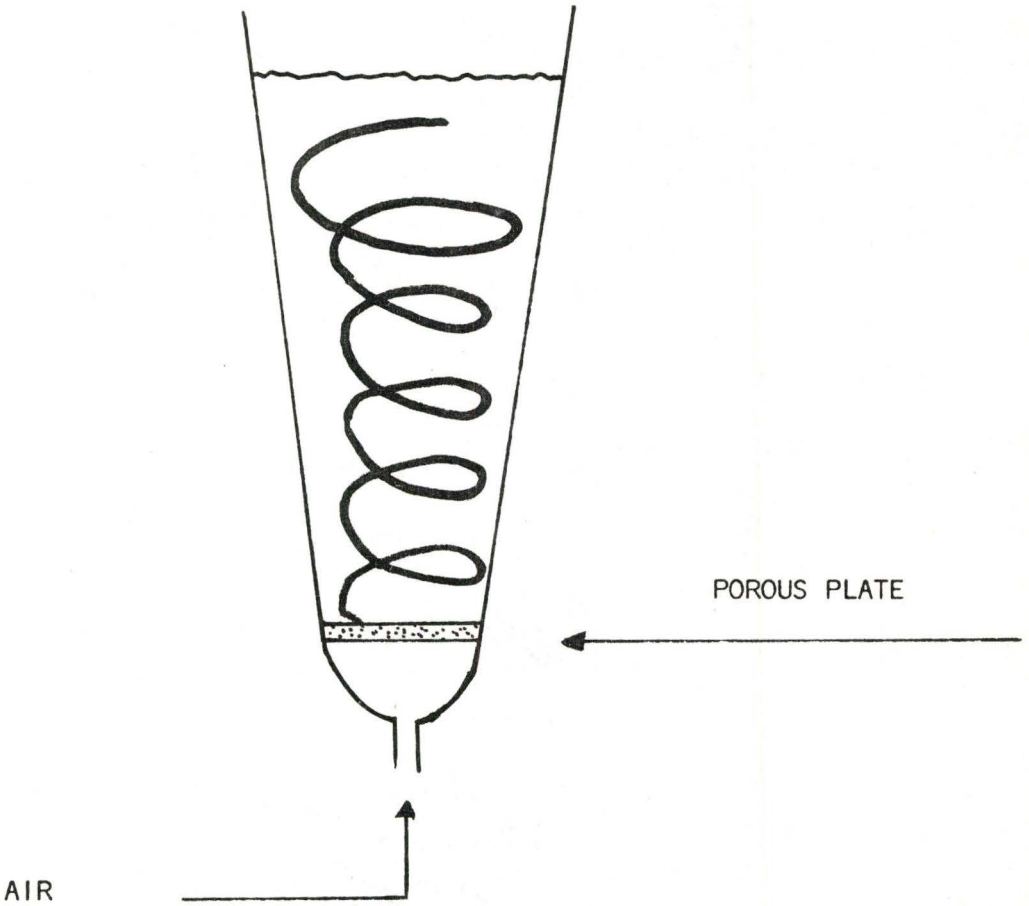
3.2 Organic Carbon Source

Simple, single substrates (glucose, soluble starch, glutamic acid and phenol) were used as a source of organic carbon. The substrate degradation process may be defined as a biochemical process in which micro-organisms change soluble organic substrate into final products following a network of possible reaction paths, all of which may consist of several combinations of consecutive and parallel reactions. The strength of organics was measured in terms of organic carbon, specific substrate concentration and the total oxygen demand.

3.3 Microbial Culture

The reactors were seeded with an activated sludge inoculum obtained from the Burlington Skyway sewage treatment

FIGURE # 3



BIOLOGICAL REACTOR.

plant. Each inoculum was acclimated aerobically to the particular substrate in the presence of nutrients. Generally, acclimatization of the sludge was considered to be achieved when the organisms grew actively on the new substrate.

3.4 Nutrient Media

For adequate nutrition of micro-organisms, a C:N:P ratio of 40:5:1 is sufficient (Eckenfelder and O'Connor, 1961). The following stock solutions were made to provide nutrients:

Nutrient	Stock Solution mg/l	ml of Stock Solution added/1000 mg of C
$(NH_4)_2SO_4$	2500	10
$FeCl_3 6H_2O$	1	2
K_2HPO_4	50	5
$MgSO_4 . 7H_2O$	50	5

Other trace elements were assumed to be supplied by the tap water, which was used as a dilution medium for the substrate. In addition, 10 ml/l of the phosphate buffer (pH 7.0) was used to control the pH of the experiments. This was supplemented when necessary with addition of sodium hydroxide.

3.5 Operating Conditions

The metabolic pathways of the substrate are probably most dependent on process reactants (the species of sludge, and the composition of organic substrate), and the system environment (pH, concentration of trace elements, temperature, pressure, etc.). In this study, two independent variables: the initial carbon

concentration and initial solids concentration, were studied for each substrate. The initial organic soluble carbon ranged from 100 mg/l- 1500 mg/l and the initial concentration of solids ranged from 200mg/l to 2000 mg/l. The operating levels of the two factors are illustrated in Table 4 in Appendix 1. For each experiment the acclimated heterogenous population of sludge was harvested by centrifugation and washed in 0.05 M phosphate buffer (pH 7). The washed cells were then resuspended in fresh medium containing the same carbon source on which they had been growing. The operating temperature was not controlled and fluctuated between 24°C - 27°C. Similarly, the air flow through the reactor was not controlled, but was approximately 1 litre/minute. This flow gave a measurable dissolved oxygen level in the reactor.

The pH was controlled by adding phosphate buffer (pH 7). In some cases this was not satisfactory, and the pH had to be adjusted approximately to 7 with NaOH solution. The pH was maintained normally at 7 $\pm$ 1.

3.6 Variables Under Study

Preliminary testing indicated several variables which seemed to affect the residual level of organic carbon. The influence of the initial level of organic carbon (C_C) and the initial suspended solids (C_B) appeared to be the most significant variables. These were assumed to be mutually independent, in an operational sense, and controllable over a discrete range, typical

of that used in the activated sludge process. These two variables were investigated in a two-variable experimental analysis. The mathematical model considered in this study was:

$$C_R = f(C_C, C_B)$$

where: C_R is the concentration of residual organic carbon.

From the preliminary experiments it became apparent that there are other variables affecting the residual carbon concentration, specificly sludge characteristics (eg. species, sludge age), the kind of substrate, pH, sludge loading, air flow rate, and temperature of the batch. In this study pH, air flow rate and temperature were controlled while the effect of the kind of substrate and the sludge loading were investigated. The sludge organisms were acclimated to the particular substrate but no specific study on composition or sludge age was attempted.

CHAPTER 4

EXPERIMENTAL RESULTS AND INTERPRETATION

4.1 Preliminary Runs

To obtain preliminary information on the range of organic residuals and the removal of organic soluble carbon for the specific substrates used in this study, preliminary batch tests were carried out. The results obtained in six of the preliminary experiments are illustrated in Figures 4 through 9.

Since the specific removal rate is defined to be the velocity of organic carbon removal per unit mass of micro-organisms, it is evident that the magnitude of this parameter will be dependent upon organic carbon concentration (C_C). The type of substrate removal curve was found to be dependent on the initial food:microorganisms ratio. These curves resemble the two-phase kinetic mass transfer model as described by Chudoba (1969). If the ratio of food (COD): micro-organisms is greater than two, the removal rate is not constant, but has a lower initial rate. High initial phenol concentration in a system can inhibit the removal of carbon (see Figure 9) although the sludge was acclimated to phenol.

In general, from these studies it may be concluded that:

(1) the decrease in concentration of substrate, TOD and soluble organic carbon follows an arithmetic linear decrease with time;

(2) until the substrate had been exhausted, the removal curves for organic carbon, substrate and total oxygen demand appeared to have a similar pattern;

(3) the relationship between substrate removal and initial solids concentration was found to be linear;

(4) while the substrate concentration reaches zero following "zero-order" kinetics, the concentration of organic carbon and TOD reaches relatively steady-state levels after the substrate had been exhausted;

(5) steady-state organic carbon levels of approximately 20-70 ppm of carbon were observed in all runs. The absence of measurable substrate indicated that this residual carbon was qualitatively different from the carbon source, and

(6) the minimum value of residual carbon was observed in the breakdown point of the carbon removal curve where the rate of carbon removal becomes zero. Beyond this point a small increase in the carbon concentration was observed. (Probably organic acids and other by-products of metabolism were released into the medium.)

4.2 Organic Carbon Released by Micro-organisms

To obtain information on whether activated sludge could release organic carbon into the medium, as was postulated in the previous section, activated sludge was harvested from the culture medium by centrifugation, washed three times with 0.05 M phosphate buffer (pH 7), and resuspended in 0.05 M phosphate buffer. The suspension was aerated, but no external source of soluble organic carbon was added. Organic carbon

FIGURE # 4

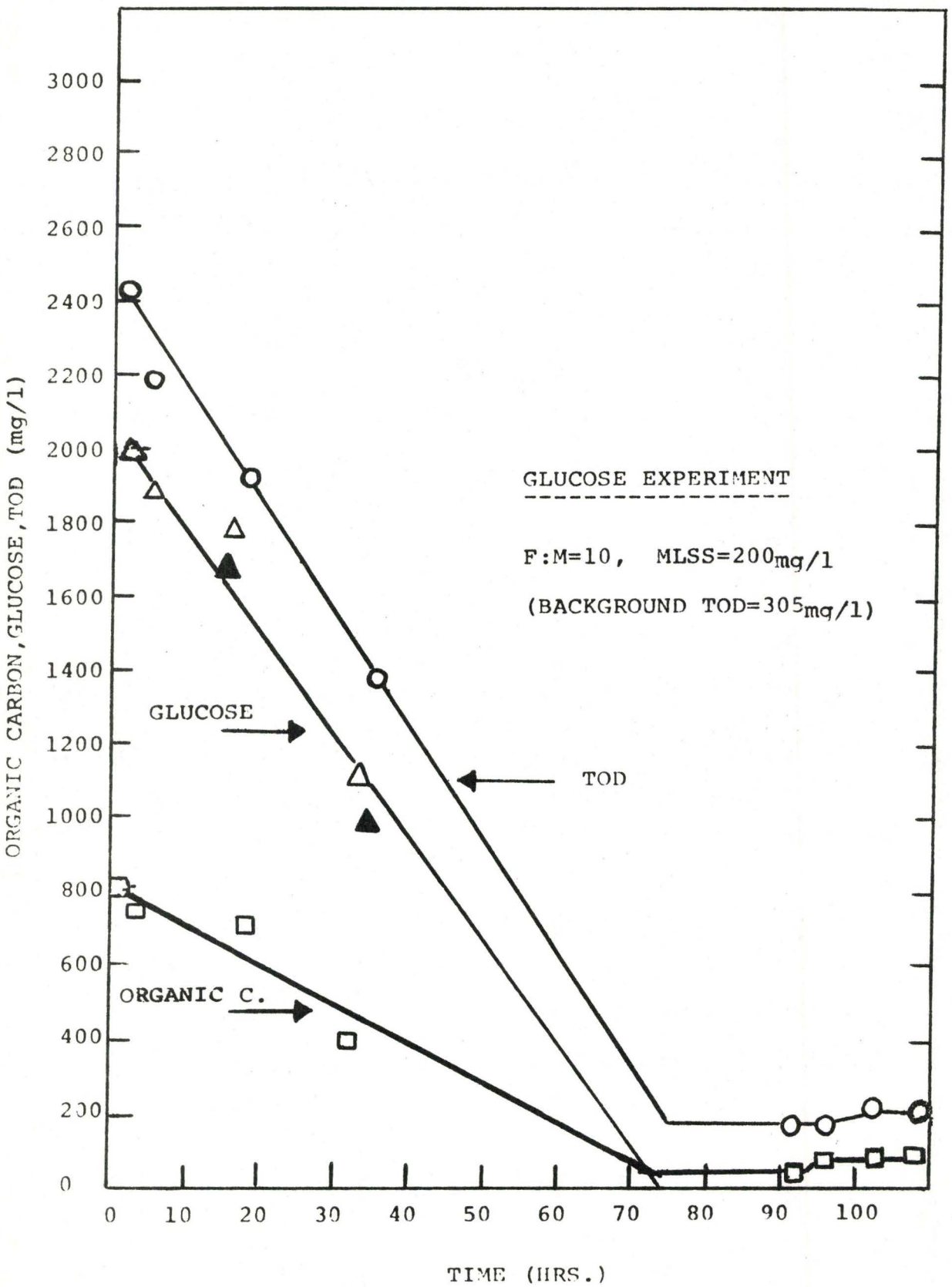

GLUCOSE EXPERIMENT
F:M=10, MLSS=200mg/l
(BACKGROUND TOD=305mg/l)
GLUCOSE
TOD
ORGANIC C.
ORGANIC CARBON, GLUCOSE, TOD (mg/l)
TIME (HRS.)
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
0
10
20
30
40
50
60
70
80
90
100

FIGURE # 5

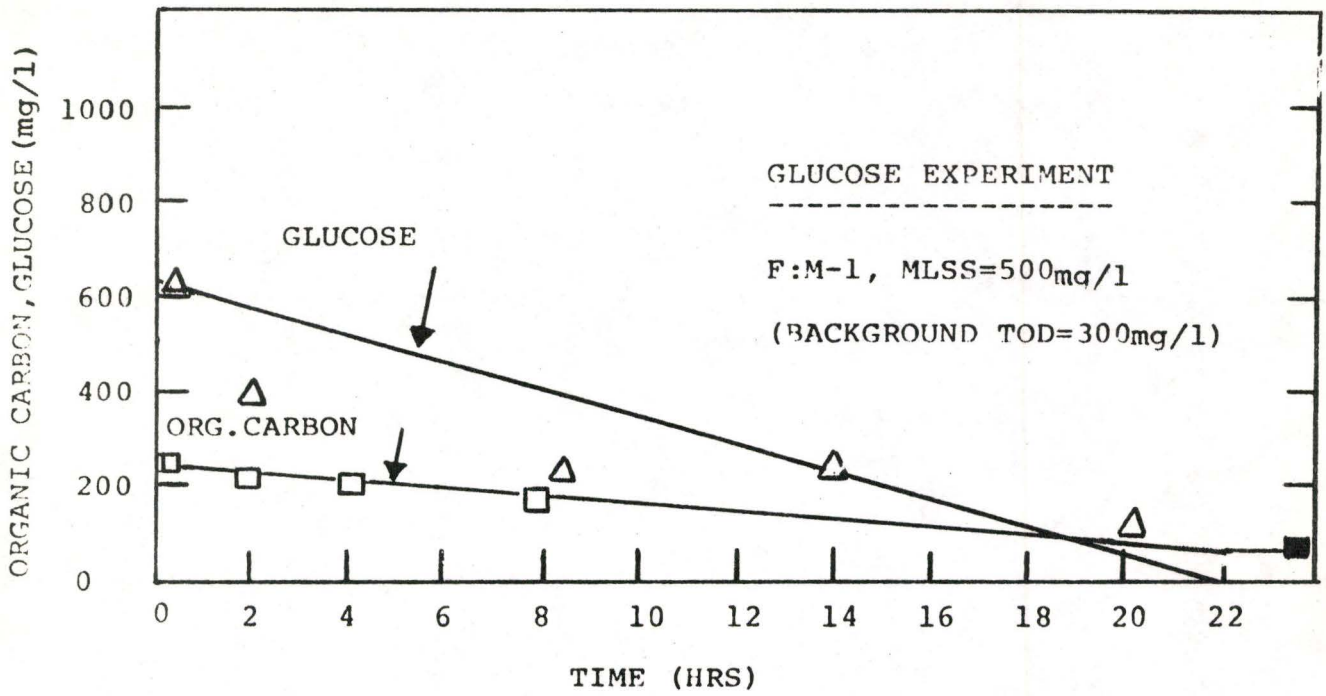


FIGURE # 6

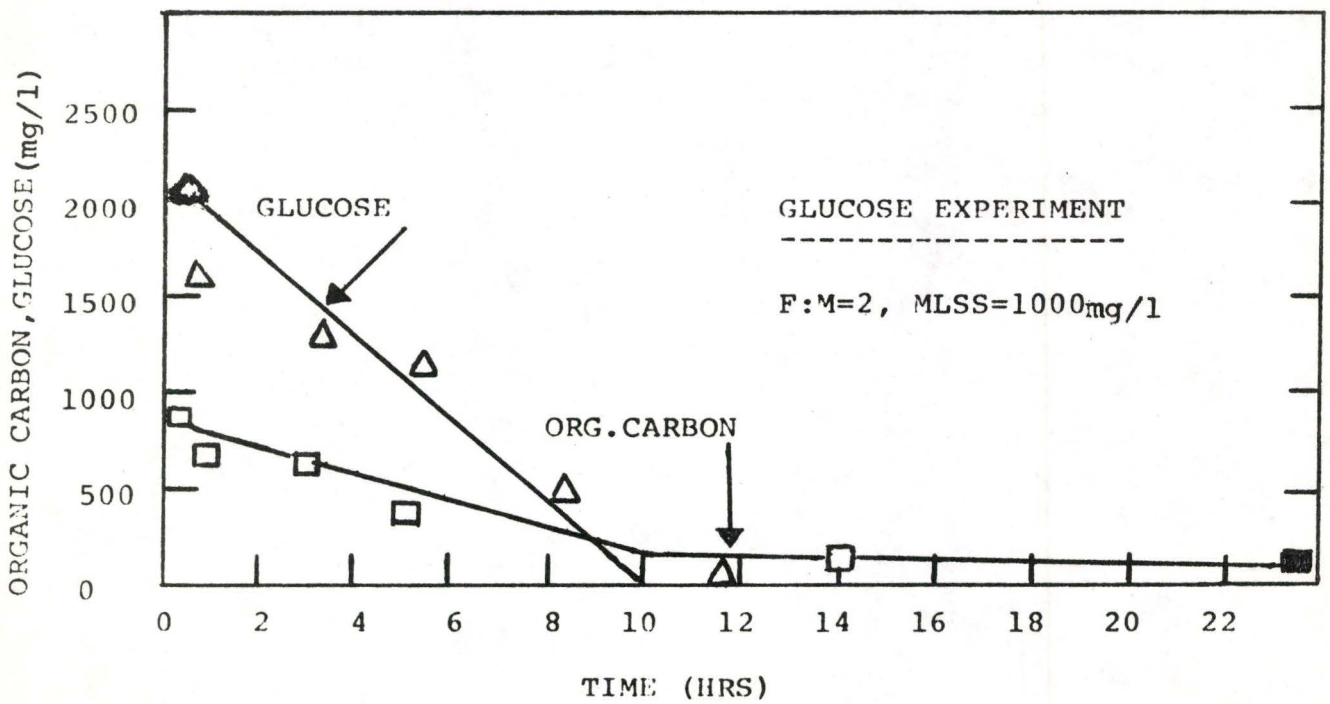


FIGURE # 7

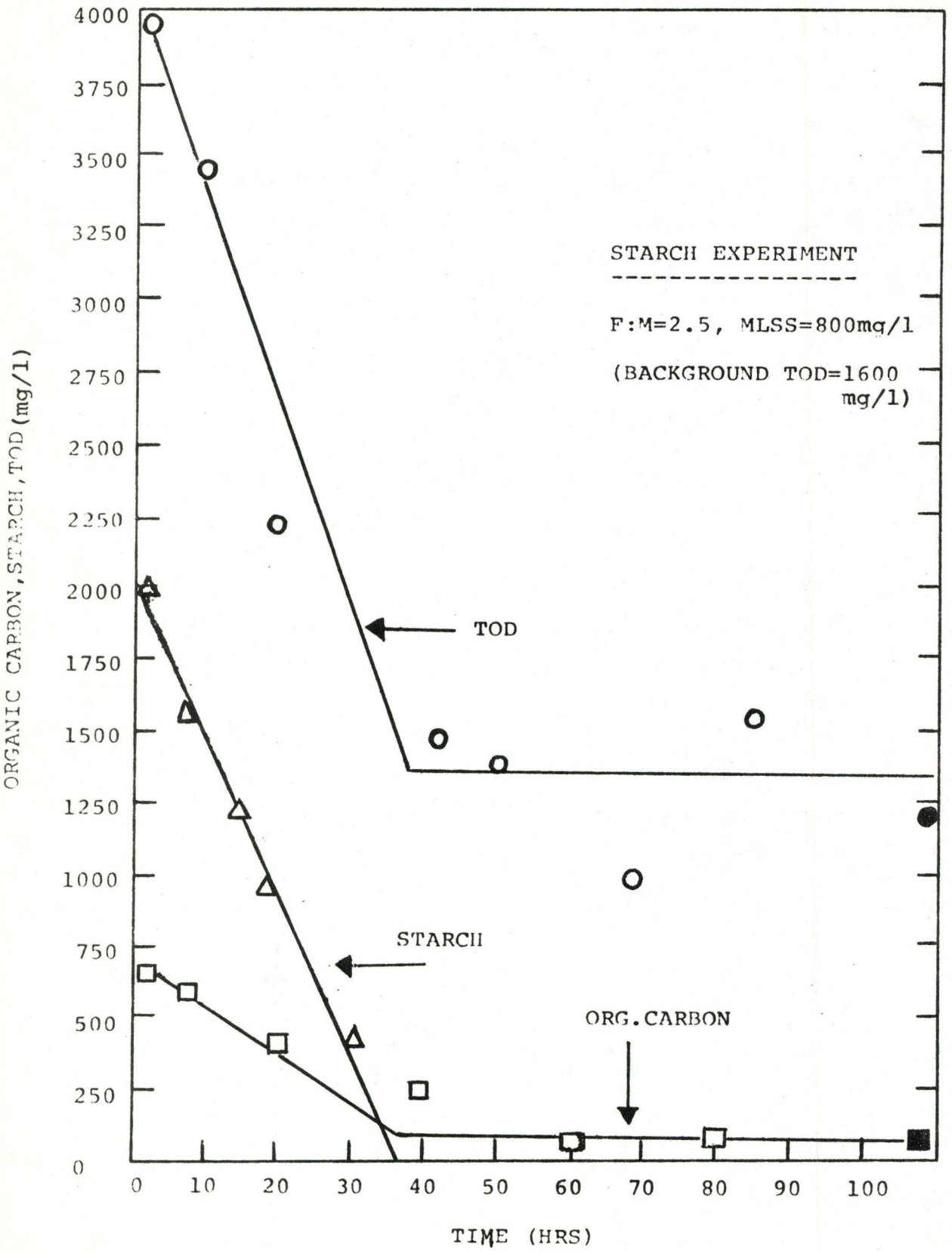

STARCH EXPERIMENT
F:M=2.5, MLSS=800mg/l
(BACKGROUND TOD=1600 mg/l)
TOD
STARCH
ORG.CARBON
ORGANIC CARBON, STARCH, TOD (mg/l)
TIME (HRS)
4000
3750
3500
3250
3000
2750
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
0
10
20
30
40
50
60
70
80
90
100

FIGURE # 8

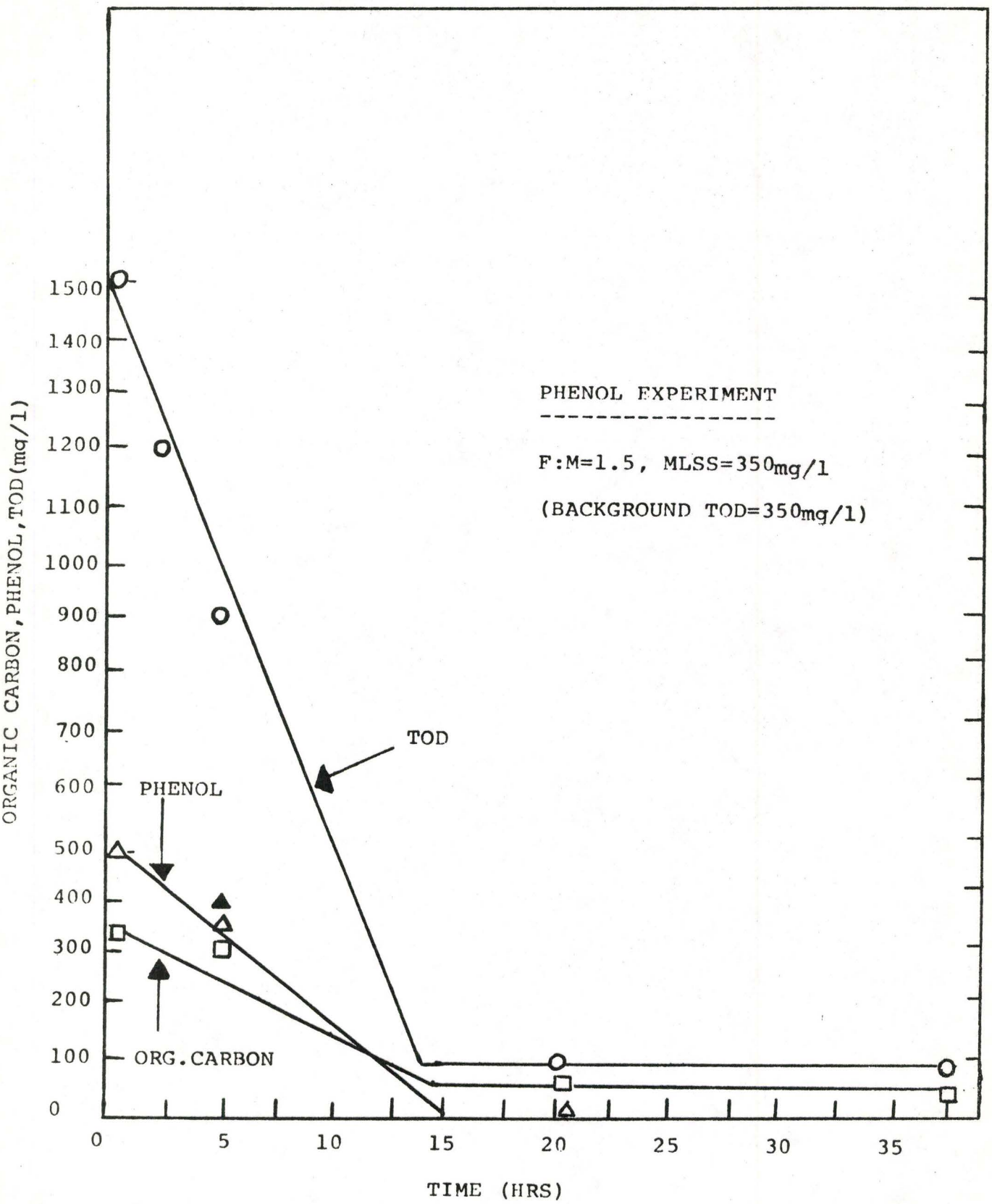

PHENOL EXPERIMENT
F:M=1.5, MLSS=350mg/l
(BACKGROUND TOD=350mg/l)
TOD
PHENOL
ORG. CARBON
ORGANIC CARBON, PHENOL, TOD (mg/l)
TIME (HRS)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
0
5
10
15
20
25
30
35

FIGURE # 9

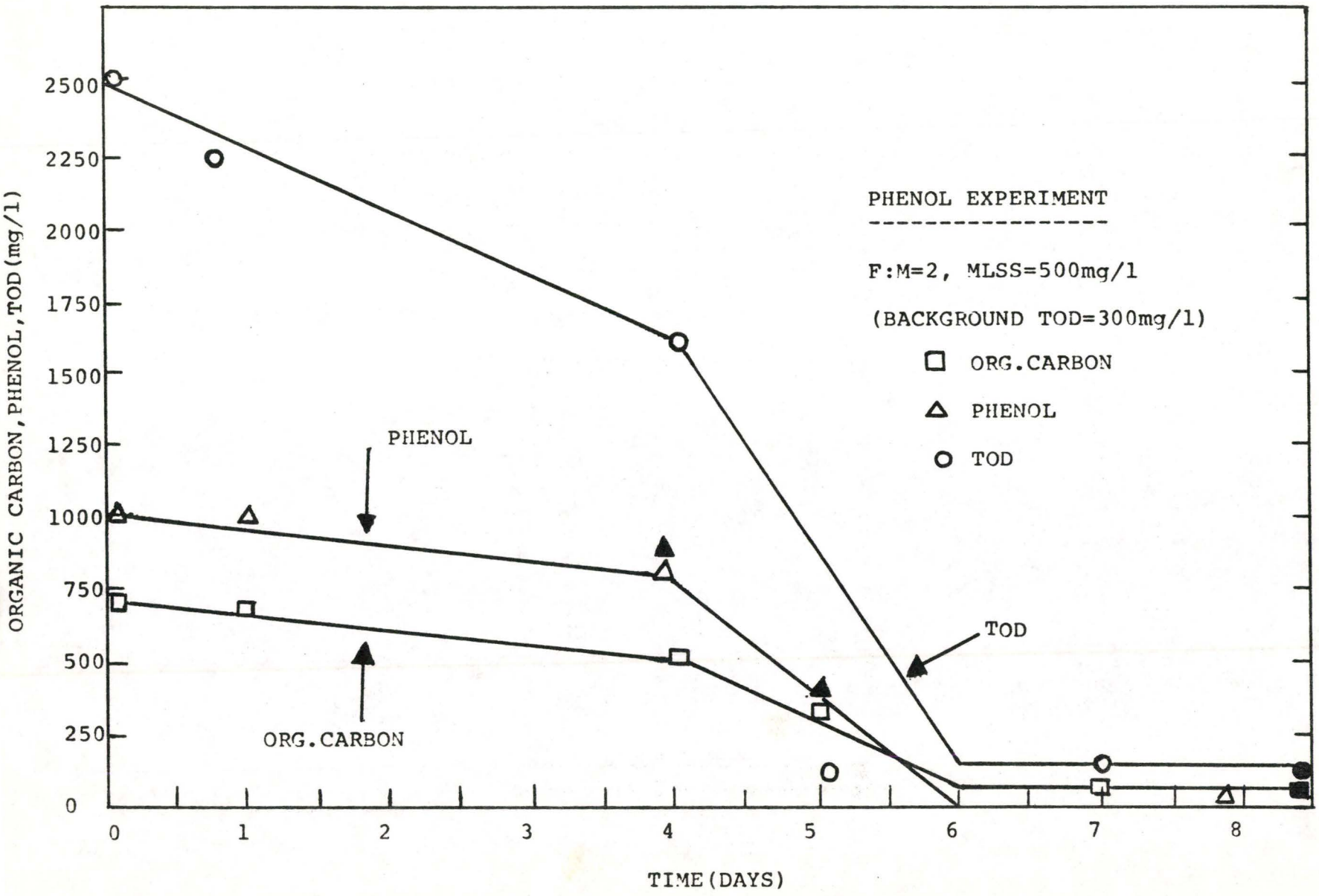

PHENOL EXPERIMENT
F:M=2, MLSS=500mg/l
(BACKGROUND TOD=300mg/l)
ORG.CARBON
PHENOL
TOD
PHENOL
TOD
ORG.CARBON
ORGANIC CARBON, PHENOL, TOD (mg/l)
TIME (DAYS)
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
0
1
2
3
4
5
6
7
8

FIGURE # 10

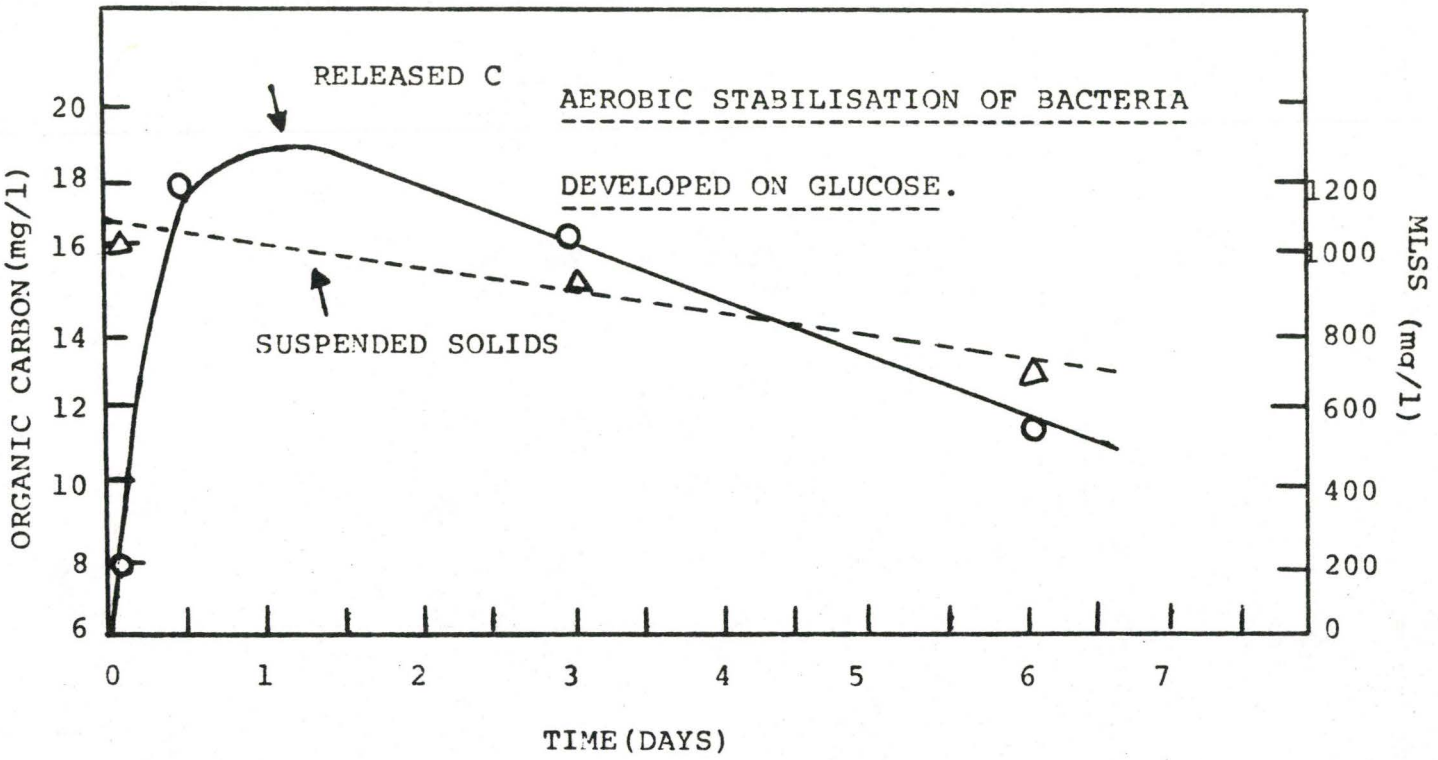
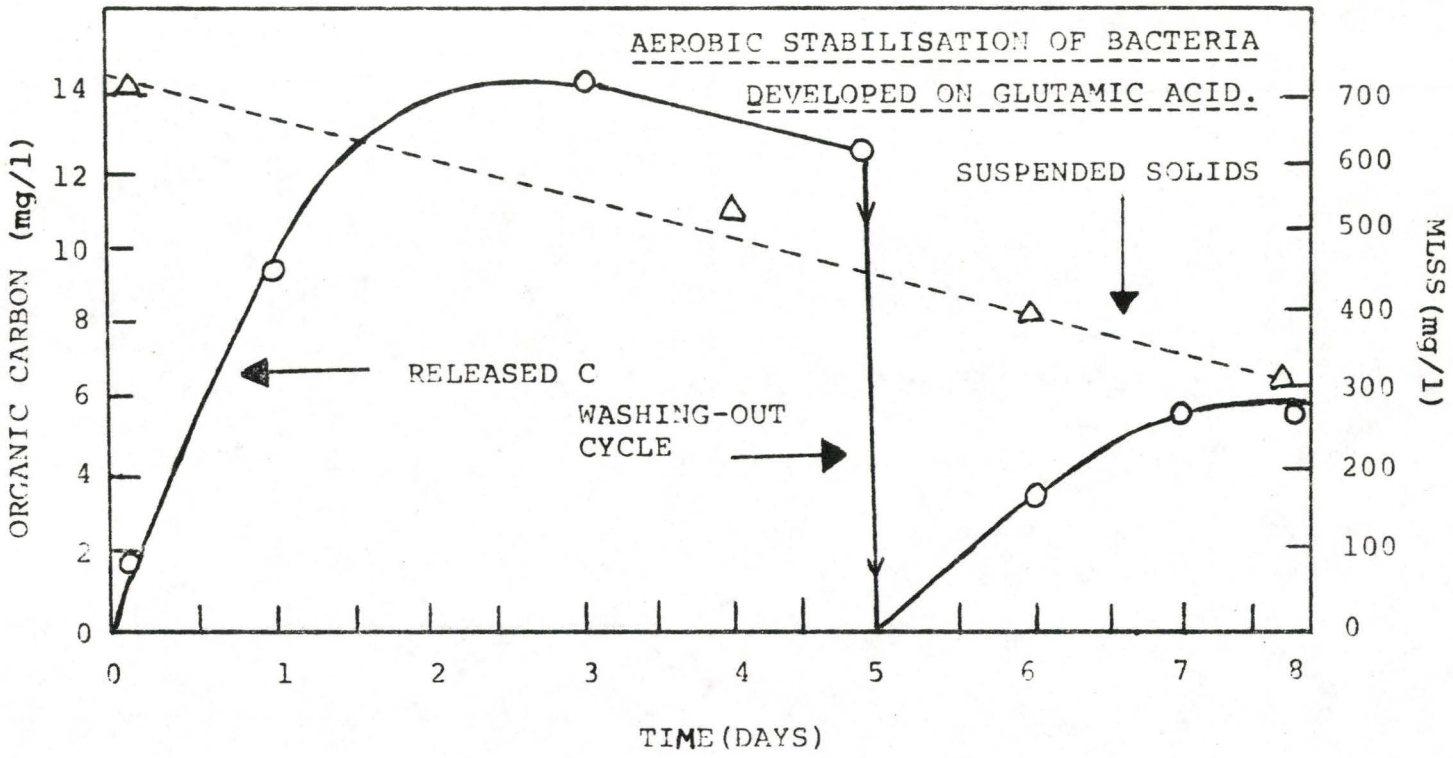

RELEASED C
AEROBIC STABILISATION OF BACTERIA
DEVELOPED ON GLUCOSE.
SUSPENDED SOLIDS
ORGANIC CARBON (mg/l)
MLSS (mg/l)
TIME (DAYS)
20
18
16
14
12
10
8
6
1200
1000
800
600
400
200
0
0
1
2
3
4
5
6
7

FIGURE # 11


AEROBIC STABILISATION OF BACTERIA
DEVELOPED ON GLUTAMIC ACID.
SUSPENDED SOLIDS
RELEASED C
WASHING-OUT
CYCLE
ORGANIC CARBON (mg/l)
MLSS (mg/l)
TIME (DAYS)
0
2
4
6
8
10
12
14
100
200
300
400
500
600
700
1
3
5
7

released into the medium was monitored periodically, and the graphical interpretation of the change in organic carbon concentration with time is presented in Figures 10 and 11. Activated sludge growing on glucose and glutamic acid were investigated separately in these experiments. In the case of the experiment illustrated in Figure 11, the sludge was harvested again during the run, rewashed and resuspended in the fresh buffer. A further measurable increase in organic carbon concentration occurred during the second resuspension.

From these tests it can be concluded that the sludge released soluble organic carbon into the medium even without an addition of external food. During the period of aeration of the sludge, a substantial decrease in concentration of suspended solids occurred. Further measurable increases in organic carbon concentration occurred following the second resuspension.

4.3 Response of Sludge to Change in Substrate

A series of runs was carried out to ascertain whether changing the nature of the substrate would produce a significant change in process response measured as residual concentration of organic carbon. As indicated in Table 1, the glucose-phenol variation lead to a failure of the process in the fourth cycle, as indicated by an extremely high level of residual carbon. Broken bacterial cells were observed by microscopy. No evaluation was made as to whether a change of microbial type would be required to utilize the different substrate as postulated by Hashimoto (1970).

4.4 Determination of Protein

A determination of the soluble protein in the reactor, as described in Appendix 11, proved negative evidence of protein in the medium, even after concentration of the liquid 1:20 by evaporation and the concentration of carbon was 500 - 1000 ppm. The sensitivity of the Biuret method, used for the determination of protein, was about 0.07 mg of protein per ml (using a standard protein).

This fact might lead to the conclusion that bacteria do not release their cell contents (protoplasmic contents) into the medium due to lysing. Moreover, the negative finding of protein in the liquid portion may exclude the presence of filtrable viruses.

4.5 Determination of Carbohydrates

Qualitative tests to detect the presence of soluble carbohydrates in the reactor were performed as outlined in Appendix 11. In all cases, positive results confirmed the presence of carbohydrates in liquid media. These carbohydrates had non-reducing action with respect to the Fehling test.

4.6 Nature of the Residual Organics

The residual portion of the batch process seemed to follow qualitatively the nature of activated sludge (colour of the concentrated samples, odour, etc.). Concentrated samples appeared yellowish in colour, but if the sludge had a characteristic colour, so did the liquid sample.

TABLE #1

RESPONSE OF THE PHENOL ACLIMATED SLUDGE TO THE VARIATION IN THE SUBSTRATE

CYCLE #	TYPE OF SUBSTRATE	CONC. OF SUBSTRATE (mg/l)	RESIDUAL ORG. CARBON (mg/l)
1	Glucose	2000	125
2	Phenol	2000	22
3	Glucose	2000	24
4	Phenol	2000	3000

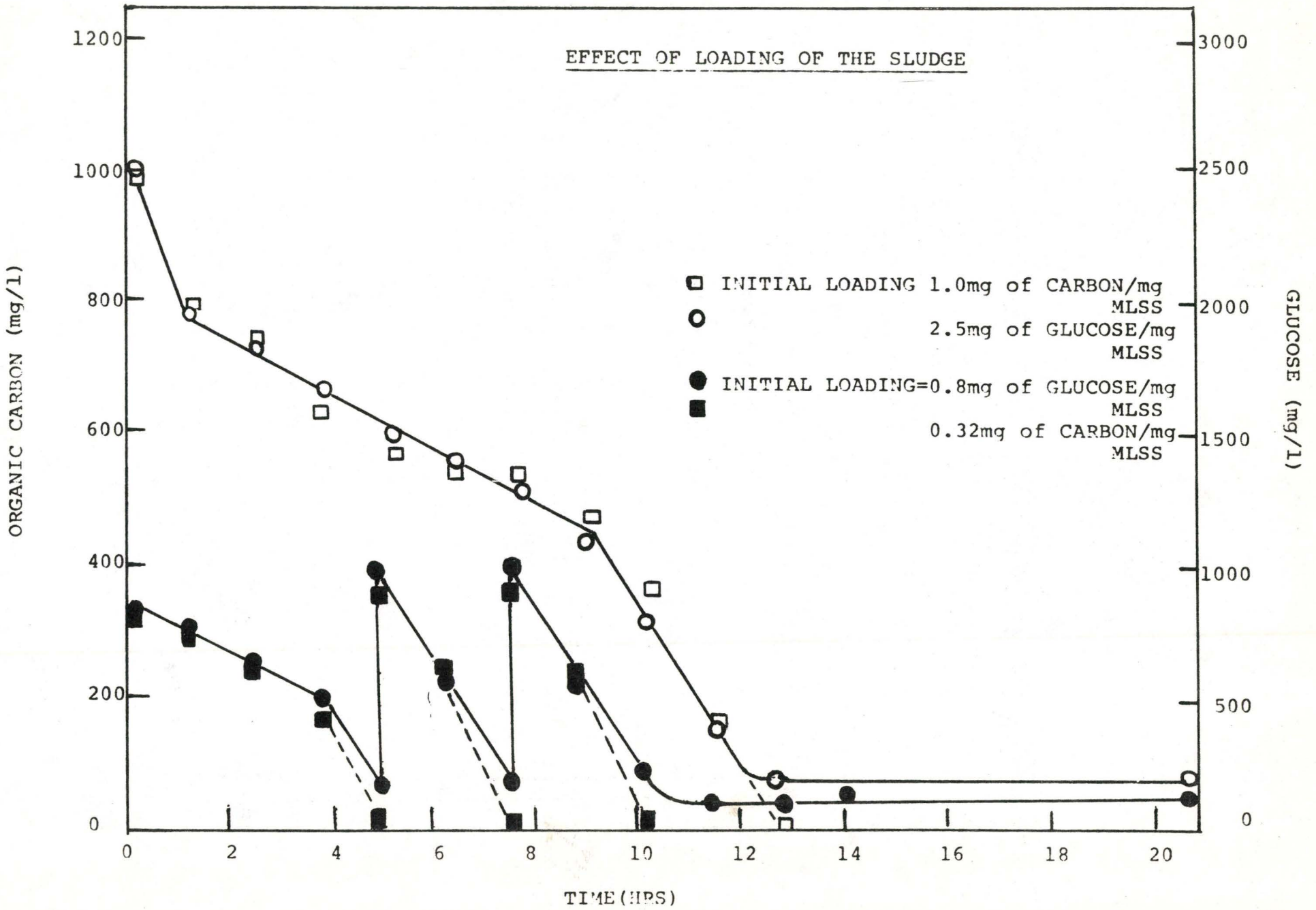
The total oxygen demand of the residual portions was very low compared with the TOD of pure substrates, suggesting the residual carbon to be highly oxidized. The ratio of C:TOD varied from 1:1 - 2:1, which suggests stoichiometric combinations, such as carboxyl, etc.

Dialysis experiments were carried out with three liquid residual portions of the glucose runs, to ascertain the diffusion characteristics of the solutes through a parchment membrane. About 15% of the organic carbon consisted of material with a relatively high molecular weight, because migration did not occur through the parchment membrane against a 0.05 M phosphate buffer.

4.7 Effect of Loading of the Sludge

During this work it was noticed that lowering the substrate concentration lead to lower values of residual carbon (by 20-30%). When the glucose concentration was 2000 mg/l and the suspended solids was 1000 mg/l, the residual carbon level was 50 mg/l. When the same experiment was repeated at the same total loading, but with the substrate's being introduced four times over a period of 1 hour, the level of residual carbon was only 37 mg/l. Another glucose experiment, illustrated in Figure 13, was carried out with an initial concentration of carbon 1000 mg/l, and the initial solid concentration was 1000 mg/l. At first the substrate was introduced in a single feeding, where in the second only a third of the substrate was introduced

FIGURE # 12


EFFECT OF LOADING OF THE SLUDGE
INITIAL LOADING 1.0mg of CARBON/mg MLSS
2.5mg of GLUCOSE/mg MLSS
INITIAL LOADING=0.8mg of GLUCOSE/mg MLSS
0.32mg of CARBON/mg MLSS
ORGANIC CARBON (mg/l)
GLUCOSE (mg/l)
TIME(HRS)
0
200
400
600
800
1000
1200
500
1000
1500
2000
2500
3000
0
2
4
6
8
10
12
14
16
18
20

initially. After the glucose disappeared, another third was added and a similar procedure followed for the last third. In the first instance, the residual level of carbon was observed to be 60 mg/l, compared with the second run, where the concentration of organic carbon was 47 mg/l. A similar increase in residual carbon concentration could be produced by changing one substrate to another. A much higher decline in the level of residual carbon was observed by introducing less soluble substances in the form of colloidal sizes (i.e. starch) instead of perfectly diluted substances.

4.8 UV-spectra Evaluation

All the liquid portion of residual organics investigated exhibited ultraviolet absorption in the region 220 - 280 mμ, with no sharply definite maximum, as illustrated in Figures 13 through 16. The absence of a distinct maximum in these spectra suggested the presence of a complex of compounds containing UV sensitive chromophor groups, rather than a few constituents. From Figures 13 through 16 it can be noticed that the absorption curves of the liquid portions of the reactors had approximately the same pattern to those of a washed sonicated suspension. It appears from the spectra that liquid portions of the reactors seem to be constituted from more compounds than those from hydrolysed micro-organisms which exhibited relatively sharper peaks.

Generally, groups absorbing UV radiation are unsaturated and seldom independent of the effect of other resonating groups.

Thus it is very complicated to assign molecular structure based on UV spectroscopy. The sharp maximum in absorption would suggest the pure compound (eg. phenol with an explicit peak at 265 mμ), while after contact with activated sludge a fairly rounded absorption peak is obtained from 240 - 290 mμ (Figure 14).

Most proteins exhibit a distinct UV absorption maximum at 280 mμ. Nucleic acid, however, absorbs much more strongly at 260 mμ than at 280 mμ (Baker et al., 1967). The absorption at 263 mμ may also suggest that the compounds are derivates of aliphatic alpha, beta unsaturated acids (Hashimoto, 1970).

4.9 Characterization of Micro-organisms

Activated sludge acclimated to glucose formed flocs of a light colour, which in the case of changing the pH turned to blue or pink. The settling characteristics of this sludge were fairly poor. Filamentous micro-organisms and yeast spores were presented in large quantities (Appendix VI). Both rod and cocci bacteria were observed to be present, using a microscope equipped with phase contrast.

Activated sludge developed on glutamic acid formed light-coloured, good settling flocs and was mainly filamentous in nature (Appendix VI). Activated sludge acclimated to phenol contained an extremely low number of filaments and yeast with a predominance of bacteria (Appendix VI). The sludge exhibited good settling characteristics.

FIGURE # 13

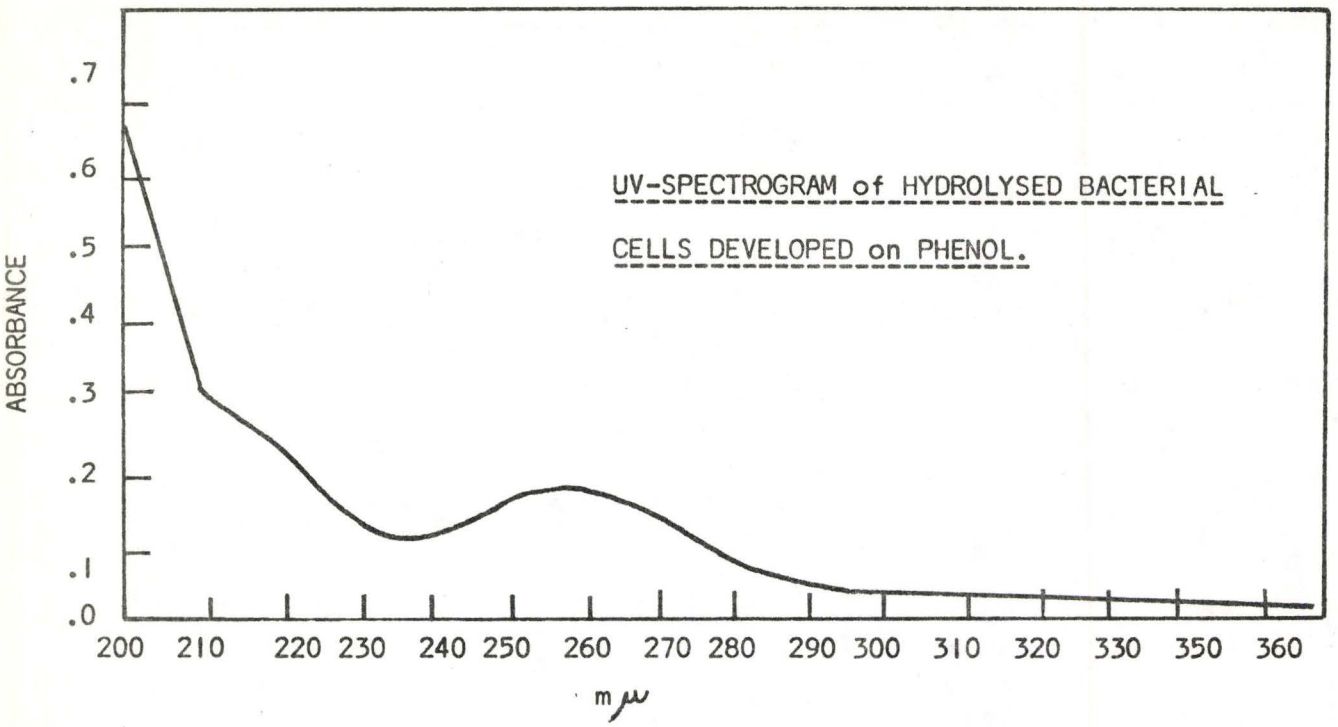


FIGURE # 14

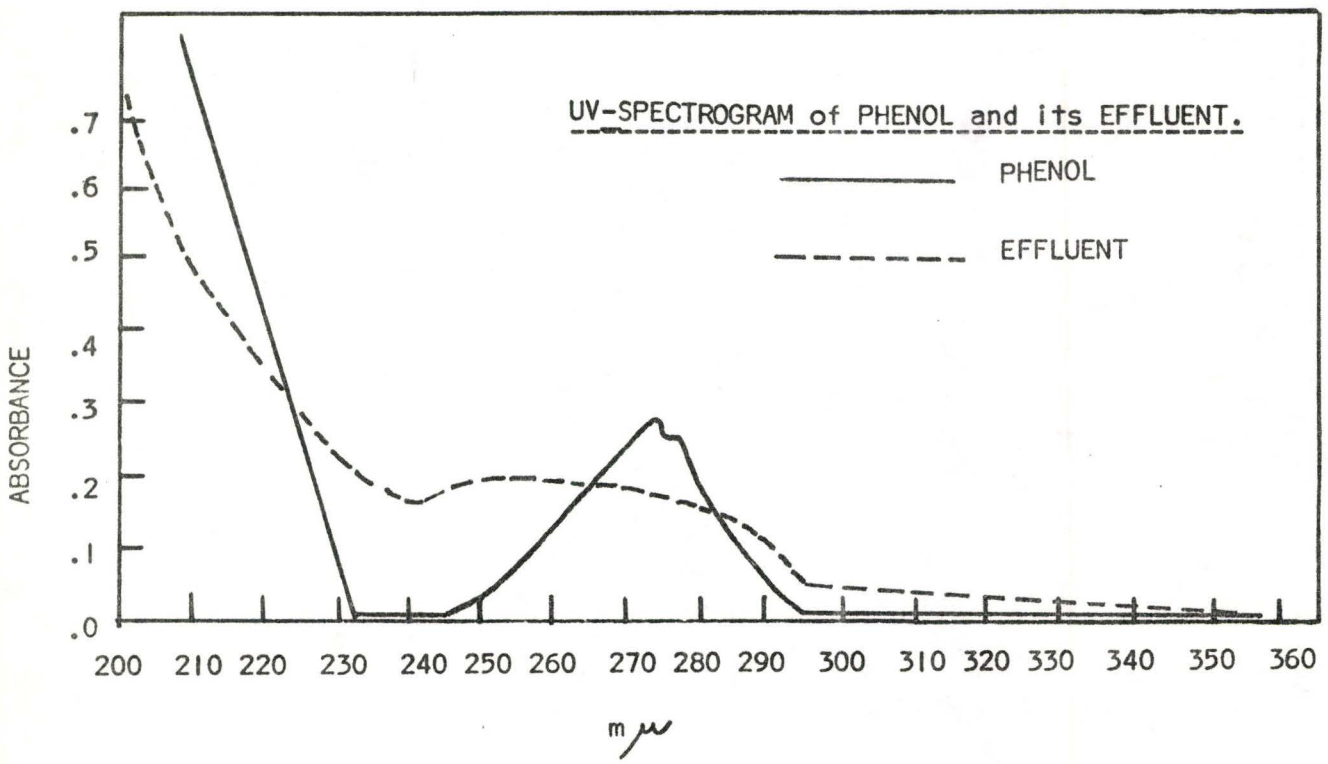


FIGURE # 15

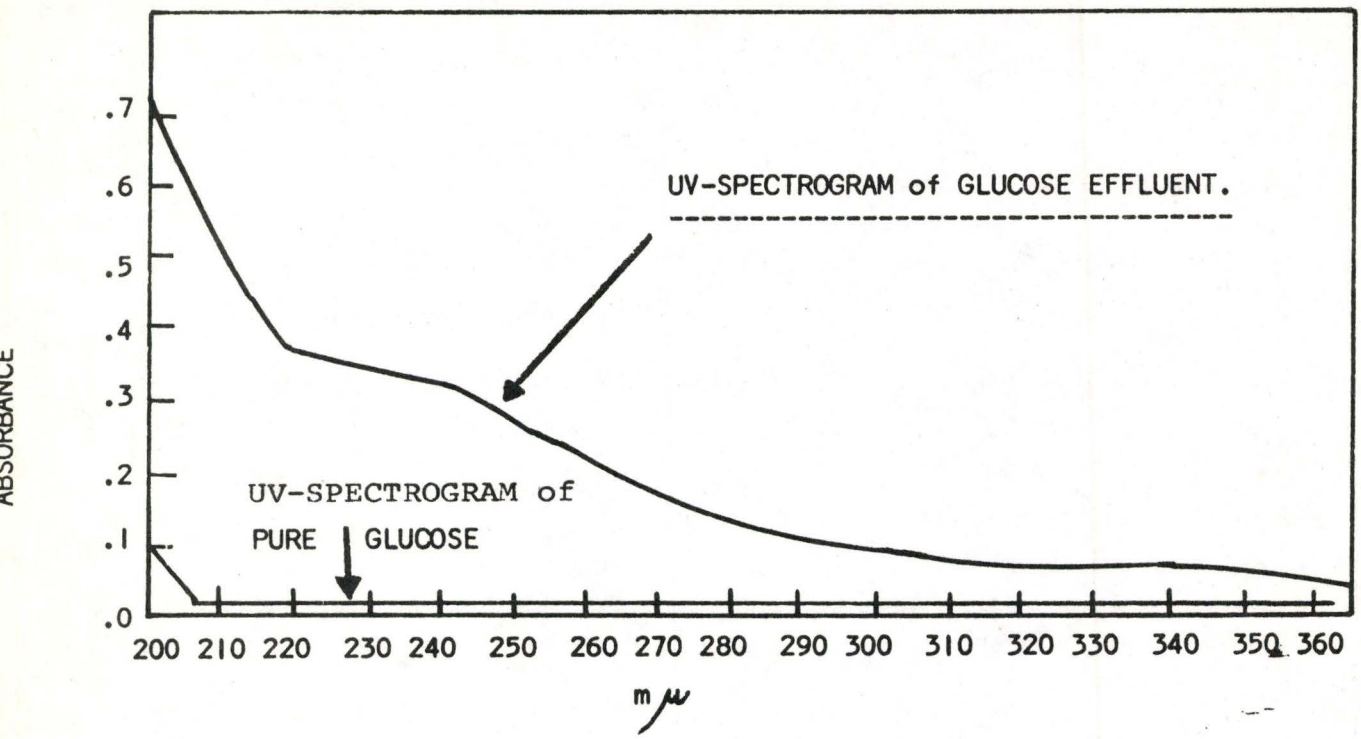
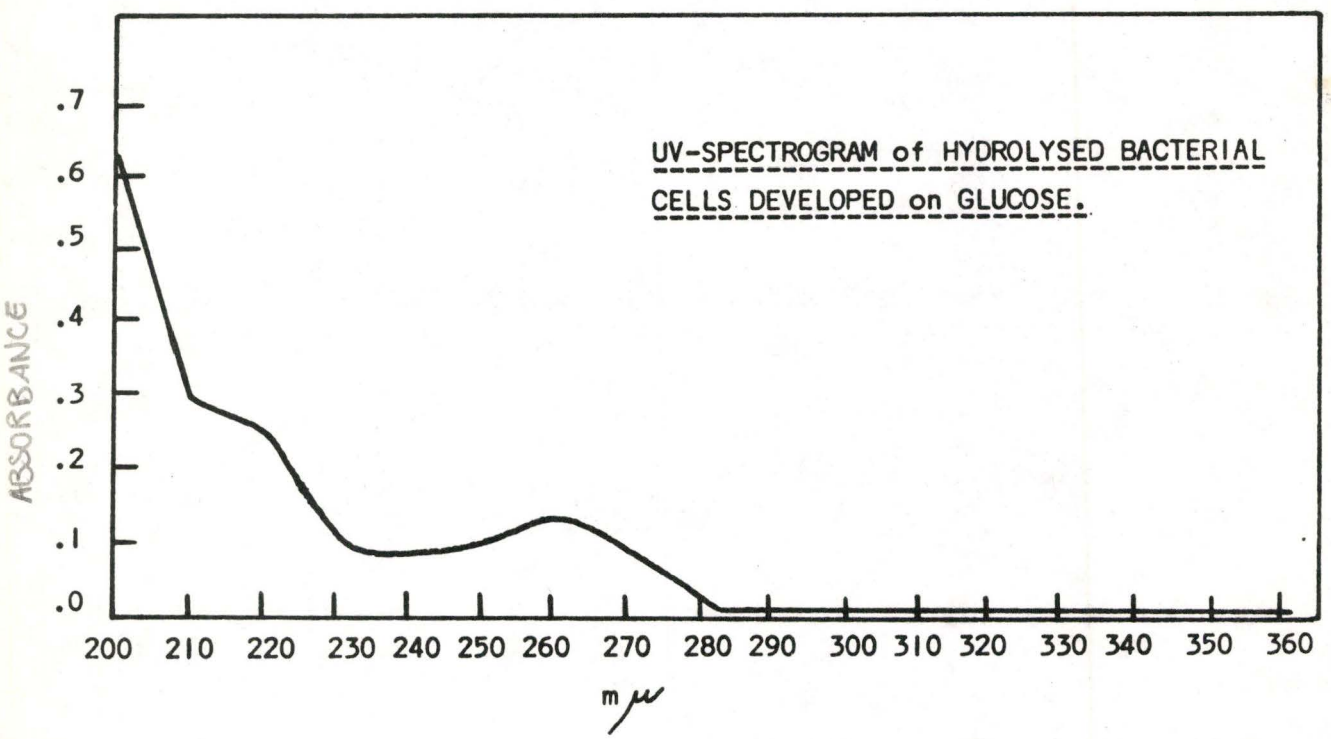


FIGURE # 16



4.10 Experimental Design

From the results of the preliminary experiments it was concluded that the level of residual soluble extra-cellular organic carbon appeared to be linearly dependent on the initial organic carbon concentration. The exception occurred with phenol experiments which were strongly influenced by the solids concentration. Therefore a number of batch experiments were performed using acclimated mixed microbial cultures and four different organic substrates to define a relationship between the initial carbon concentration, solids concentration, and the residual carbon concentration over a range of practical interest. With this information it might be possible to evaluate optimum reactor conditions to obtain a minimum concentration of residual carbon. Further, it would be possible to obtain qualitatively the effect of different substrates on the residual carbon concentration. The design of experiments is described in Appendix 1 and the table of responses is illustrated in Table 2. Some of the runs were repeated several times in a random order to find the experimental error. The experimental design matrix with corresponding responses of the batch reactor is illustrated in Table 2. With this design, the second order effect in the residual level of carbon (C_R) with respect to the initial level of carbon (C_C) and solids (C_B) could be considered. Applying the method of least squares, illustrated in Appendix 111, the

estimates of the coefficients of the response surface were statistically evaluated at the 95% confidence level. From the experimental design second order effects and their interactions could be estimated. A model of the form

$$Y=b_0+b_1X_1+b_2X_2+b_3X_1^2+b_4X_2^2+b_5X_1X_2$$

could be obtained for all substrates. If the non-linear terms were insignificant, then the residual error, when employing a linear correlation of the form

$$Y=b_0+b_1X_1+b_2X_2$$

should be insignificant when compared to pure experimental error. From this comparison, the details of which are included in Appendix V, it was concluded, that, for the region defined by the range of this design, the responses were linear functions of the initial concentration of carbon and solids on the basis of 95% confidence.

To test whether one general model was representative of the response obtained from all single substrates, the composite regression was compared with the single substrate regressions applying the "null" hypothesis of the equality of the regression coefficients. The procedure is outlined in Appendix V. The resulting correlations are presented in table 3. On the basis of 95% confidence the response surface, developed for the composite model was inadequate to represent the response for starch, a coloidial suspension, as at the 95% confidence level the coefficients b_0 and b_1 were significantly

TABLE #2

EXPERIMENTAL DESIGN MATRIX AND RESPONSES.

Note: Y Residual organic carbon (mg/l)

X_1 Initial substrate carbon concentration (coded)

X_2 Initial solids concentration (coded)

TABLE 2A - GLUCOSE RUNS

TRIAL #	X_1	X_2	Y
1	-1	-1	6;12
2	0	-1	22
3	1	-1	45
4	-1	0	10
5	0	0	24;25;29;30
6	1	0	52
7	-1	1	12;24
8	0	1	29;59
9	1	1	30;60;31

TABLE 2B - STARCH RUNS

TRIAL #	X_1	X_2	Y
1	-1	-1	24;30
2	0	-1	--
3	1	-1	58
4	-1	0	13;30
5	0	0	107;72;130
6	1	0	200
7	-1	1	18;29;45
8	0	1	87;69
9	1	1	145;135

TABLE 2C - PHENOL RUNS

TRIAL #	X_1	X_2	Y
1	-1	-1	26;17
2	0	-1	23
3	1	-1	53
4	-1	0	44;37;16
5	0	0	22;47;17;22;21
6	1	0	33
7	-1	1	34;16
8	0	1	70
9	1	1	96;100

TABLE 2D - GLUTAMIC ACID RUNS

TRIAL #	X_1	X_2	Y
1	-1	-1	16
2	0	-1	30;35
3	1	-1	54
4	-1	0	13
5	0	0	37
6	1	0	50
7	-1	1	33;40
8	0	1	--
9	1	1	--

different. With phenol no significant difference in coefficients was observed. For glucose and glutamic acid no significant difference in the coefficients b_0 and b_1 was observed. However, the coefficient b_2 was judged statistically different.

Despite the differences which appeared to exist between the postulated models for the soluble substrates, the soluble substrate data were pooled to determine if a composite model for soluble substrates would be representative of the individual soluble substrates. As illustrated in Appendix V no significant difference at the 95% confidence level occured. This led to the postulation of the overall correlation for the soluble substrates given in Table 3.

TABLE #3

MATHEMATICAL MODELS DEVELOPED FROM EXPERIMENTAL DESIGN

Note: C_R Residual organic carbon (mg/l)

C_C Initial carbon concentration (mg/l)

C_B Initial solids concentration (mg/l)

SUBSTRATE:	MATHEMATICAL MODEL:
All substrates	$C_R = 2.80+0.036C_C+0.014C_B$
All soluble substrates	$C_R = 9.10+0.021C_C+0.009C_B$
Glucose	$C_R = 5.15+0.024C_C+0.005C_B$
Glutamic acid	$C_R = 10.82+0.024C_C+0.007C_B$
Phenol	$C_R = 3.87+0.022C_C+0.019C_B$
Starch	$C_R = 7.74+0.077C_C+0.010C_B$

CHAPTER #5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

For the material and methods used in this study, it has been concluded that:

(1) using organisms from the activated sludge process, residual organic material was produced in batch reactors using simple substrates. This material is not readily bio-degradable,

(2) the residual organic matter is qualitatively different from the substrate and is present in the liquid medium, even when the substrate is eliminated,

(3) the concentration of residual organic carbon varied from 20 to 70 mg/l during this study. For a given substrate this concentration was dependent upon the initial carbon concentration and the solids concentration;

(4) for the three soluble substrates glucose, glutamic acid and phenol no significant difference in residual organic carbon concentration could be attributable to the variation in substrate. For this case the residual organic carbon concentration could be expressed as:

$$C_R = 9.1 + 0.021 C_C + 0.009 C_B$$

(5) for the colloidial substrate starch a significant difference was observed. In this case the residual organic

carbon could be expressed as:

$$C_R = 7.74 + 0.077C_C + 0.01C_B$$

(6) the liquid medium from the activated sludge process contains carbohydrates. At least a portion of the residual organics in the liquid medium has a relatively high molecular weight. No existence of soluble protein was detected. The total oxygen demand of the residual matter was relatively low as the TOD:C ratio varied from 1 to 0.5 during this study;

(7) higher instantaneous loading of the activated sludge apparently could increase the concentration of the residual organic matter; and

(8) UV-spectra of sonicated micro-organisms and the liquid media from the same reactor exhibited similar patterns.

5.2 Recommendations

It is recommended that:

(1) in order to achieve a minimum level of residual organic matter

(a) biological reactor design be based upon a series of laboratory studies over a range of suspended solids to determine the optimum level for a given waste; and

(b) influent concentrations be reduced to a minimum and that instantaneous shock loads be avoided;

(2) specific analytical methods applicable for trace

organics present in activated sludge effluents should be developed; and

(3) techniques for the determination of the level of viable micro-organisms in the total solids be developed.

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APPENDIX 1

EXPERIMENTAL DESIGN

The statistical design of experiments has been used as a research tool to obtain all relevant information from the planned number of data points. If the design is sound, then even relatively quick methods of analysis can yield a great deal of pertinent information.

A 3 level with 2 variables factorial design (3^2 design) was chosen in order to analyze for a second order polynomial response surface (Davies, 1967). The design is illustrated geometrically in Figures 17 and 18, and was employed for the concentration of substrate carbon (X_1) and concentration of micro-organisms (X_2).

Properties of this design:

$n = 9$ + replications of the centre point

$$\frac{X_1}{n} = \frac{X_2}{n} = \frac{X_1X_2}{n} = 0$$

if the predicted response surface polynomial is:

$$Y = b_o + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

Thus: $Y = b_o + b_{11} = b_{22}$

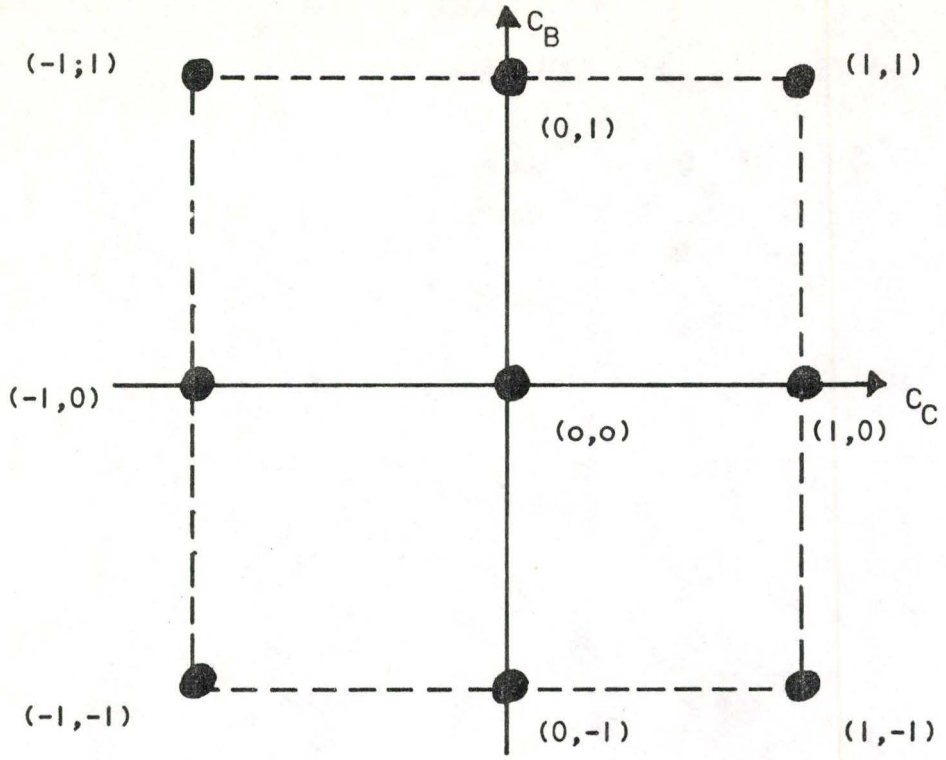
Coding Equations:

Since the range of variable X_1 was chosen:

X_1 (100 mg/l - 1500 mg/l)

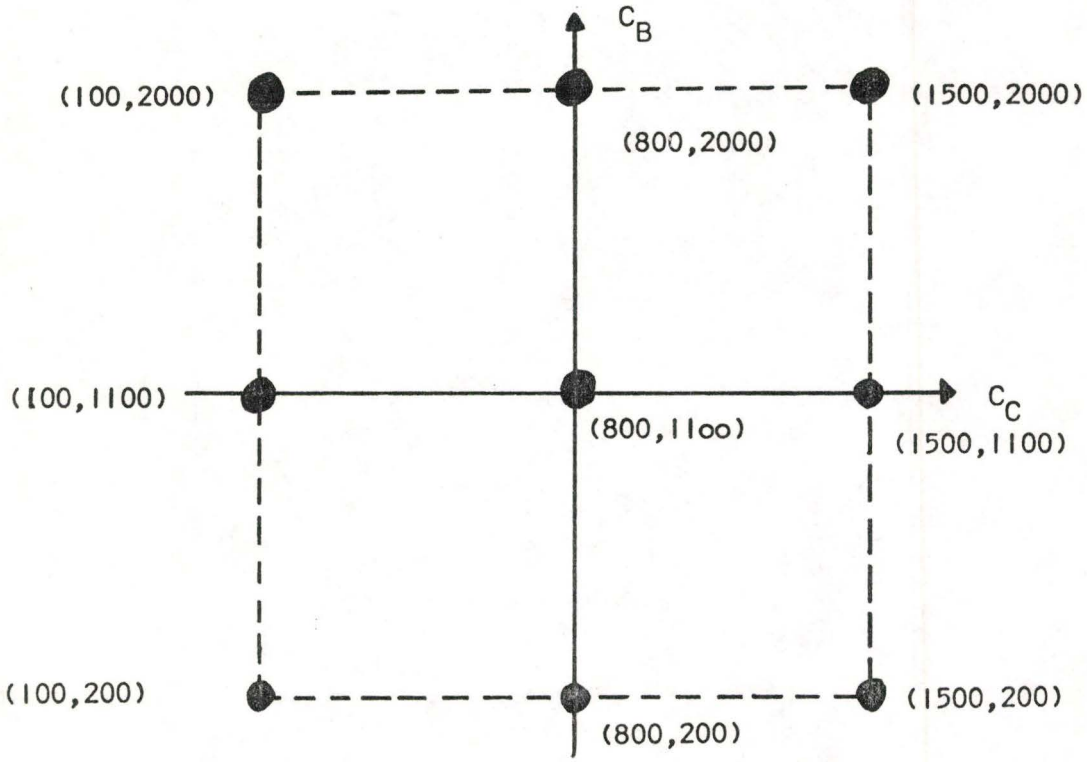
and variable X_2:

FIGURE # 17



VARIABLE COORDINATE SYSTEM

FIGURE # 18



VARIABLE NON-CODED SYSTEM

TABLE #4

UNCODED EXPERIMENTAL DESIGN MATRIX

TRIAL #	X_1	X_2
1	100	200
2	800	200
3	1500	200
4	100	1100
5	800	1100
6	1500	1100
7	100	2000
8	800	2000
9	1500	2000

X_2 (200 mg/l - 2000 mg/l)

the following coding equations can be derived:

$$X_1 = \frac{X_1 - 800}{700}$$

$$X_2 = \frac{X_2 - 1100}{900}$$

where $X_1 = 800$ and $X_2 = 1100$. The uncoded design matrix is as described in Table 4.

Two-Factor Interaction

Since there are two variables involved in the design of these experiments, only one interaction, the substrate concentration and the solids concentration interaction, can be calculated. Basically this value determines the difference in response between two levels of one factor when the second one is changed from the minimum to the maximum level. Possibly this can be understood better from Figure 19, where the two diagonals of the design matrix represent the high - and the low - level conditions. Interaction is defined as:

$$1(X_1, X_2) = \frac{1}{2}(Y_1 + Y_9 - Y_3 - Y_7)$$

The interaction is sometimes easily computed by vector multiplication using the following method. Multiplication vector $X_{1,2}$ is obtained by multiplication vector X_1 by vector X_2 from design matrix:

$$\underline{X_1} \cdot \underline{X_2} = \underline{X_{1,2}}$$

$$2.\underline{1}(X_1, X_2) = \underline{X_{1,2}} \cdot \underline{Y} \quad \text{where } \underline{Y} \text{ is the response vector.}$$

In this particular design:

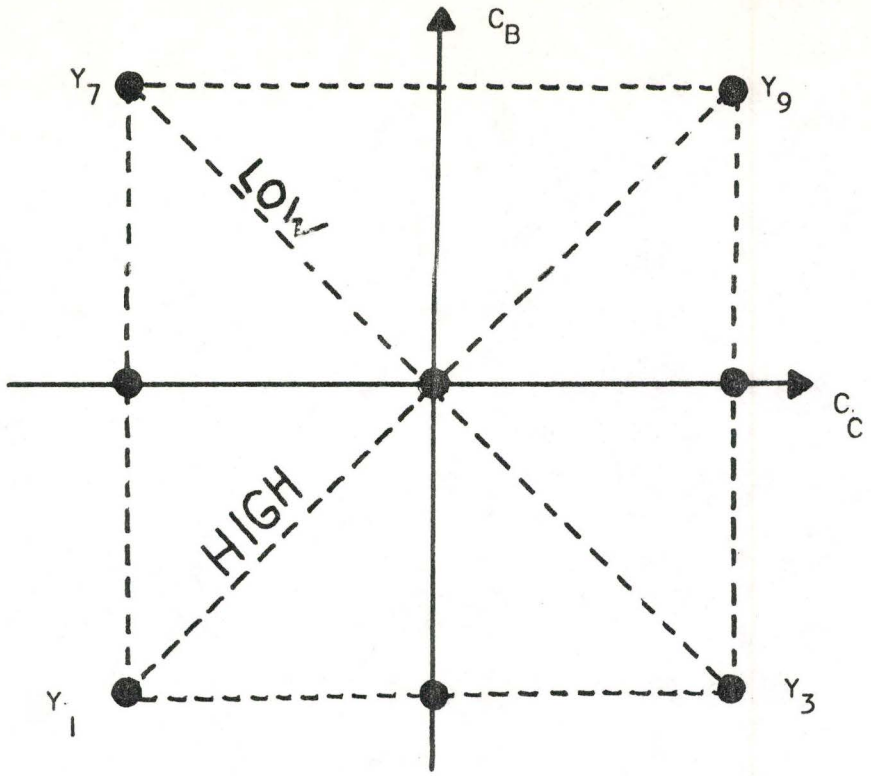
$$\underline{X_{1,2}} \cdot \underline{Y} = \underline{2I(X_1, X_2)}$$

$$\begin{bmatrix} 1 \\ 0 \\ -1 \\ 0 \\ 0 \\ 0 \\ -1 \\ 0 \\ 1 \end{bmatrix} \times \begin{bmatrix} Y_1 \\ Y_2 \\ Y_3 \\ Y_4 \\ Y_5 \\ Y_6 \\ Y_7 \\ Y_8 \\ Y_9 \end{bmatrix} = \begin{bmatrix} Y_1 \\ \\ -Y_3 \\ \\ \\ \\ -Y_7 \\ \\ Y_9 \end{bmatrix}$$

Average interaction X_1 and X_2 is:

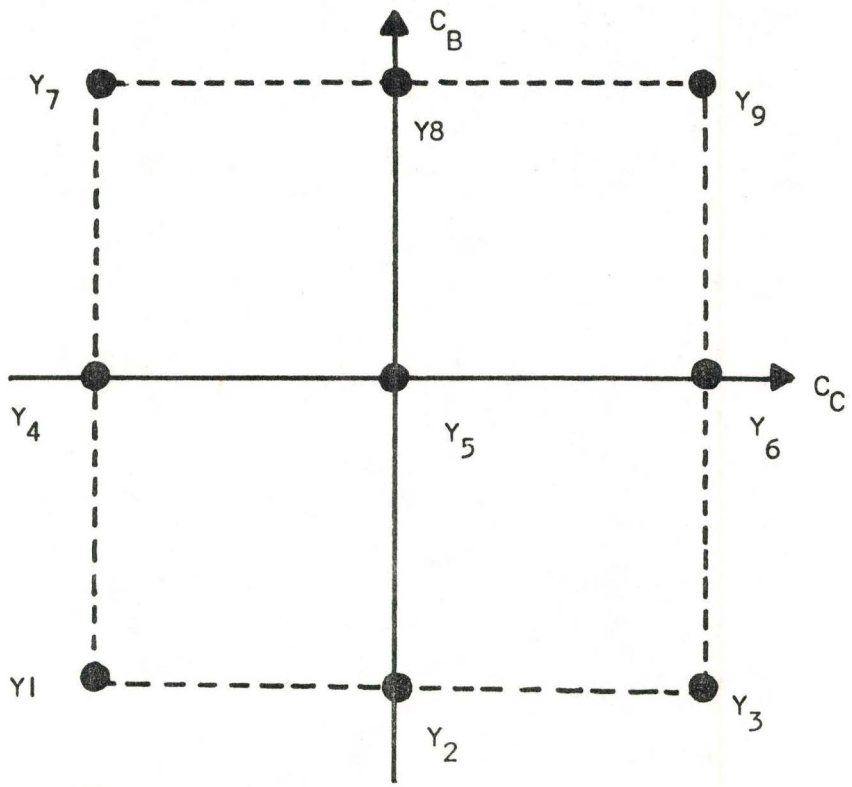
$$I(X_1, X_2) = \frac{1}{2}(Y_1 - Y_3 - Y_7 + Y_9)$$

FIGURE # 19



GRAPHICAL INTERPRETATION of INTERACTION X_1 vs. X_2

FIGURE # 20



DEPENDENT VARIABLE of the DESIGN(C_C)

APPENDIX 11

ANALYTICAL METHODS

Determination of Suspended Solids Concentration

The suspended solids concentrations were determined gravimetrically using Sartorius "weight-constant" membrane filters (47 mm DIA; 0.45 micron pore) in conjunction with a 6 stall vacuum filtration apparatus. Preparatory to each run, the membrane filters were individually washed with 100 ml of distilled water, placed in a tagged aluminum foil dish, placed in a mechanical convection oven at $103^{o}C$ for 0.5 hour, and dessicated to room temperature. Filter papers were individually removed, weighed to the nearest 10^{-4} gm on a Mettler type N$\underline{o}$H - 15 balance and mounted on the filtration apparatus to accept a known volume of reactor contents. After filtration, each membrane filter was returned to its foil dish, redried and reweighed.

Determination of Soluble Organic Carbon

Samples from individual runs were analysed at intervals for total organic soluble carbon after the suspended solids had been removed by membrane filtration. An infrared analyser, the Beckman model IR 315, was used. Inorganic carbon presented in the samples was converted to CO_2 by acidifying with HCl and the CO_2 was liberated by means of a five minute nitrogen purge.

Twenty microlitre aliquots were injected by a syringe into the analyser until three successive determinations produced output signals differed by less than 1% of full scale output. The organic carbon was determined by a linear interpolation between 2 points derived from standard solutions of sodium oxalate in distilled water. These standard solutions were capped and stored at $4^{o}C$ between runs.

Determination of Total Oxygen Demand

TOD was measured simultaneously with the substrate and organic carbon determination. The instrument, the Ionic's model 225 TOD Analyser, was used. The analyser was an automatic instrument capable of graphically recording the output on a continuous basis. The samples, previously filtered by the membrane filtration, were placed in a sampling flask connected to an automatic injection valve with a teflon tube. After combustion of the sample, the depletion of oxygen in the carrier gas was measured by means of a silver-lead fuel cell detector and recorded. The TOD value of the sample was obtained by means of a seven point standard curve derived from the sodium oxalate standard. The dissolved oxygen was removed by means of a five minute nitrogen purge.

Determination of Glucose

Glucose, as a reducing sugar, was measured using Fehling's solution. Basically the aldehyde group of glucose reacts according to the following equation:

$$R - CHO + 2CuO = Cu_2O + R - COOH$$

The Fehling's solution contained in 500 ml:

13.37 g of $CuSO_4.5H_2O$

86.5 g of sodium potassium tartrate

and 30 g NaOH

The solution was prepared fresh before the analysis, and was standardized by titration with a glucose standard. The boiling Fehling solution was titrated till the blue colour disappeared. As 1 ml of Fehling's solution corresponded to 0.005 g of glucose, the concentration of the glucose in the reactor was calculated from the proportionality of the sample volume and the volume of the Fehling's solution.

Determination of Starch

The method of starch determination is based on photometric determination of the absorbance of the blue colour produced by amylose upon the addition of an iodine-iodide complex. The sample was alkalyzed with 0.5 ml of N-NaOH, warmed 3 minutes, cooled, neutralized with 0.5 ml of N-HCl. The sample was placed in a 50 ml flask and 0.1 g of sodium potassium tartrate + 0.5 ml of iodine solution was added.

The iodine solution contained 2 mg of I_2/ml and 20 mg of KI/ml. The sample was made up to 50 ml and allowed to stand for 20 minutes, and measured at 680 millimicron. The reference solution was an iodine solution of equal concentration. The

concentration starch in the sample was determined by a 10 point calibration curve.

Determination of Phenol

The Bromometric method, suggested for higher concentrations of phenols was used. The method is based on the bromation of monohydroxy-benzenes separated from the sample after steam distillation. The consumption of bromine proportional to the amount of phenol present is given by the difference in the amount of the reagent at the beginning and the end of the reaction. The unreacted excess of the reagent is estimated by adding potassium iodide and titrating with sodium thio-sulphate.

pH Measurement

The pH was measured periodically in the reactor with a model 76 Beckman pH meter or pH indicator paper with a narrow range.

Determination of Protein

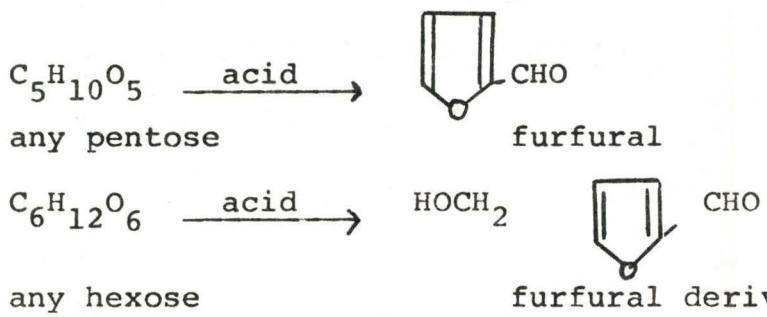
The filtrates were examined for protein by the Biuret method. Basically the substances containing two or more peptide bonds form a purple complex with copper salts in alkaline solution. The optical density of this complex is determined with a spectro-photometer in the region of 540-560 mμ. The concentration of protein in the sample is obtained by reference to a calibration curve established with a clear solution of serum protein

(VERSATOL) which is basically pooled human serum with a normal electrophoretic pattern featuring the albumin to globulin ratio 1:2.

There are practically no substances other than protein normally present in biological materials which give the Biuret reaction to an extent sufficient to cause significant interference (Layne 1970).

Determination of Carbohydrates

Qualitative tests for carbohydrates are based on the formation of furfural (or its derivates) which is a product of the breakdown of carbohydrates by means of concentrated strong acids. This procedure was adapted from that given by Fairbarne (1953). The furfural is made to condense with various aromatic compounds to produce characteristic colours. For example:



The blue-green coloured reaction product of furfural-anthrone was used as a qualitative proof of presence of carbohydrates. Anthrone was dissolved in a concentration of H_2SO_4 (1:1000) and kept at 4^oC. 5 ml of anthrone was added to 2 ml of sample to give a two-phase density stratified mixture in a test tube.

The precipitates remained in the upper layer. The sky-blue or green ring of the furfural-anthrone complex was created with the intensity proportional to the concentration of carbohydrates.

Ultraviolet Spectroscopy

A UV analysis was performed to determine the presence of certain functional groups which would have the necessary bounding energy.

The Unicam SP 1800 Spectrofotometer with a slave recorder was used. The instrument is able to cover the ultraviolet and visible spectral regions, and the wave length selection is carried out automatically. Samples were run in the range of wave length from 400 - 200 millimicron against the tap water with nutrient media.

Microscopic Examination

Microscopic examinations were made using an Olympus microscope with a phase contrast attachment. Sufficient resolution was available to permit the observation of different micro-organisms growing on different substrates. Some of these observations were recorded on film, using a camera attachment.

APPENDIX 111

CORRELATION-REGRESSION ANALYSIS OF EXPERIMENTAL DESIGN

References: Draper-Smith (1966)

The interpretation of the results of the experimental design was performed on an IBM 7040 computer using a library program by Wood (1967).

Definitions:

bi	parameter of the model
d. f.	degree of freedom
F	F value of the hypothesis test for goodness of fit (M. S. due to lack of fit/M. S. of the experimental error)
MS	mean square
R	multiple regression coefficient
SS	sum of squares
Y	dependent variable
X	independent variable

Models Under Consideration:

1. Second-order model with two independent variables

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2$$

11. First-order model with two variables and cross product

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_1X_2$$

111. First-order model with two variables

$$Y = b_o + b_1X_1 + b_2X_2$$

lV. First-order model with one variable

$$Y = b_o + b_1 X_1$$

Characterization of the Computing Routine:

A second-order polynomial model was chosen, as there was very little information about the studied response surface. It was assumed that it could be approximated by a polynomial of a low order.

In order to estimate the parameters of the considered models, the library computer program (Wood 1967) was applied. The statistics calculated by this program included the number of observations, the number of independent variables, the residual degrees of freedom, the maximum and minimum value of each variable as well as its range, the standard error and t-value for each coefficient, the F-value of the hypothesis that all of the coefficients are jointly equal to zero, the multiple correlation coefficient squared, the total sum of squares, the residual sum of squares, and the residual mean square. The goodness of fit was estimated by an F-test comparing the residual mean square to an estimate of the pure error variance obtained from replicants. An analysis of variance was carried out, and the simplest of all the four considered models which was statistically acceptable was chosen as the mathematical model expressing the studied problem (see Appendix V). Analysis of variance tables for the selected models and regression

coefficients are outlined in Appendix V. To test the significance of the differences between the combined and single substrate models, on the bases of 95% confidence, the "null" hypothesis of the equality of regression coefficients was applied as outlined in Appendix V. Similar procedure was carried out for comparisson of the regression model obtained by "pooling" the data from the soluble substrates against the single substrates.

APPENDIX 1V

PURE EXPERIMENTAL ERRORS

For a given type of experiment a combined variance of all sets, which were repeated in random, was judged as a pure error of a given type of experiment. For n sets of repeated results the combined variance is given by the weighted formula:

$$S^2 = \frac{v_1 s_1^2 + v_2 s_2^2 \ldots + v_n s_n^2}{v_1 + v_2 \ldots + v_n}$$

where: s^2 ..... combined variance

N_i ..... number of repetitions of i set

s_i ..... variance of i set

v_i ..... degree of freedom of i set $(N_i - 1)$

d.f. .... degree of freedom of combined variance

TABLE #5

PURE ERRORS OF EXPERIMENTS

Substrate	Error combined variance	d.f.
All soluble substrates	128	18
All substrates	191	24
Glucose	130	9

TABLE #5 - CONT'D.

Substrate	Error combined variance	d.f.
Glutamic acid	18	2
Phenol	156	7
Starch	384	6

APPENDIX V

SELECTING THE "BEST" REGRESSION MODEL FOR COMBINED DATA

To test whether model (B) $:Y=b_0+b_1X_1+b_2X_2$ would be an adequate representative of model (A): $Y=b_0+b_1X_1+b_2X_2+b_3X_1^2+b_4X_2^2+b_5X_1X_2$ the criterion of reliability was the F-statistic. The ratio of the residual error of (B) to the pure error of experiment was compared to the critical value derived on the basis od 95% confidence level. The pure error of experiment is illustrated in Appendix IV.

ANALYSIS OF VARIANCE OF THE COMPOSITE MODEL

Model: $Y=b_0+b_1X_1+b_2X_2$

$b_0=2.8$

$b_1=0.036$

$b_2=0.014$

Source	d.f.	SS	MS
Residual	6	382	19
Pure error	24	4161	191

F=0.1 crit. F=2.51

∴ non-linear terms are not significant

Further possible simplification would be omitting b_2

Model (C) $Y=b_0+b_1X_1$

The criterion of reliability used was the F-test comparison of the residual error with and without b_2

Source	Residual		
	d.f.	SS	MS
(C)	7	1368	196
(B)	6	381	63.6
(C) - (B)	1	991	991

$F = 991/196 = 5.06$ $F_{crit.} = 4.05$

$\therefore$ b_2 is signif.

Model: $Y=b_0+b_1X_1+b_2X_2$ accepted

Comparisson of the combined and single substrates models.

The equality of the regression coefficients is compared for two regression models on the basis of the equality of the corresponding coefficients. The "null" hypothesis is applied for 95% confidence. The t-test was used as the criterion of associated significances.

Note:

$\hat{B}_{il}$ regression coefficient of the i-coded variable X_i of the first model (table 6)

$\hat{B}_{i2}$	regression coefficient of the i-coded variable X_i of the second model (table 6)
s_p^2	pooled error variance of two compared regressions
x_i	i^{th}-coded variable
B_i	estimator of the coefficient $\hat{B}_i$
t	Student's t-value
d.f.	degrees of freedom
Q	abbreviation for $\sqrt{\frac{1}{\sum (x_{i1}-x_{i2})^2} + \frac{1}{\sum (x_{i1}-x_{i2})^2}}$

Tested H_o:hypothesis $B_{i1}-B_{i2} = 0$ against $B_{i1}-B_{i2} \neq 0$

If the inequality:

$$(B_{i1}-B_{i2})-t.s_p.Q \leq \hat{B}_{i1}-\hat{B}_{i2} \leq (B_{i1}-B_{i2})+t.s_p.Q$$

is satisfied, H_0 is accepted.

TABLE #6

CORRELATION COEFFICIENTS FOR CODED VARIABLES

Type of experiment	B_0	B_1	B_2
Glucose:	29.9	16.8	4.9
Glutamic acid:	37.8	16.8	6.3
Phenol:	41.7	15.1	16.9
Starch:	80.3	53.9	9.0
.....			
Composite model:	47.4	25.3	12.9
Composite soluble substrates model:	36	14.8	8.2

Composite model versus Glucose:

$(B_{01}-B_{02}) = 8.5$

$s_p = 7.3$

d.f. =12

t = 2.18

Q = .48

$8.5-9.81 < B_{01}-B_{02} < 8.5+9.81$

H_0:accepted for B_0

$(B_{11}-B_{12}) = 3.9$

Q = .575

$3.9-9.15 < B_{11}-B_{12} < 3.9+9.15$

H_0:accepted for B_1

$(B_{21}-B_{22}) = 17.5$

Q = .575

$17.5-9.15 < B_{21}-B_{22} < 17.5+915$

H_0:rejected for B_2

Composite model versus Glutamic acid:

$(B_{01}-B_{02}) = 8.5$

$s_p = 7.65$

d.f. =10

Q = .49

$8.5-8.5 < B_{01}-B_{02} < 8.5+8.5$

H_0:accepted for B_0

$(B_{11}-B_{12}) = 6.6$

Q = .6

$6.6-10.4 < B_{11}-B_{12} < 6.6+10.4$

H_0:accepted for B_1

$(B_{21}-B_{22}) = 9.6$

Q = .48

$9.6-8.21 < B_{21}-B_{22} < 9.6+8.21$

H_0:rejected for B_2

Composite model versus Phenol:

$(B_{01}-B_{02})=10.2$

$s_p = 13.9$

d.f. =12

Q = .48

t = 2.18

$10.2-14.5 < B_{01}-B_{02} < 10.2+14.5$

H_0:accepted for B_0

$(B_{11}-B_{12})=-4.0$

Q = .575

$-4-17.5 < B_{11}-B_{12} < -4+17.5$

H_0:accepted for B_1

$(B_{21}-B_{22})= 5.7$

Q = .575

$5.7-17.5 < B_{21}-B_{22} < 5.7+17.5$

H_0:accepted for B_2

Composite model versus Starch:

$(B_{01}-B_{02})=-38.6$

$s_p = 22.5$

d.f. = 11

t = 2.2

Q = .48

$-38.6-23.7 < B_{01}-B_{02} < -38.6+23.7$

H_0:rejected for B_0

$(B_{11}-B_{12})= 3.9$

Q = .575

$3.9-28.5 < B_{11}-B_{12} < 3.9+28.5$

H_0:accepted for B_1

$(B_{21}-B_{22})=-32.9$

Q = .63

$-32.9-31.5 < B_{21}-B_{22} < -32.9+31.5$

H_0:rejected for B_2

Analysis of variance of the composite soluble substrates model

Model: $Y=b_0+b_1X_1+b_2X_2$

$b_0=9.1$

$b_1=0.021$

$b_2=0.009$

Source	d.f.	SS.	MS.
Residual	6	370	19
Pure Error	18	2320	128

F=0.15 crit.F=2.66

Comparison of the"composite soluble substrate" models with the single substrate models.

"Pooled substrate model" vs. Glucose:

$(B_{01}-B_{02})= 6.1$

$s_p = 7.25$

d.f. =12

t = 2.18

Q = .48

$6.1-9.75 < B_{01}-B_{02} < 6.1+9.75$

H_0:accepted for B_0

$(B_{11}-B_{12})= 2.0$

Q = .575

$2-9 < B_{11}-B_{12} < 2+9$

H_0:accepted for B_1

$(B_{21}-B_{22})= 3.3$

Q = .575

$3.3-9 < B_{21}-B_{22} < 3.3+9$

H_0:accepted for B_2

"Composite substrates" model vs. Glutamic acid:

$(B_{01}-B_{02})=-1.8$

$s_p = 7.55$

d.f. =10

t = 2.23

Q = .49

$-1.8-8.3 < B_{01}-B_{02} < -1.8+8.3$

H_0:accepted for B_0

$(B_{11}-B_{12})=-2.0$

Q = .6

$-2.0-10.2 < B_{11}-B_{12} < -2+10.2$

H_0:accepted for B_1

$(B_{21}-B_{22})= 1.9$

Q = .48

$1.9-8.10 < B_{21}-B_{22} < 1.9+8.10$

H_0:accepted for B_2

"Composite substrates" model vs. Phenol:

$(B_{01}-B_{02})=-5.7$

s_p =13.75

d.f. =12

t = 2.18

Q = .48

$-5.7-14.3 < B_{01}-B_{02} < -5.7+14.3$

H_0:accepted for B_0

$(B_{11}-B_{12})=-0.3$

Q = .575

$-0.3-17.2 < B_{11}-B_{12} < -0.3+17.2$

H_0:accepted for B_1

$(B_{21}-B_{22})=-8.7$

Q = .575

$-8.7-17.2 < B_{21}-B_{22} < -8.7+17.2$

H_0:accepted for B_2

If the single substrates were treated individually, their regressions would be as following:

Analysis of variance table for the selected model

Model: $Y=b_0+b_1X_1+b_2X_2$

Glucose experiments:	Source	d.f.	SS	MS
b_0=5.15	Residual	15	1346	90
b_1=0.024	Pure error	9	1070	130
b_2=0.005				
	F=0.69	crit.F=3.00		

Glutamic acid experiments:	Source	d.f.	SS	MS
b_0=10.82	Residual	6	220	36.6
b_1= 0.024	Pure error	2	36	18
b_2= 0.007				
	F=2.04	crit.F=19.33		

Phenol experiments:

b_0=3.87

b_1=0.0216

b_2=0.0188

Source	d.f.	SS	MS
Residual	16	3883	203
Pure error	7	1092	156

F=2.43 crit.F=3.68

Starch experiments:

b_0=7.74

b_1=0.077

b_2=0.01

Source	d.f.	SS	MS
Residual	13	13532	1040
Pure error	6	2304	384

F=2.70 crit.F=3.98

Analysis of variance of the model IV

Model: $Y=b_0+b_1X_1$

Glucose experiments:

b_0=10.4

b_1=0.032

Source	d.f.	SS	MS
Residual	19	3532	186
Pure error	9	1170	130

F=1.42 crit.F=3.14

Glutamic acid experiments:

b_0=19.2

b_1=0.0213

Source	d.f.	SS	MS
Residual	7	388	55
Pure error	2	36	18
F=3.05	crit.F=19.3		

Phenol experiments:

Model is not accepted

Source	d.f.	SS	MS
Residual	12	6727	562
Pure error	8	1248	156
F=3.62	crit.F=3.25		

Starch experiments:

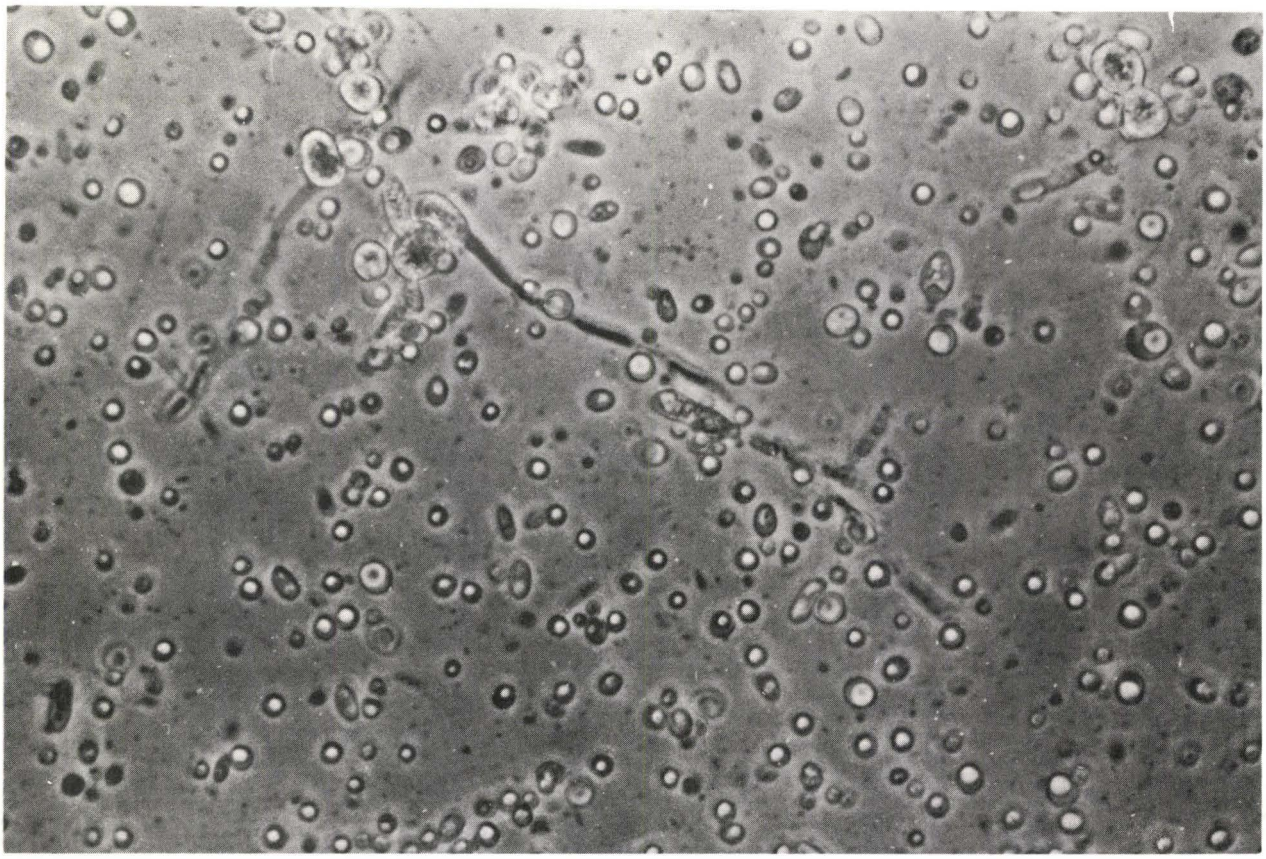
Model is not accepted

Source	d.f.	SS	MS
Residual	11	16938	1540
Pure error	8	3072	384
F=4.01	crit.F=3.31		

APPENDIX V1

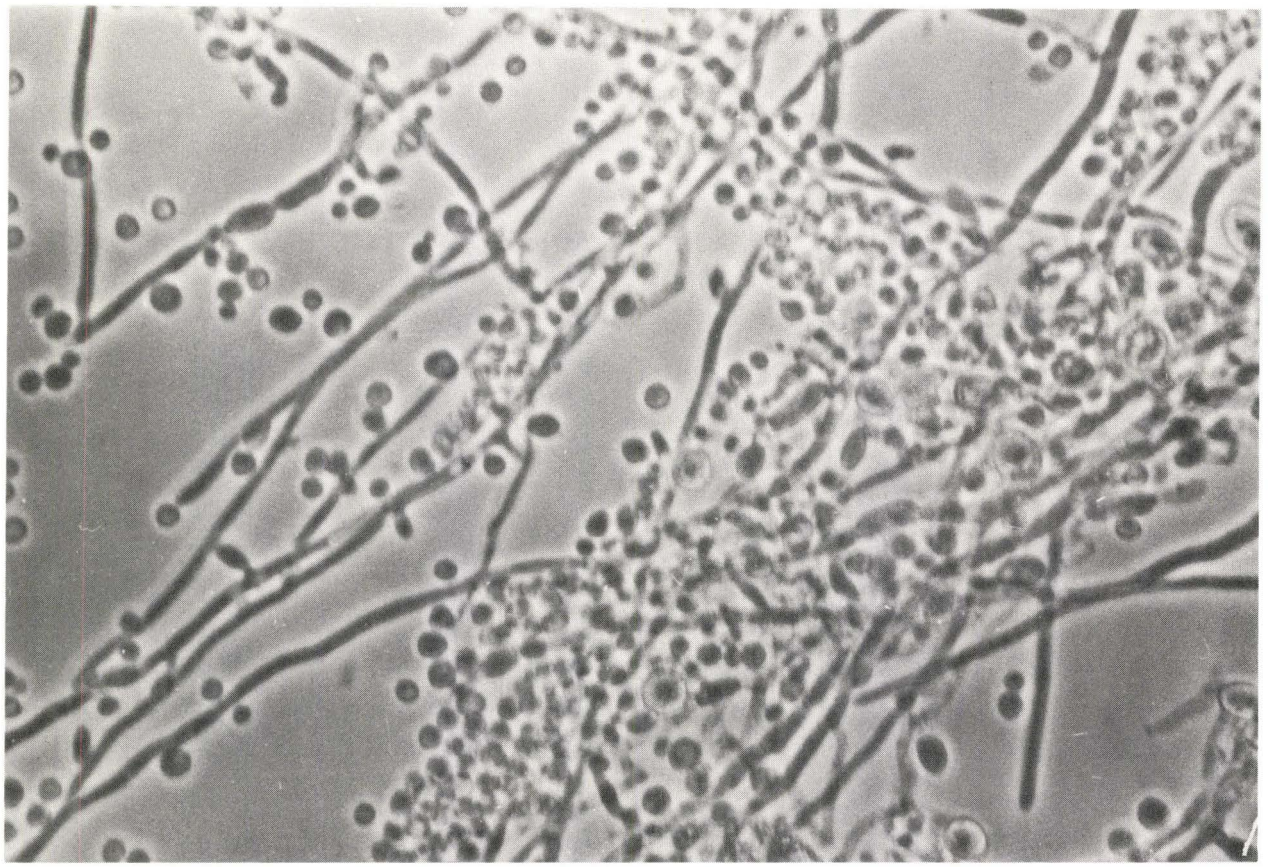
PICTURES OF THE MICROBIAL CULTURES DEVELOPED ON PURE SUBSTRATES

PICTURE # 1



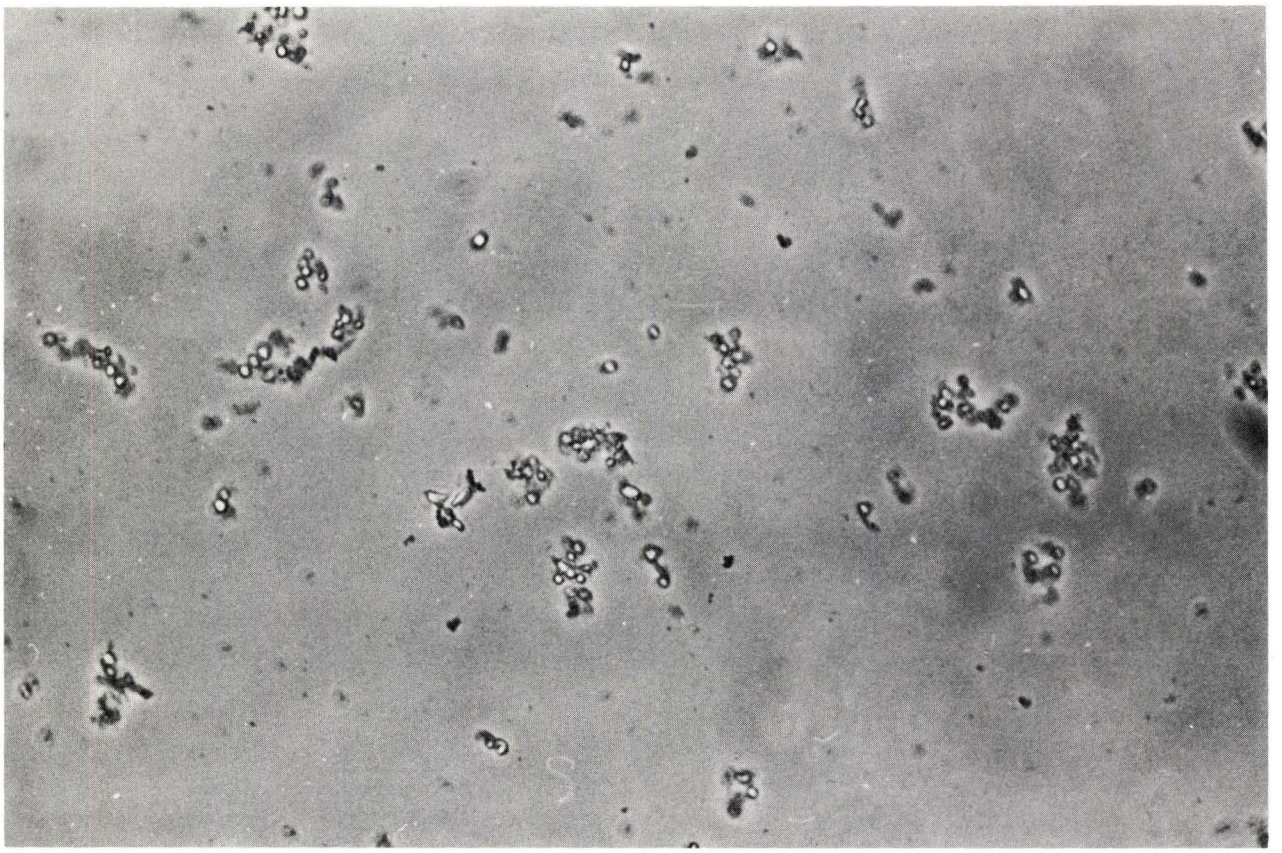
CULTURE DEVELOPED on GLUCOSE (200x)

PICTURE # 2



CULTURE DEVELOPED on GLUTAMIC ACID (200x)

PICTURE # 3



CULTURE DEVELOPED on PHENOL (200x)

APPENDIX V11

NOMENCLATURE

Term:	Meaning:
ABS	Alkyl Benzene Sulfonate
Background TOD	Total oxygen demand of nutritious elements at zero time
b_i	Regression coefficient i independent variable obtained by the method of least squares
BOD_5	Biochemical oxygen demand in 5 days. The amount of dissolved oxygen in ppm required by organisms for the aerobic decomposition of organic matter in water.
C_B	Concentration of micro-organisms (mg/l)
C_C	Concentration of substrate carbon (mg/l)
C_R	Concentration of residual soluble organic carbon (mg/l)
COD	Chemical oxygen demand. Total oxygen consumed by the chemical oxidation of materials in water
d.f.	degree of freedom
F	Fisher's F - statistical distribution
F/M	Food to micro-organisms ratio. The ratio of initial conc. of substrate to the initial solids concentration
I	Abbreviation for the interaction
mg/l	Milligrams per litre
MLSS	Mixed liquor suspended solids (mg/l)

Term:	Meaning:
mμ	Millimicron, 1×10^{-9} meter
MS	Mean square
n	Number of observations
pH	The negative logarithm of hydrogen ion concentration
ppm	Abbreviation for parts per million
R	Multiple regression coefficient
SS	Sum of squares
s^2	the variance
s_p^2	the pooled variance
TOD	Total (theoretical) oxygen demand. Total oxygen in mg/l for complete oxidation of material in water
X	Independent variable
Y	Dependent variable