CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL-339 (r. 82/08)

Canada

MICROBIAL ACTIVITY AND BIOMASS IN AEROBIC SLUDGE DIGESTION

by

Walter Andres Sanchez-Bain

Submitted in partial fulfillment
of the requirements for the degree of
Master of Applied Science

Department of Civil Engineering

School of Graduate Studies

University of Ottawa

Ottawa, Canada

April, 1982.

© W.A. Sanchez-Bain, Ottawa, Canada, 1982

UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ABSTRACT

The purpose of this investigation was to contribute to the data base of technical information necessary for a more comprehensive mathematical formulation of the aerobic sludge digestion process. The main focus was the determination of the best measure of microbial activity and its relation to active mass in the mixed liquour.

Four activity measures were evaluated. These were ATP, plate counts, dissolved oxygen uptake rate, and activity of dehydrogenase enzymes. Their variation with digestion time was monitored for batch and semi-continuous flow regimes at three different operating temperatures : 10° C, 20°C, and 30°C.

It was found that ATP offers the best estimate of activity, while plate counts can result in erroneous and misleading interpretations of the metabolic state of the mixed liquour population.

ATP was found to parallel the decrease in biodegradable volatile suspended solids (BVSS) during batch digestion, when corrected for cell lysis. It was concluded that the decrease in ATP does reflect a decrease in active biomass from the endogenous metabolism of the bacterial population, and that the ATP content per active cell mass remains constant with detention time thus allowing the estimation of active fraction of the sludge (F_A).

On this basis, preliminary investigations on active fraction variations revealed that essentially different types of cell decay occurred between batch and semi-continuous flow regimes. A

first order rate of decrease in F_A with detention time was obtained in all batch units whereas this fraction remained essentially constant in the semi-continuous units. The above observations have direct implications in process design in that it is common practice to extrapolate endogenous decay coefficients from one type of flow regime to another. Analysis of the experimental data demonstrated that such extrapolations cannot be used to accurately predict percent reductions in solids. This is particularly true at low temperatures of operation, where batch coefficients were found to be up to 5 times smaller than the semi-continuous ones.

Analysis of temperature effects on kinetic coefficients indicated that coefficients determined on a BVSS basis in semi-continuous units do not follow an Arrehnius relationship, as is usually assumed. These type of coefficients were shown to be dependent on the rapidity of BVSS destruction rather than on the magnitude of solids reductions. Sharp differences in ultimate biodegradability of the sludge with temperature of operation make the use of such coefficients inadequate for the comparison of process performance among units.

Active mass was found to be a useful parameter in the monitoring and design of aerobic sludge digestion processes. However further studies are required to fully determine its behaviour during digestion and its relation to sludge stability.

ACKNOWLEDGMENTS

The author expresses his sincerest appreciation to his thesis supervisor, Dr. Ronald L. Droste, for his professional guidance and encouragement through the course of this investigation and preparation of this manuscript.

Special appreciation is expressed to Dr. Richard S. Tobin of Health and Welfare Canada for his advice on various aspects of this study.

The author would also like to thank Mr. Francisco Aposaga for his technical support and assistance with laboratory analysis.

Financial assistance for this research was supplied by a grant from the National Research Council, grant # NSERCA0993.

TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES AND ILLUSTRATIONS	vii
GLOSSARY	xii
CHAPTER 1. INTRODUCTION	1
1.1 Background of Investigation	1
1.2 Justification and Scope	4
1.3 Objectives	5
CHAPTER 2. LITERATURE REVIEW	6
2.1 Aerobic Sludge Digestion	6
2.1.1 Process Description	6
2.1.2 Process Kinetics	15
2.2 Activity Indicators	22
2.2.1 Adenosine Triphosphate (ATP)	23
2.2.2 Dissolved Oxygen Uptake Rate (DOUR)	33
2.2.3 Dehydrogenase Activity	36
2.2.4 Bacterial Plate Count	37
2.2.5 Direct Counting of Bacteria	39
CHAPTER 3. EXPERIMENTAL CONSIDERATIONS	42
3.1 Measures of Activity	42
3.2 Biomass Estimation	44
CHAPTER 4. EXPERIMENTAL PROCEDURE	49
4.1 Work Organization and Apparatus	49
4.1.1 Overall Organization	49

4.1.2 Set Up 50

4.1.3 Feed Sludge 51

4.1.4 Daily Operation 52

4.2 Analytical Methods 52

4.2.1 ATP Assay 52

4.2.2 DOUR 59

4.2.3 Plate Count 59

4.2.4 AOE-Dehydrogenase Activity 60

4.2.5 Overall Monitoring of the Units 61

CHAPTER 5. EXPERIMENTAL RESULTS 62

5.1 Batch Reactors 62

5.1.1 Solids Variations 62

5.1.2 Plate Counts 66

5.1.3 AOE-Dehydrogenase Activity 66

5.1.4 ATP and DOUR 72

5.2 Semi-Continuous Reactors 73

5.2.1 Solids Variations 73

5.2.2 Activity Measures 77

CHAPTER 6. DISCUSSION 84

6.1 Activity Parameters 84

6.1.1 AOE-Dehydrogenase Activity 84

6.1.2 Plate Counts 84

6.1.3 ATP and DOUR 86

6.2 Active Fraction Determination 90

6.3 Active Fraction Variations 101

6.4 Design Considerations 106

6.4.1 Solubilization of Volatile Organics 106

6.4.2 Extrapolation of Decay Coefficients 107

6.4.3 Temperature Effects 109

6.5 Active Mass as Process Variable 112

CHAPTER 7. CONCLUSIONS 120

CHAPTER 8. SUGGESTIONS FOR FUTURE WORK 123

REFERENCES 125

APPENDIX A. KINETIC MODEL DERIVATIONS 133

A.1 Adams et al. Model 133

A.2 Benefield and Randall's Model 135

APPENDIX B. SELECTED LABORATORY PROCEDURES 140

B.1 ATP Extraction Procedure 140

B.2 ATP Measurement Procedure 141

B.3 DOUR Procedure 143

B.4 AOE-Dehydrogenase Activity Procedure 144

APPENDIX C. REGRESSION ANALYSIS 147

C.1 Fitting of ATP and BVSS vs. Time Curves 147

C.2 Fitting of ATP vs. BVSS Curves 149

C.3 Fitting of F_A vs. Time Curves 151

C.4 Fitting of M_A vs. Time for Batch Reactors 153

C.5 Fitting of M_A vs. Time for Semi-Continuous Reactors 155

C.6 Fitting of BVSS vs. Time for Batch Reactors 157

C.7 Fitting of BVSS vs. Time for Semi-Continuous Reactors 159

APPENDIX D. EXPERIMENTAL DATA 161

APPENDIX E. CORRECTION OF TSS AND TS FROM FEED VARIATIONS 187

APPENDIX F. CORRECTION OF BVSS FOR SOLUBILIZATION FROM CELL LYSIS 196

LIST OF TABLES AND ILLUSTRATIONS

Figure		Page
2.1	Minimum SRT Values for Aerobic Digestion	8
3.1	Activity Concentration Versus Active Mass	47
4.1	Modifications to Scintillation Vials	55
4.2	Typical Bioluminescence Decay Curve	56
4.3	Typical ATP Calibration Curve	58
5.1	Solids Variations in 10°C Batch Run	63
5.2	Solids Variations in 20°C Batch Run	64
5.3	Solids Variations in 30°C Batch Run	65
5.4	Activity Data for Batch Run at 10°C	67
5.5	Activity Data for Batch Run at 20°C	68
5.6	Activity Data for Batch Run at 30°C	69
5.7	Viability and Dehydrogenase Enzyme Activity Data for Batch Reactors	71
5.8	Solids Concentrations for Semi-Continuous Units at 10°C	74
5.9	Solids Concentrations for Semi-Continuous Units at 20°C	75
5.10	Solids Concentrations for Semi-Continuous Units at 30°C	76
5.11	Viability and Dehydrogenase Enzyme Activity Data for Semi-Continuous Reactors	78
5.12	ATP and DOUR Variations in Semi-Continuous Units	79
5.13	Viable Cells and % Activity in Semi-Continuous units	80
5.14	ATP Content per Cell in Semi-Continuous Units	81
5.15	ATP Content per Cell Reported in the Literature	82
6.1	BVSS and ATP vs. Time, for 10°C Run	92
6.2	BVSS and ATP vs. Time, for 20°C Run	93

Figure		Page
6.3	BVSS and ATP vs. Time, for 30°C Run	94
6.4	Solubilization of Organic Solids in Batch Reactors	95
6.5	ATP vs. BVSS, 10°C Run	97
6.6	ATP vs. BVSS, 20°C Run	98
6.7	ATP vs. BVSS, 30°C Run	99
6.8	Active Fraction Variations in Batch Units	102
6.9	Active Fraction Regression Analysis in Batch Units	103
6.10	Active Fraction Variations in Semi-Continuous Units	105
6.11	Comparison Between Batch and Semi-Continuous Decay Coefficients	108
6.12	Temperature Effects on Decay Rate Coefficients	111
6.13	Active Mass Variations in Batch Units	114
6.14	Active Mass Regression Analysis in Batch Units	115
6.15	Active Mass Regression Analysis in Semi-Continuous Units	116
6.16	Batch and Semi-Continuous Reactor Preformance at 30 days Detention Time	118
A.1	Complete-Mix Aeration Basin	133
A.2	Changes in TSS during Aerobic Digestion	136
C.1	ATP,BVSS vs. Time Curves, Regression Parameters for Batch Reactors	147
C.2	ATP,BVSS vs. Time Curves, Regression Data for Batch Reactors	148
C.3	ATP vs. BVSS Curves, Regression Parameters for Batch Reactors	149
C.4	ATP vs. BVSS Curves, Regression Data for Batch Reactors	150

Figure		Page
C.5	F_A vs. Time Curves, Regression Parameters for Batch Reactors	151
C.6	F_A vs. Time Curves, Regression Data for Batch Reactors	152
C.7	M_A vs. Time Curves, Regression Parameters for Batch Reactors	153
C.8	M_A vs. Time Curves, Regression Data for Batch Reactors	154
C.9	M_A vs. Time Curves, Regression Parameters for Semi-Continuous Reactors	155
C.10	M_A vs. Time Curves, Regression Data for Semi-Continuous Reactors	156
C.11	BVSS vs. Time, Regression Parameters for Batch Reactors	157
C.12	BVSS vs. Time, Regression Data for Batch Reactors	158
C.13	BVSS vs. Time, Regression Parameters for Semi-Continuous Reactors	159
C.14	BVSS vs. Time, Regression Data for Semi-Continuous Reactors	160
D.1	Solids Concentrations in Batch Reactor at 10°C	161
D.2	Solids Concentrations in Batch Reactor at 20°C	162
D.3	Solids Concentrations in Batch Reactor at 30°C	162
D.4	Activity Concentrations in Batch Reactor at 10°C	163
D.5	Activity Concentrations in Batch Reactor at 20°C	163
D.6	Activity Concentrations in Batch Reactor at 30°C	164
D.7	Specific Activities for Batch Reactor at 10°C	164
D.8	Specific Activities for Batch Reactor at 20°C	165
D.9	Specific Activities for Batch Reactor at 30°C	165
D.10	Viability and Dehydrogenase Enzyme Activity Data for Batch Reactor at 10°C	166

Figure		Page
D.11	Viability and Dehydrogenase Enzyme Activity Data for Batch Reactor at 20°C	166
D.12	Viability and Dehydrogenase Enzyme Activity Data for Batch Reactor at 30°C	167
D.13	Solids Concentrations in Semi-Continuous Reactors at 10°C, 10 Days Detention Time	168
D.14	Solids Concentrations in Semi-Continuous Reactors at 10°C, 20 Days Detention Time	169
D.15	Solids Concentrations in Semi-Continuous Reactors at 10°C, 30 Days Detention Time	170
D.16	Solids Concentrations in Semi-Continuous Reactors at 20°C, 10 Days Detention Time	171
D.17	Solids Concentrations in Semi-Continuous Reactors at 20°C, 20 Days Detention Time	172
D.18	Solids Concentrations in Semi-Continuous Reactors at 20°C, 30 Days Detention Time	173
D.19	Solids Concentrations in Semi-Continuous Reactors at 30°C, 10 Days Detention Time	174
D.20	Solids Concentrations in Semi-Continuous Reactors at 30°C, 20 Days Detention Time	175
D.21	Solids Concentrations in Semi-Continuous Reactors at 30°C, 30 Days Detention Time	176
D.22	Activity Measures in Semi-Continuous Reactors at 10°C	177
D.23	Activity Measures in Semi-Continuous Reactors at 20°C	178
D.24	Activity Measures in Semi-Continuous Reactors at 30°C	179
D.25	Dehydrogenase Enzyme Activity Data for Semi-Continuous Reactors at 10°C	180
D.26	Dehydrogenase Enzyme Activity Data for Semi-Continuous Reactors at 20°C	181
D.27	Dehydrogenase Enzyme Activity Data for Semi-Continuous Reactors at 30°C	182

Figure	Page
D.28 COD Data for Batch Reactors	183
D.29 COD Data for Semi-Continuous Reactors at 10°C	183
D.30 COD Data for Semi-Continuous Reactors at 20°C	184
D.31 COD Data for Semi-Continuous Reactors at 30°C	184
D.32 PH Data for Batch Reactors	185
D.33 PH Data for Semi-Continuous Reactors at 10°C	185
D.34 PH Data for Semi-Continuous Reactors at 20°C	186
D.35 PH Data for Semi-Continuous Reactors at 30°C	186
E.1 Solids Corrections for Semi-Continuous Reactors at 10°C	190
E.2 Solids Corrections for Semi-Continuous Reactors at 20°C	192
E.3 Solids Corrections for Semi-Continuous Reactors at 30°C	194
F.1 BVSS Corrections for Batch Reactor at 20°C	198
F.2 BVSS Corrections for Batch Reactor at 30°C	198

GLOSSARY

A	- activity concentration in mixed-liquor
AOE	- acridine orange epifluorescence
ASE	- activated sludge extract agar
ATP	- adenosine triphosphate, µg/l or µg/mg VSS
BFSS	- biodegradable fixed suspended solids, mg/l
BOD	- biochemical oxygen demand, mg oxygen/l
BVSS	- biodegradable volatile suspended solids, mg/l
CPM	- counts per minute
D	- fraction of degradable active mass in influent that appears in effluent
DO	- dissolved oxygen, mg oxygen/l
DOUR	- dissolved oxygen uptake rate, mg oxygen/ hr or mg oxygen/ mg VSS-hr
F_A	- active fraction of microorganisms in mixed-liquor
FSS	- fixed suspended solids, mg/l
HRT	- hydraulic retention time, days
K	- stabilization rate coefficient, day^{-1}
K_e	- endogenous decay rate coefficient, day^{-1}
K_T	- reaction rate coefficient at temperature T
K_{20}	- reacton rate coefficient at 20°C
M	- total suspended solids concentration in mixed-liquor, mg/l
M_A	- active mass concentration in mixed-liquor, mg/l
MLVSS	- mixed-liquor volatile suspended solids, mg/l
NBVSS	- non-biodegradable volatile suspended solids, mg/l
OSLR	- organic solids loading rate, Kg VS or VSS/m^3/d
P_B	- base value or activity concentration per active cell mass

P_S - specific activity content in mixed-liquor

PL - power level, $kW/10^3m^3$

Q - volumetric flowrate, l/d or m^3/d

r - correlation coefficient

R_A - specific rate of decay of living cells, day^{-1}

R_D - specific rate of decay of dead cells, day^{-1}

SRT - mean solids retention time, day^{-1}

T - temperature, °C

TDS - total dissolved solids, mg/l

TF - triphenyl formazan

TGE - tryptone glucose extract agar

TS - total solids, mg/l

TSS - total suspended solids, mg/l

TTC - 2,3,5 triphenyl tetrazolium chloride

V - reactor volume, l or m^3

VS - volatile solids, mg/l

VSS - volatile suspended solids, mg/l

X_a - active fraction of sludge

θ - thermal coefficient

subscripts :

e - effluent

ea - effluent active

ead - effluent active degradable

o - influent

oa - influent active

oad - influent active degradable

CHAPTER 1

INTRODUCTION

1.1 Background of Investigation

All conventional wastewater treatment operations and processes produce large quantities of sludge that cannot normally be disposed of without some means of pretreatment. This waste material is in the form of a liquid or semisolid liquid which typically contains from 0.25 to 12 % solids depending on the characteristics of the raw wastewater and treatment processes that generated it (41).

Sludge is composed largely of pollutants responsible for the offensive and noxious nature of untreated wastewater and therefore must be stabilized prior to disposal so as to minimize its harmful effects. In this context 'stabilize' refers to :

i) Reduction of the pathogenic organism content of the sludge,

ii) Reduction or elimination of offensive odours,

iii) Reduction or inhibition of the potential for putrefaction,

iv) Reduction of the solids quantity that must be handled subsequently.

Traditionally, sludge stabilization or sludge digestion as it is more frequently termed, has been accomplished by anaerobic digestion. This process produces a stable sludge but has several disadvantages (3) :

i) Resulting supernatant is high in nutrients and organics due

to solubilization in anaerobic metabolism.

ii) Anaerobically digested sludges are in general difficult to dewater by mechanical means.

iii) The process is very sensitive and therefore results in frequent upsets.

iv) The process is efficient only under controlled conditions of temperature and pH and therefore requires sophisticated equipment and adequate supervision.

Because of the above undesirable characteristics, other methods of sludge stabilization have been introduced, the most popular one being aerobic digestion. This process has been used extensively in small scale wastewater treatment plants due to its simplicity of operation and minimal need of supervision. High operating costs related to he energy requirements of maintaining aerobic conditions in the stabilizing reactors encouraged its initial application to small treatment systems only. However in recent years aerobic digestion has been shown particularly suitable for industrial sludge treatment, the reasons for this are its low capital investment, simplicity of operation, ability to handle dilute solids concentrations, and greater immunity to potentially toxic industrial wastes (67). Also this process is being increasingly used in larger systems to stabilize waste activated sludge separately from sludges resulting from primary clarification. The reason for this is that the enormous volumes of dilute waste activated sludge generated are extremely difficult to be either anaerobically stabilized or economically dewatered.

In spite of its increasing popularity, only limited attention has been given to the improvement of process kinetics description and design procedures. This has resulted in a lack of technical information regarding design criteria and contradictory predictions on the effects of some environmental factors related to the process.

At the root of these problems is a controversy regarding what parameters to use for the control and design of the process. Up to date, volatile suspended solids (VSS) or their biodegradable part (BVSS) have been used extensively as process variables. These mass measurements have generally been assumed to be an accurate determination of the active microbial mass in the mixed liquour, with their ease of determination being the main reason for their popularity in this and other biological treatment processes. However in recent years increasing recognition of their limitations has led to the examination of several alternative measures of activity or active biomass (65, 62, 45). The most relevant ones being adenosine triphosphate (ATP), dissolved oxygen uptake rate (DOUR), dehydrogenase enzyme activity and plate counts. Although some authors have proposed their use in aerobic digestion design (8), experimental investigations on their applicability have concentrated almost exclusively on the activated sludge process.

For this process the measures above have been claimed to be more direct and accurate estimators of microbial activity, as compared to VSS. Consequently, their use in aerobic digestion would be expected to result in better monitoring and control of

the process, and in terms of design, a better reflection of the rate of mass reduction through endogenous respiration.

1.2 Justification and Scope

This investigation was intended to contribute to the data base of technical information required to formulate and improve process design criteria for the aerobic digestion of waste activated sludges.

There is presently a need for a more comprehensive kinetic model of the process, but before its formulation is attempted, a clear definition of the basic process variables is necessary. As mentioned earlier, there has been a recent trend to substitute organic solids concentrations by more direct measures of activity or active mass. Nevertheless their widespread applicaton in biological processes has been hampered by two principal factors:

i) Existing confusion regarding advantages and disadvantages of each measure, with conflicting reports on their merits as reliable estimators of metabolic activity; and

ii) A lack of definition of active mass determined from these activity measures, the transition from activity to biomass often necessitating the use of gross or unverified assumptions that have significant impact on the sensitivity and accuracy of the estimations.

Due to the limited resources available and the extent of the topic, it was deemed necessary to restrict this investigation to the study of activity measures and their application to the

aerobic digestion process. It is believed that the results of this study will lay the basis for a more comprehensive mathematical formulation of the process.

1.3 Objectives

With the development of a process model in mind as the ultimate goal, this investigation was restricted to the attainment of the following objectives:

i) a critical review and evaluation of the present measures of activity,

ii) analysis of the relationship between activity measures and biomass, and

iii) a preliminary investigation on the application of activity or active mass information in process description and design.

CHAPTER 2

LITERATURE REVIEW

2.1 Aerobic Sludge Digestion

2.1.1 Process Description

Aerobic digestion may be defined as the conversion of degradable organic wastewater sludges by aerobic biological mechanisms to oxidized end products (1). The biological reactions which occur and their extent are dependent upon the type and condition of the feed sludge, which is either primary, secondary (waste activated sludge) or mixtures of the two. With sludge from primary clarification, the organic substrates are first utilized by aerobic microorganisms for synthesis and respiration. With secondary sludges, the supply of available substrate is at or near depletion depending on the process detention time, and therefore in the absence of an external source of carbon, the microorganisms begin to consume their own protoplasm to obtain energy for cell maintenance reactions. When this occurs, the microorganisms are said to be in the endogenous phase of the growth cycle. Eventually some organisms undergo lysis releasing their protoplasm into solution which is then utilized by other bacteria. The net result is a decrease in the unoxidized portion of the microbial population or sludge mass (41). Only about 75 to 80 percent of the cell tissue can be oxidized. The remaining 20 to 25 percent is composed of inert compounds and organic matter

that is not biodegradable. This residual portion is commonly termed non-biodegradable solids (NBVSS) and is estimated by the VSS concentration of the mixed liquour in a batch reactor after extended periods of digestion, in excess of 30 days. Conventional aerobic digesters can be operated as batch or flow-through reactors, with the semi-continuous (fill and draw) type being the most popular one.

The main parameters that control the process have been described by Stein et al. (58) as :

i) Hydraulic retention time and solids retention time
ii) Loading criteria
iii) Oxygen requirements
iv) Energy requirements
v) Environmental conditions
vi) Characteristics of feed sludge

Hydraulic Retention Time (HRT) and Solids Retention Time (SRT)

Experimental research evaluating the importance of SRT as a process variable has almost solely considered batch or nonrecycle continuous flow digestion systems. For these conditions SRT is equal to HRT or aeration time. While the other factors are important, most system designs have been based on aeration time alone. This fact was pointed out by Hegg and Rakness (27) in their review of aerobic digestion design considerations. In their work, they recommended minimum SRT values for various types of sludges which are tabulated below.

Fig. 2.1 Minimum SRT Values for Aerobic Digestion

Sludge Classification	SRT (days)
High Rate Activated Sludge	20-25
Conventional Activated Sludge	15-25
Step Aeration Activated Sludge	15-25
Contact Stabilization Activated Sludge	15-20
Extended Aeration Activated Sludge	15-20
Primary Clarification	20-30
Primary + Waste Activated Sludge	20-30
Trickling Filter Humus	20-30

The values in this table also represent recommended HRT values, since SRT is always equal to or greater than HRT.

Ahlberg and Boyko (3), based on a review of seven operating aerobic digestion systems in Canada, suggested a minimum total SRT value of 45 days. By 'total SRT' it is meant both the solids retention time of the sludge during digestion plus the SRT of the sludge in its source process. The authors also cautioned that selection of design SRT values must consider the process operating temperature, as low temperatures can significantly lower stabilization rates. In summary, typical SRT values reported in the literature range from 10 to 35 days for the digestion of waste activated sludge using diffused aeration with temperatres between 15 to 30°C.

Loading Criteria

Organic solids loading rate (OSLR) represents loading on a volumetric basis, usually per cubic metre of sludge under aeration. Units are typically in kilograms volatile solids or volatile suspended solids per cubic metre aeration volume per day (kg VS or VSS/m^3/d).

Several studies have been conducted to examine the relationship between OSLR and process efficiency, usually expressed as percent reduction in biodegradable solids. Jaworski et al. (30) operated laboratory scale digesters on 1.75 to 1.00 (dry solids basis) mixtures of primary and waste activated sludges within ranges of 0.39 to 4.70 kg VS/m^3/d. Lawton and Norman (35) also used mixed sludges with OSLR values between 0.68 to 2.27 kg VS/m^3/d. Cohen

and Fullerton (16) conducted plant and pilot scale digestion tests on waste activated sludge using both diffused air and pure oxygen aeration. For the diffused air tests OSLR was varied from 0.42 to 3.0 kg VSS/m^3/d. In pure oxygen experiments the OSLR values varied from 1.33 to 9.61 kg VSS/m^3/d.

Two general observations from these studies were that increasing OSLR lowers the degree of stability obtained within a given time period and that the rate of stabilization, expressed as the mass of volatile solids destroyed per unit time, increases with increasing organic loading rates.

In their review of seven aerobic digestors, Ahlberg and Boyko (3) reported design loadings between 0.38 to 1.28 kg VS/m^3/d and actual loadings ranging as low as 0.06 to 0.43 kg VSS/m^3/d. In his examination of aerobic design criteria, Loehr (37) reported prevalent OSLR values ranging from 1.62 to 2.27 kg VS/m^3/d.

In summary, experimental and full scale OSLR values encountered in the literature range between 0.4 to 5.0 kg VSS/m^3/d for conventional batch or continuous flow aerobic digestion. Higher values have been applied for pure oxygen aeration or elevated digestion temperature processes.

Oxygen Requirements

The oxygen requirements that must be satisfied during aerobic digestion are those of endogenous metabolism and, with mixed sludges, the BOD of the primary sludge component.

Various investigators including Loehr (37) and Reynolds (51) have presented equations relating theoretical oxygen requirements

to the mass of volatile solids destroyed.

By approximating cell mass with the molecular formula $C_5H_7NO_2$, endogenous respiration may be represented by the equation :

$$C_5H_7NO_2 + 7\ O_2 \longrightarrow 5\ CO_2 + NO_3 + 3H_2O + H^+ + \text{energy}$$

From this equation the oxygen requirement for the complete oxidation of cell tissue is equal to 7 mol/mol of cells or about 2 kg/kg of cells. The oxygen requirement for the complete oxidation of the organic matter contained in primary sludges has been reported to vary from about 1.7 to 1.9 kg/kg destroyed (41).

Benefield and Randall (8) presented a theoretical estimation based on the active degradable mass reduction, BOD of primary sludge and nitrogenous oxygen demand. They recommended, as average values, air requirements of 0.25 to 0.33 l/sec/m^3 (15-20 cfm/1000 ft^3) of tank capacity for activated sludges and 0.41 to 0.50 l/sec/m^3 (25-30 cfm/1000 ft^3) for primary plus activated sludge mixtures.

As an approximate rule for process design it has been recommended to have sufficient aeration capacity to maintain a specified minimum dissolved oxygen concentration in the process. The most common range given has been 1.0 to 2.0 mg/l (41). Loehr (37) reported the minimum range to be 0.5 to 1.0 mg/l. However if diffused aeration is selected for both oxygen transfer and mixing, more often the amount of air required is a function of the amount needed for mixing rather than oxygen supply (19).

Oxygen Requirements for Mixing

Adequate mixing must be achieved in order to keep the solids in suspension and to maintain optimum oxygen transfer efficiency. Mixing requirements are commonly expressed in terms of power level (PL) which is defined as the power per unit volume under aeration, and has units of $kW/10^3m^3$ (8). The majority of installations have diffused aeration systems. This result is from the early applications of the aerobic digestion process to package activated sludge treatment systems already equipped with diffused aeration. These devices have been subject to frequent clogging of diffusers and air headers in many installations because aeration must be terminated prior to decanting supernatant and withdrawing digested sludge (52). Ahlberg and Boyko (3) also pointed out that this method of operating the digester results in solids deposition and thus in a loss of digester capacity. They recommended that aeration devices should be capable of maintaining a solids concentration of 6 percent in suspension and of resuspending this concentration following a long settling period.

Benefield and Randall recommend as a rule of thumb, a power level of 14 to 20 $kW/10^3m^3$ of digester volume for solid levels in digester below 20,000 mg/l and 20-40 $kW/10^3m^3$ for total suspended solids above the 20,000 mg/l.

Environmental Conditions

Temperature and pH play an important role in the operation of aerobic digesters. Many studies have monitored pH during

digestion (35, 48, 50) or intentionally varied pH to ascertain its influence on process performance (48). However, attempts to use this parameter as a design variable in full scale operations have not been reported. It is generally agreed that pH will drop due to the growth of a significant population of nitrifying organisms and lowering of the buffering capacity of the sludge as a result of air stripping (41). Drops to a pH of 5 have been reported for batch and continuous flow reactors with long solids retention times (48, 50).

In contrast to the anaerobic digestion process, conventional aerobic digestion systems are usually designed with an uncovered and unheated aeration basin. Despite this apparent disregard for digesting sludge temperature, the process is very sensitive to it (41). The stabilization rate coefficient of the process (K) is generally believed to follow an Arrhenius relationship such as (1, 38) :

$$K_T = K_{20}\, \theta^{(T-20)} \qquad (2.2)$$

where K_T and K_{20} are the reaction rate coefficients at temperatures T and 20°C respectively, and θ is a thermal coefficient determined from a plot of ln(K) versus T.

Values of θ ranging from 1.02 to 1.07 have been reported by Adams et al. (1) although a very limited amount of data was used in their determination.

Several authors have disputed the above relationship, in particular for temperatures above 20°C (50, 24). An experimental determination of the temperature effects is recommended instead. Nonetheless, it is generally agreed that sludge temperatures below

15°C necessitate longer stabilization times, and that digesters should be designed on the basis of the minimum stabilization rate realized during winter conditions (1).

Characteristics of Feed Sludge

Both sludge type and condition affect the digestion process. Primary sludge contains a large fraction of degradable organic matter which is degraded by the active biomass before endogenous metabolism can prevail. Waste activated sludge, on the other hand, is composed essentially of microbial mass with no or insignificant amounts of easily degradable substrate.

More subtle differences occur with the condition of the sludge prior to digestion. Loehr (37) commented on these differences and pointed out that the type of process (extended aeration, high rate, contact stabilization, and conventional activated sludge) influences the initial sludge SRT and the level of activity. With this reasoning, Ahlberg and Boyko (3) proposed to consider sludge stability on the basis of total SRT, not just SRT within the aerobic digestion process.

However Cohen and Fullerton (16) found that when the SRT in their activated sludge system ranged between 4.2 and 25.6 days, very little effect on VSS reduction was detected. They concluded that VSS reduction was more sensitive to environmental conditions during digestion, such as temperature and pH, than to sludge prehistory. They also found better correlation between digester SRT and percent VSS reduced, than between activated sludge system SRT and VSS reduction.

2.1.2 Process Kinetics

The reduction of cell material during aerobic digestion is the result of several concurrent and interdependent processes such as endogenous metabolism, predation through phagocytosis or cell lysing, and solubilization of cell material from lysis of dead cells (24). However it is customary to simplify the concept of digestion by assuming it as a process in which endogenous metabolism is the only phenomenon responsible for the mass reduction. A fraction of the organism mass disappears to provide energy for maintenance of the remaining mass.

The process is usually described by empirically modelling the decrease in suspended solids concentrations (i.e., total, volatile, or mixture of the two) with detention time, the most popular approach being the one presented by Adams et al. (1).

This model utilizes biodegradable suspended solids (BVSS) as a process variable. BVSS are defined by the authors as the difference between total VSS and their non-degradable portion, which is assumed constant throughout the aeration period. The destruction of volatile degradable organics is approximated by first order kinetics as follows:

$$d(BVSS)/dt = - K(BVSS) \qquad (2.3)$$

where the endogenous decay coefficient, K, is usually determined from a semi-log plot of the effluent to initial BVSS concentration ratio versus aeration time, in batch flow reactors.

The effluent VSS concentration at a given detention time is

estimated from a mass balance around the digester, yielding in the case of batch-fed reactors the equations :

$$\frac{BVSS_e}{BVSS_o} = e^{-Kt} \qquad (2.4)$$

with

$$VSS_e = VSS_o e^{-Kt} + (1-e^{-Kt})NBVSS \qquad (2.5)$$

And in the case of continuously-fed complete-mix reactors:

$$\frac{BVSS_e}{BVSS_o} = \frac{1}{1+Kt} \qquad (2.6)$$

with

$$VSS_e = \frac{VSS_o + Kt\ (NBVSS)}{1 + Kt} \qquad (2.7)$$

where $BVSS_e$ = degradable VSS in effluent, (mg/l)

$BVSS_o$ = degradable VSS in influent, (mg/L)

NBVSS = non degradable portion of VSS, (mg/l)

VSS_e = effluent total VSS at time t, (mg/l)

VSS_o = influent total VSS at time t, (mg/l)

t = detention time, (d)

The derivations of the above equations are presented in Appendix A.

As previously stated, the authors proposed the determination of the rate coefficient, K, from batch BVSS reduction data. They performed a series of laboratory-scale digestion experiments and verified that such a determination could be used to adequately predict the performance of complete-mix continuous-flow systems.

However this approach has been subject to several criticisms. Randall et al. (49) and Benefield and Randall (8) argued that the

rate coefficient as determined above is based on how rapidly the biodegradable volatile solids are destroyed rather than on the magnitude of solids reductions. They also remarked that since the amount of biodegradable solids varies with digester conditions such a coefficient could be very misleading if used to compare the performance between units.

The former authors also pointed out that contrary to the common belief, a considerable amount of fixed suspended solids (FSS) degradation takes place during the digestion process, with average FSS reductions of up to 28 % being reported for biological sludges. These reductions were explained as solubilization of solids within microbial cells during cellular lysis. This type of solids reduction is not accounted for in the above model.

High solubilization of volatile solids from cell lysis was also reported by Randall et al. (49) at temperatures of operation in excess of 30°C. Such phenomena could result in misleading changes in VSS concentrations during digestion and thus affect the determination of the design parameters.

Other criticisms exist for this model. The majority refer to the use of volatile solids as process variable.The use of this type of solids has been refuted as being an incomplete measure of metabolic activity, responding only slowly to variations in activity, and subject to misrepresentation from the accumulation of inert organics in flow-through reactors (43,62).

These problems led Benefield and Randall (8) to attempt to incorporate a more direct measure of activity in their proposed model. The authors assumed that only the degradable active fraction of total suspended solids (TSS) decreased during digestion. This decrease was approximated by first order kinetics as follows:

$$(dM/dt)_g = -K_e \; D \; M_{oad} \qquad (2.8)$$

where $(dM/dt)_g$ = rate of mass decrease, (mg/l)/d

K_e = decay rate coefficient, d^{-1}

M_{oad} = influent active degradable suspended solids concentration, mg/l

D = fraction of degradable active mass in influent that appears in effluent

And for a complete-mix flow-through reactor, the detention time was derived as :

$$t_d = \frac{(1-D)}{D} \left(\frac{1}{K_e}\right) \qquad (2.9)$$

or in terms of active mass :

$$t_d = \frac{M_o - M_e}{D \; M_{oad} \; K_e} \qquad (2.10)$$

where M_o = influent TSS, mg/l

M_e = effluent TSS, mg/l

t_d = detention time, d

The above derivations are presented in detail in Appendix A.

The authors remarked that Equation 2.10 reflects the importance of the physiological state of the biomass in design. However they experienced some difficulty in providing reliable

design procedures.

For instance, due to their inability of determining the degradable active biomass in the mixed liquour, they assume it to be 77 % of the total active mass (M_{oa}) based on the work of Kountz and Forney (34). M_{oa} is then estimated as the product of the active fraction of the sludge (X_a) and the TSS concentration in the mixed liquour. The authors recommend the estimation of the active fraction (X_a) from activity measures such as ATP or DOUR but no procedure is given for this, nor are effects of temperature variation considered. As for the fraction of degradable active mass remaining (D), it is assumed to vary between 0.1 and 0.3 but no explanation or supporting experimental data are given.

Furthermore, from an inspection of equation (2.9) and with the definition of fraction D, it can be shown that this equation can be expressed as :

$$\frac{M_{ead}}{M_{oad}} = \frac{1}{1 + Kt} \qquad (2.11)$$

with

$$M_{ead} = D\, M_{oad} \qquad (2.12)$$

where M_{ead} and M_{oad} represent the effluent and influent degradable active TSS (refer to Appendix A).

This is nothing more than a mass balance around the digester in terms of active degradable suspended solids (compare to Equation 2.6). The authors however recommend the determination of K_e in terms of TSS only. This is in agreement with their basic assumption that only the degradable active mass decreases during

digestion, but contradicts their repeated observations that significant reductions in FSS occur from solubilization of dead cells during the process (49, 50, 8).

A different design approach was presented by Grady and Lim (24). The authors recognized two different forms of decay during digestion, decay of living cells at a specific rate R_A and decay of dead cells at a rate R_D. However they avoided this differentiation in their model on the basis of the difficulty in assessing the viability of the sludge. Instead they proposed to model the decrease in biodegradable TSS as the sum of two reductions :

i) Reduction of biodegradable BVSS at a rate :

$$d(BVSS)/dt = -K_v(BVSS) \qquad (2.13)$$

ii) Reduction of biodegradable FSS at a rate :

$$d(BFSS)/dt = -K_f(BFSS) \qquad (2.14)$$

Both types of solids reductions are treated in exactly the same manner as in the model presented by Adams et al. (1), with TSS concentrations at any time t given by the sum of VSS and FSS concentrations at this time.

Although this approach does account for solubilization of fixed solids from cell lysis, it is subject to the same criticisms presented for Adams' model. It should be noted too that data for only 1 batch run at an unspecified temperature was presented to defend the assumption of first order kinetics for the reduction of FSS. The authors do warn against the varaibility in the extent of these solids reductions with type of sludge and

operating conditions. They remark that the magnitudes of such reductions are related to the number of dead cells in the influent sludge and that this number is in turn a function of the prehistory of the sludge. More experimental studies would be required to properly assess the merits of such a procedure in predicting the decrease of FSS.

In summary, the literature review on aerobic digestion kinetics indicated the existence of several controversies with regard to process modelling and in particular with regard to what parameters to use in the determination of the kinetic constants and in the assessment of the degree of stability achieved under a set of operating conditions. This situation stresses the need for continuing investigation in this area with special attention given to a clear definition of the process variables required for the assessment of sludge stability.

2.2 Activity Indicators

Parameters capable of reflecting the concentration and activity of the activated sludge microorganisms have been investigated by several researchers. Weddle and Jenkins (65) presented an excellent discussion on this topic. In their review of the different methods investigated at the time, they discarded the use of DNA, organic nitrogen content and protein concentration as activity indicators on the basis that these elements were partially retained by non-living organisms. Techniques with the property of being specific to living organisms were reported to be:

i) ATP measurements

ii) oxygen uptake rate measurements

iii) dehydrogenase enzyme activity

iv) bacterial plate counts

The authors performed experimental tests on the above techniques as well as VSS measurements and concluded that although the four parameters above reflected the viable organism content of the activated sludge, VSS was an excellent index of activity and more sophisticated indicators were not necessary. It should be pointed out however that their study was specific to the activated sludge process and as such it was concerned with the relation between activity and net growth rates of microorganisms. No investigation between activity and endogenous decay was attempted.

The four parameters listed above are still considered the best

indicators of biological activity in that they are general to all microorganisms and non-conservative (i.e., they exclude dead cells). A brief review of these parameters is presented below. Also included is an enumeration technique which is based on epifluorescence microscopy and activity of dehydrogenase enzymes that has not yet been applied in the wastewater treatment field.

2.2.1 Adenosine Triphosphate (ATP)

ATP is a highly reactive compound. It is defined as the usual form of chemical energy that increases the energy state of the reactants in biosynthesis. Its universal presence in all living forms and its rapid degradation upon cell death makes this compound an excellent indicator of biological activity.

ATP in Wastewater Treatment

Many researchers have investigated the application of ATP measurements to the activated sludge process. Patterson et al. (45) proposed the use of ATP as a measure of metabolic activity and biomass. In addition to describing their analytical technique in great detail, the authors demonstrated the specificity of ATP as an indicator of cell viability. Activated sludge samples were filtered and dried at 103°C for one hour and showed no residual ATP. They also demonstrated the sensitivity of this parameter as a measure of metabolic activity by first starving a batch activated sludge process and subsequently adding a slug organic load. The results indicated that the sludge ATP pool (μg

ATP/mg VSS) was affected by the metabolic activity of the activated sludge microorganisms, displaying a rapid and significant increase in response to the increase in substrate loading. Also the ATP pool was only gradually reduced as the organisms entered the endogenous growth phase during starvation. A similar experiment giving similar results was performed on a full scale contact stabilization system. The authors also found consistent ATP pool values in the range of 1.4 to 2.0 µg ATP/mg VSS for the various activated sludge samples that were taken.

Brezonik and Patterson (10) examined the response of activated sludge ATP to stresses imposed on the sludge environment and demonstrated the utility of ATP measurements in determining changes in sludge activity. The activated sludge was subjected to varying temperatures, changes in dissolved oxygen, increased substrate loadings and addition of heavy metals while monitoring ATP variations. The authors concluded that ATP was a sensitive and rapid indicator of metabolic activity.

As mentioned previously, Weddle and Jenkins (65) also investigated ATP as a measure of activity and biomass concentration. In their laboratory and pilot plant experiments they observed that the ATP content per viable cell remained constant with respect to growth rate over a growth rate range of 0.03 to 6.4 d^{-1}. The ATP content per cell was reported to be between 10^{-8} and 10^{-9} µg ATP/cell or 0.1- 1 % ATP on a dry weight basis. It was concluded that since the ATP content of viable organisms remained constant over a wide range of growth rates, ATP measurements were a useful tool for the determination

of the viable organism content of activated sludge.

Chiu et al. (15) demonstrated that ATP concentrations responded rapidly to cell viability and changed with population shifts and phase growth. Their study also showed that organic loading rate exerted a profound effect on activated sludge ATP content. Microbial populations in continuous systems operated at high loading rates had a high metabolic activity while those species predominating at low loading rates had a low metabolic activity.

Interpretation of ATP Measurements

As mentioned in the above section, ATP measurements have been proposed as both a means of determining microbial biomass and level of metabolic activity. According to Hamilton and Holm-Hansen (25) the conversion of ATP data into reliable estimates of heterotrophic biomass depends on two assumptions :

i) Microbial cells of different species contain a relatively constant amount of ATP per cell.

ii) The ATP content of cells of any one species does not fluctuate significantly with age or exposure to changing nutrient or substrate conditions.

Many questions have arisen as to the validity of these assumptions for various types of microbial cultures.

D'Eustachio and Johnson (17) reported the endogenous ATP content of 13 species of gram-negative and gram-positive aerobic bacteria. Their data showed a range from 2.2 to 10.3 $x10^{-10}$ µg ATP/cell, with a mean of 4.7 $x10^{-10}$ µg ATP/cell and a standard deviation of 2.6 $x10^{-10}$ µg ATP/cell across all species analysed.

Hamilton and Holm-Hansen (25) examined seven species of marine bacteria and found ATP values ranging from 0.2 to 7.8 $x10^{-9}$ µg ATP/cell with a mean ATP content of 1.5 $x10^{-9}$ µg/cell. Under starvation conditions (endogenous metabolism) the authors reported a continual decrease in ATP concentration that paralleled the decline in number of viable cells. The ATP content per cell during these conditions ranged from 0.2 to 0.5 $x10^{-9}$ µg/cell.

Chapelle and Levin (14) presented ATP levels in 19 bacterial species during the stationary growth phase. ATP content was found to vary among the species by approximately an order of magnitude with an overall mean ATP level of 1.97 $x10^{-10}$ µg ATP/cell. However some of the reported data showed higher ATP values resulting from longer extraction times and this suggests that their extraction of cellular ATP may have been incomplete.

Patterson et al. (45) reviewed previously reported ATP data for bacterial cultures and concluded that the endogenous ATP pool was reasonably constant for different bacterial species. They proposed that an estimate of viable biomass could be obtained from ATP values by using a conversion factor of 2 µg ATP/mg dry weight of cell material. They also showed, through substrate addition tests, that ATP measurements reflected the metabolic activity of activated sludge.

In a later study Brezonik and Patterson (10) questioned whether this increase in ATP pool levels following substrate addition indicated an increase in cell population or an actual increase in ATP content per cell. Further analysis of their data

confirmed their hypothesis that substrate addition effects an increase in ATP concentration per cell. The authors then concluded that ATP is a specific measure of sludge activity and that under constant environmental conditions it can be used to estimate viable biomass in sludge.

Kao et al. (32) confirmed the findings of Patterson et al. (44) and Brezonik and Patterson (10). They also reported that significant differences in cell yield and ATP accumulation existed between pure and mixed cultures. ATP concentrations mesured in cells from mixed cultures were appreciably smaller than those measured in pure cultures. This interaction between bacterial species may thus have a profound effect on the system analysed.

In summary, ATP is generally believed to be a sensitive and reliable measure of metabolic activity. As for the estimation of biomass, the literature is inconclusive. Several factors have been recognized to affect the ATP concentration in the cell. These include growth rate, substrate composition and concentration, predominance of species in mixed cultures and environmental stress. Reports on the extent of variation from these factors are conflicting and their analysis is complicated by the different ways in which the ATP pool is expressed. For instance both ATP content per cell and ATP content per dry weight of cell material have been claimed to be constant throughout the growth cycle. This in itself is a contradiction since cells are known to change in size (and thus in weight) as they proceed through growth. As noted by Roe (53), it is more likely for the

ATP content per cell mass to be constant than the content per cell, as ATP and cell synthesis are intimately related. In addition, ATP concentrations per weight of active mass are more pertinent to the wastewater treatment field since the main interest lies in the variation of cell mass during biological treatment rather than variation in cell number. Values of 1.1 and 2.5 µg ATP per mg active MLVSS have been reported by Upadhyaya and Eckenfelder (62) and Nelson and Lawrence (43) respectively, for activated sludge processes treating domestic wastes. However the estimation of active mass by means of an universal ATP pool such as these seems at the present time not justifiable. Any conversion factor of this type should be restricted to the set of environmental and operating conditions in which it was estimated.

ATP Assay

The most sensitive method to measure ATP is by the bioluminescent firefly-luciferin-luciferase enzyme reaction (14,55). McElroy (40) found that luminescence in fireflies has an absolute requirement for ATP and that this reaction is unique among bioluminescent phenomena. He summarized this complex reaction by the following equations :

i) The initial reaction leading to light emission is luciferase catalyzed and involves luciferin and ATP :

$$LH_2 + ATP + E \xrightarrow{Mg^{++}} E\text{-}LH_2\text{-}AMP + PP_i \qquad (2.15)$$

where LH_2 = luciferin

E = enzyme luciferase

$E\text{-}LH_2\text{-}AMP$ = enzyme-luciferin-adenosine monophosphate complex (or luciferyl-adenylate)

PP_i = inorganic pyrophosphate.

di) The complex is rapidly oxidized to oxyluciferyl-adenylate

$$E\text{-}LH_2\text{-}AMP + O_2 \longrightarrow E\text{-}L\text{-}O^* + \text{products} \qquad (2.16)$$

where L = dehydroluciferin

$E\text{-}L\text{-}O^*$ = oxyluciferyl-adenylate.

iii) The oxyluciferyl-adenylate is in an exited state which results in the release of a quantum of light :

$$E\text{-}L\text{-}O^* \longrightarrow E\text{-}L\text{-}O^- + h\nu \qquad (2.17)$$

The total amount of light emitted during the reaction is a function of the concentrations of luciferase, luciferin, oxygen, magnesium ions and ATP. The peak light intensity is reached almost instantaneously, whereas the measurement of total light emission requires a longer period of time.

Two basic approaches exist for the measurement of ATP :

i) measurement of the intensity of initial flash, and

ii) measurement of the number of photons produced over a definite time interval after the initiation of the reaction.

The instruments that have been used to measure this light consist essentially of a photomultiplier tube, an amplifier and a recorder. Although several researchers have constructed their own instruments (14,29), four commercial instruments are available (13, 15, 32, 61, 62):

i) The Chem-Glow photometer (American Instrument Co.)

ii) The 760 Luminescence biometer (E.I. Dupont de Nemours,Inc.)

iii) The ATP-photometer (SAI Technology Company)

iv) The Vitatec IIs (Vitatec Corporation).

In addition to these instruments which are designed specifically for measuring luminescent reactions, scintillation counters have been widely used (10, 11, 14, 26, 44, 45, 54, 57, 60). No major modifications of these counters are needed and with current models it is possible to measure as little as 10^{-12} moles of ATP.

The sensitivity and accuracy of the ATP assay depends mainly upon:

i) the quality of the firefly reagent,

ii) the preparation of the ATP standard solution, and

iii) the extraction procedure.

Firefly reagent

Tobin et al. (61) tested three commercial luciferin-luciferase preparations : Dupont reagent kit, Sigma lypophilized preparation, and the Worthington lypophilized preparation. Their investigation indicated that the Dupont purified reagent was fifty percent more sensitive than the others and had a slower reaction decay. Afghan (2) reported that the crude reagent preparations from firefly extracts should be avoided since they are 50 to 100 fold less sensitive than the purified preparations and give inconsistent and inaccurate results.

ATP Standards

Tobin et al. (61) also investigated the stability of ATP standard solutions. Crystalline trihydrated ATP disodium salt was dissolved and diluted into 14 different buffer and pH conditions.

From their tests, the authors selected 10^{-2} M glycine at pH 10 as the optimum buffer solution since it was found to be stable for over 6 months at room temperature.

Extraction Procedure

Investigations on the extraction of ATP from living cells has been extensive (13, 14, 29, 32,36, 44, 45, 61). The extraction procedure consists in concentrating the cells (by filtration or centrifugation) when samples contain low levels of biomass, lysing the cells to release the ATP into an extraction solution, and separating the dead cell material and other debris from the solution. Problems with the procedure arise from the presence in the samples of heavy metals, organic materials, and high salt concentrations. These materials can produce interferences by both inhibiting the luciferase activity and catalysing non-enzymatic hydrolysis of ATP (61).

The different extracting media investigated have included :

perchloric acid (25, 36)

boiling ethanol (25)

boiling water (25)

butanol with sonication (36)

butanol plus octanol (15, 32)

trichloro acetic acid (36)

boiling TRIS buffer (10, 14, 36, 44, 45, 60, 61, 62, 65)

As shown above, the last medium has been widely used, and it is generally reported that the boiling TRIS buffer procedure is the simplest to perform and gives reproducible results.

However, extensive research by the Analytical Methods Research Section of Fisheries and Environment Canada (13, 61) and Dearborn Chemical Company Limited (13) has recently shown that the above procedure results in losses of ATP through hydrolysis, binding of ATP on particulate material and incomplete extraction of ATP from microorganisms. Both groups above independently developed alternate extraction procedures which appeared to solve many of the difficulties associated with the existing methods. The Analytical Methods Research Section proposed two procedures : boiling glycine and homogenizing techniques; while Dearborn Chemical Co. concentrated its efforts on an acetone-TRIS extraction technique. These three , with a later addition of an acetone-TRIS-Mg EDTA procedure, were compared and evaluated in a recent study by Dearborn Environmental Consulting Services (13). From this study it was concluded that the boiling glycine and homogenizing techniques were superior to the other procedures in terms of precision and accuracy.

In the boiling glycine technique, the ATP is extracted in boiling alkaline glycine buffer containing magnesium disodium ethylene diamine tetracetate (Mg-EDTA). The extracts are cooled and then centrifuged to remove debris. This method results in complete extraction and no hydrolytic loss of ATP. However it is not satisfactory for sediments containing high amounts of humic acid substances as well as high metal concentrations.

In the homogenizing method, the ATP is extracted from the sample by homogenization in trisodium phosphate solution containing chloroform ($CHCl_3$). This method is more versatile

than the previous one in that it can be used for sediment samples. The extracts of sediment samples are centrifuged. The extracts of aqueous samples and the supernatant of sediment extracts are filtered under vacuum for clarification and the removal of dissolved chloroform. The clarified sediment extracts are diluted prior to analysis to prevent the interference of soluble humic substances with the bioluminescence assay.

Nevertheless, for the assay of waters, wastewaters, and activated sludges, the boiling glycine procedure is recommended as the preferred extraction method because it is more practical for field applications than the homogenizing technique.

2.2.2 Dissolved Oxygen Uptake Rate (DOUR)

In aerobic systems, such as the activated sludge process or aerobic digestion, the removal of substrate and/or reduction of endogenous mass is accompained by biological oxidation of part of this material. In this oxidation the final electron acceptor is dissolved oxygen (DO). The rate of DO uptake has been used extensively as a measure of activated sludge activity.

Patterson et al. (44) and Weddle and Jenkins (65) investigated the use of DOUR in the activated sludge process and concluded that this parameter offered a good indication of the sludge activity. The latter authors reported increases in DOUR (expressed on a VSS basis) from 8 mg DO/mg VSS-hr at a net growth rate of 0.03 d^{-1} to 120 mg DO/mg VSS-hr at a net growth rate of 6.9 d^{-1}. When expressed on a viable cell basis, DOUR for pilot

plant and laboratory data was found to be constant at 10.1 $x10^{-8}$ µg DO/cell-hr with a standard deviation of ± 6.3 $x10^{-8}$ µg DO/cell-hr for the net growth rate range tested.

Barnhart (6) monitored DOUR during laboratory and pilot-scale batch digestion experiments on a variety of industrial waste activated sludges. The author studied the variations of DOUR with aeration time and concluded that a first order relationship between these two variables existed.

Benges (9) commenting on the work of Randall et al. (50) proposed the use of DOUR as a means of assessing the rate coefficient K in aerobic batch digestion systems. He proposed the following first order relationship :

$$\ln \frac{(dO_2/dt)}{(dO_2/dt)_o} = -Kt \qquad (2.18)$$

where (dO_2/dt) = DOUR of mixed liquour at detention time t, mg O_2/hr

$(dO_2/dt)_o$ = DOUR of raw sludge, mg O_2/hr

The major stated advantages for basing the kinetic determination on DOUR were that it is a direct measure of biological activity and more accurate than TSS measurements at high concentrations.

Ahlberg and Boyko (3) examined DOUR values in seven full-scale aerobic digestion systems of both batch and continuous flow patterns. DOUR values versus total sludge age (total SRT) rather than against hydraulic retention time were reported. A first order relationship was found to closely approximate the behaviour of specific DOUR measurements with an average reaction

rate coefficient of 0.010 d^{-1} for plants where the sludge temperature was greater than 10°C. The authors proposed the use of DOUR measurements as a better alternative to VSS for the determination of sludge activity and stability.

Although both ATP and DOUR have been reported to be accurate and reliable estimates of metabolic activity, some authors have remarked that under certain operating conditions, these two measures do not vary with detention time in a similar manner.

Eikum et al. (21) reported differences in variation of these measures during the stabilization of mixed primary-chemical(alum) sludge. For semi-continuous reactors at both 12° and 18°C, specific DOUR's were reported to decrease steadily with detention time while specific ATP concentrations increased during the first ten days and then remained constant for up to 35 days of detention time.

Upadhyaya and Eckenfelder (62) have also reported discrepancies between these two parameters. In their study, they investigated the effect of VSS concentration on activity parameters by varying the mixed liquour VSS concentration in an activated sludge unit while holding the organic loading rate constant. For these conditions, a decrease in specific ATP with increasing MLVSS was observed, while specific DO uptake rates remained constant. They attributed this discrepancy to the error in the determination of the uptake rate caused by the reduction in dissolved oxygen in the test bottle as opposed to the actual uptake in the aeration tank. They also remarked that variations in floc sizes with different VSS concentrations could have an effect on the oxygen

penetration through the floc.

2.2.3 Dehydrogenase Activity

In the electron transport chain of the energy-yielding metabolism of bacteria there are several enzymes that catalyse the transfer of electrons by dehydrogenation of a substrate. The activity of these enzymes, which are collectively known as dehydrogenases, can be used as a measure of bacterial activity. In the dehydrogenase activity test, oxygen (the ultimate electron acceptor in the oxidation chain) is replaced by colourless 2,, 3, 5 triphenyltetrazolium chloride (TTC). This compound is subsequently reduced to red insoluble formazan which can be extracted from the cells and measured colorimetrically. Dehydrogenase activity is usually expressed in units of milligrams of triphenyl formazan per gram of VSS per hour (mg TF/g VSS-hr).

Weddle and Jenkins (65) measured dehydrogenase activity for activated sludge over a growth rate range of 0.02 to 6.4 d^{-1}. In their study, they compared their measured values to those presented by Ford et al. (22) and Washington (64) using temperature, time, and pH corrections developed by Ford et al. (22). The latter authors reported values of 31 to 38 mg TF/g VSS-hr as compared to 2 to 10 mg TF/g VSS-hr of Weddle and Jenkins, for growth rates between 0.2 and 1 d^{-1}. The values reported by Washington ranged from 11 to 32 mg TF/g VSS-hr for growth rates between 0.03 to 0.45 d^{-1}. No definite reason for

the discrepancies between the above results was given. However Weddle and Jenkins pointed out that the increase in dehydrogenase activity in the three studies reflected an increase in viable organism content of the activated sludge.

Patterson et al. (45) evaluated a number of sludge activity parameters and their application to toxicity measurements in the activated sludge process. Included were ATP, DOUR, and dehydrogenase enzyme activity. From their results, the authors concluded that the measurement of cellular ATP was a good indicator of sludge activity and a quantitative measure of microbial biomass. DOUR was also found to provide a direct measurement of cellular activity and to offer an advantage over the dehydrogenase enzyme activity test in that DOUR measurements did not cause drastic changes in the intracellular environment. Because of interferences with the normal metabolism of sludge microorganisms, the dehydrogenase test was reported to present certain hazards in both technique and interpretation of results.

2.2.4 Bacterial Plate Count

Plate counts necessitate the growth of bacteria and the formation of visible colonies in laboratory media. Stanier et al. (56) stated that the standard plate count procedure is the most sensitive method for viable cell determinations in pure microbial cultures. However this technique must be applied with caution to biological sludges. The heterogeneous nature and floc forming characteristics of biological sludges pose two major

problems with this method.

Dispersion of the biological flocs prior to plating has been reported to be essential, but this results in the elimination of symbiotic or parasitic associations between microorganisms. Several studies have shown three-, four-, and even nine-fold increases in bacterial counts with floc dispersion (4, 28, 42, 65)

The second difficulty with plate counting is that there is never any assurance that the growth medium selected will support colony formation of all viable cells from a mixed population. It has been generally assumed that aerobic heterotrophic bacteria form the basis of the activated sludge floc and the types of media used reflect this assumption. Dias and Bhat (18) attempted to provide a nonselective medium for bacteria in activated sludge by forming sewage agar (sewage solidified with 2 % agar and adjusted to pH 7). Prakasam and Dondero (47) prepared an activated sludge extract agar (ASE agar) by solidifying an autoclaved and filtered activated sludge sample with 2 % agar. The authors reported higher bacterial counts with this medium than with nutrient or sewage agar. Weddle and Jenkins (65) employed this medium in their research with good results. However, Upadhyaya and Eckenfelder (62) obtained consistently more numerous, clearer and easier to count colonies with tryptone glucose extract agar (TGE agar) than with nutrient or ASE media.

The interpretation of the viable bacterial count is further complicated by the existence of non-reproducing cells that remain metabolically active in the sludge. The metabolic importance of such cells has not been well documented. Only a couple of studies

were encountered during this literature survey. A study by Wooldrige and Standfast (66) showed total to viable count ratios for biological sludges in a range of 100 to 1000. The authors attributed a high degree of enzymatic activity to these 'dead cells'. Walker and Davies (63) investigated viability and respiration rate variations in activated sludge at low net growth rates. They demonstrated, in contradiction to the work done by Weddle and Jenkins (65), that respiration rates per viable cell did not remain constant but in fact increased at low growth rates. They attributed this variation to the activity of nonviable bacteria, these microorganisms been responsible for over 80 % of the total respiration at a growth rate of 0.1 d^{-1}.

From the above observations it is clear that viable counts (plate counts) are subject to considerable variations and are at most a limited estimation of the number of active organisms in activated sludge. In spite of this, their use is very popular due to the simplicity of the test (8, 62, 65).

2.2.5 Direct Counting of Bacteria

Due to the problems associated with plate counting, many alternative enumeration techniques have been investigated.

Strugger (59) proposed the use of acridine orange as a vital stain for soil bacteria and the examination of the stained specimens by fluorescence microscopy. This technique reportedly yielded a difference in fluorescence between cells and detritus, and offered the additional advantage of differentiating between

living and dead bacteria. According to the author, viable cells would fluoresce green whereas nonviable cells would fluoresce red. However this claim has been refuted (12, 20, 33). Korgaonkar and Ranade (33) demonstrated that although a fluorescence shift to red-orange occured in highly degraded cells, the shift was not obligatorily linked to viability when cells were killed by various physical treatments. This was further substantiated by the work of Donald et al. (20). Pure cultures of Klebsiella, Escherichia coli and Bacillus subtilus containing both red and green fluorescing cells, were autoclaved, boiled, or treated with formalin or mercury. After these treatments large number of green fluorescing cells were still observed. Nevertheless the authors concluded that the acridine orange epifluorescence technique (AOE) did permit a ready discrimination of bacteria from detritus and was the method of choice in estimating total microbial counts.

In a recent study, Zimmermann et al. (68) proposed a new enumeration technique involving the AOE procedure and the activity of the dehydrogenase enzymes. The technique is based on the principle that the electron transport system of respiring organisms reduces 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to INT-formazan. Respiring bacteria deposit accumulated INT-formazan intracellularly as dark red spots. Corresponding to electron transport system activity, these deposits attain a size and degree of optical density which allows them to be examined by light microscopy. However the formazan deposits did not represent a definite outline of

bacterial cells and thus it was difficult to define the nature of the counted spots if used alone. But by combining formazan detection and epifluorescence microscopy a method is obtained which allows the differentiation of bacteria form detritus and the differentiation among the bacteria between respiring and non-respiring cells.

This technique has been applied for the enumeration of active microorganisms in sea and lake waters. For these environments it has been claimed to offer the great advantages of being : i) non-selective, ii) closer to in situ conditions than with comparable methods of estimating bacterial activity, and iii) a direct and rapid count of active microorganisms. No reports on its application to the wastewater treatment field were encountered.

CHAPTER 3

EXPERIMENTAL CONSIDERATIONS

The purpose of this chapter is to outline specific points the research addressed and the theoretical considerations that underlined the experimental design.

3.1 Measures of Activity

From the literature review, a series of controversies and constraints regarding activity measures and their application to biological treatment processes became apparent. Clearly, the first step for the use of these measures in design of aerobic digesters requires a clear determination and evaluation of the merits and restrictions of each technique for this particular process. To assess these, bench scale experiments with both batch and flow-through reactors were conducted. Four activity measures were investigated : ATP, DOUR, plate count and the direct counting technique involving epifluorescence microscopy and dehydrogenase enzyme activity which will be refered to as AOE-Dehydrogenase technique.

It was also remarked in the review that the metabolic activity of the microorganisms in the mixed liquour is very dependent on sludge type, sludge prehistory, solids concentrations, and operating conditions of the process. Consideration of all these factors being impossible, it was necessary to restrict the study

to the analysis of the effects of temperature and detention time on activity. The reactors were fed with sludge from the return sludge line of a conventional activated sludge plant treating domestic sewage, and the solids concentration was fixed at 7500 mg VSS/l. Both batch and semi-continuous reactors were run for extended periods of time (up to 2 months) at different operating temperatures.

Of special interest during the experiments were the following points :

i) Applicability of the AOE-Dehydrogenase technique to wastewater samples.

ii) Relations between variations of the four activity parameters and VSS concentrations with detention time and temperature.

iii) Differences in variation beween DOUR and ATP.

iv) Comparison of cell concentrations from plate counts and the AOE-Dehydrogenase technique.

As noted above, the feed sludge was set to have a constant VSS concentration. VSS was used as the base parameter for expression and evaluation of the different measures for the following reasons :

i) All previous studies on the activity of biological sludges have reported the activity measures per weight of VSS. Therefore comparison of the experimental results with those of other authors required the use of VSS as the basis for expression.

ii) It was remarked earlier that VSS gives no indication of the reduction of fixed solids and consequently its replacement by TSS

has been recommended. The magnitude of variation of the fixed solids is nevertheless highly unpredictable and dependent on the prehistory of the sludge and on the set of operating conditions of the process itself (24). Due to this reason, the utilization of TSS in lieu of VSS was considered to be less reliable as these solids are composed in part of fixed solids. Also it should be noted that the decrease in FSS is due to the decay of dead cells and as such it has no relation to changes in metabolic activity nor active mass during the process.

3.2 Biomass Estimation

Activity measures are usually expressed as specific values (i.e., per mass of VSS) and as such they are indicators of the specific activity of the mixed liquour only. A direct measurement of active mass being impossible, it is common practice to express it as active fraction of suspended solids (F_A). This fraction is a calculated parameter, and is usually obtained from the relationship (7, 43, 53, 62) :

$$F_A = P_S / P_B \qquad (3.1)$$

where F_A = active fraction of VSS

P_S = specific activity content in mixed liquour

P_B = base value or activity measure per active cell mass.

A direct determination of the ATP content per active cell mass (P_B) in a mixed culture being impossible, it has been approximated by the specific activity content of the culture as

it approaches 100 % activity . The two procedures that have been employed are :

i) Extrapolation from plots of specific activity versus sludge age at or near 0 days (43, 53, 62)

ii) Placing a small amount of mixed liquour in an environment containing a large excess of substrate, repeating the procedure several times with a small amount of culture obtained from each preceeding step and calculating the specific activity parameter for the last step. The underlying assumption is that under such conditions the mixed liquour suspended solids will consist almost entirely of active biomass (8, 53).

The accuracy of biomass estimation is very dependent on the accuracy and variability of P_B. This value has been generally assumed to be constant for a particular waste, and independent of growth rate and metabolic state of the organisms within the practical sludge age operating ranges of the activated sludge process. However there is no experimental verification of this nor are there comments on the effects of changing environmental conditions.

Furthermore, the existing investigations have concentrated on the activated sludge process, with very little attention given to aerobic digestion. It seems unlikely that such procedures for determining P_B would give reliable results in aerobic digestion processes since it would entail its determination from a rapidly growing culture and thus from a completely different metabolic state to that of cell decay in the mixed liquour.

Before Equation 3.1 can be applied to aerobic digestion, it

must be first determined whether or not there is a constant activity concentration (in terms of µg ATP or mg O_2/l-hr for example) per active cell mass, and if there is, how it may be estimated.

It was pointed out in the previous sections that aerobic digestion is commonly described as a process in which endogenous metabolism is the only phenomenon responsible for mass reduction. Therefore the loss in mass during the process is considered to be a loss in active cell mass.

On the other hand, it can be said that for the activity concentration to be constant with active cell mass during digestion, the rate of decrease in this measure should be proportional to the rate of decrease in active mass or :

$$dA/dt = K\, dM_A/dt \qquad (3.2)$$

where A = activity concentration, µg ATP/l or mg O_2/l-hr or viable cells/l or active cells/l

M_A = active mass in mixed liquour, mg/l

K = proportionallity constant, activity units/mg active mass.

The proportionality constant above would also be the slope of a plot of activity concentration versus active mass for corresponding detention times (see Fig. 3.1), and therefore it is an estimate of the activity concentration per active cell mass (or factor P_B in Equation 3.1).

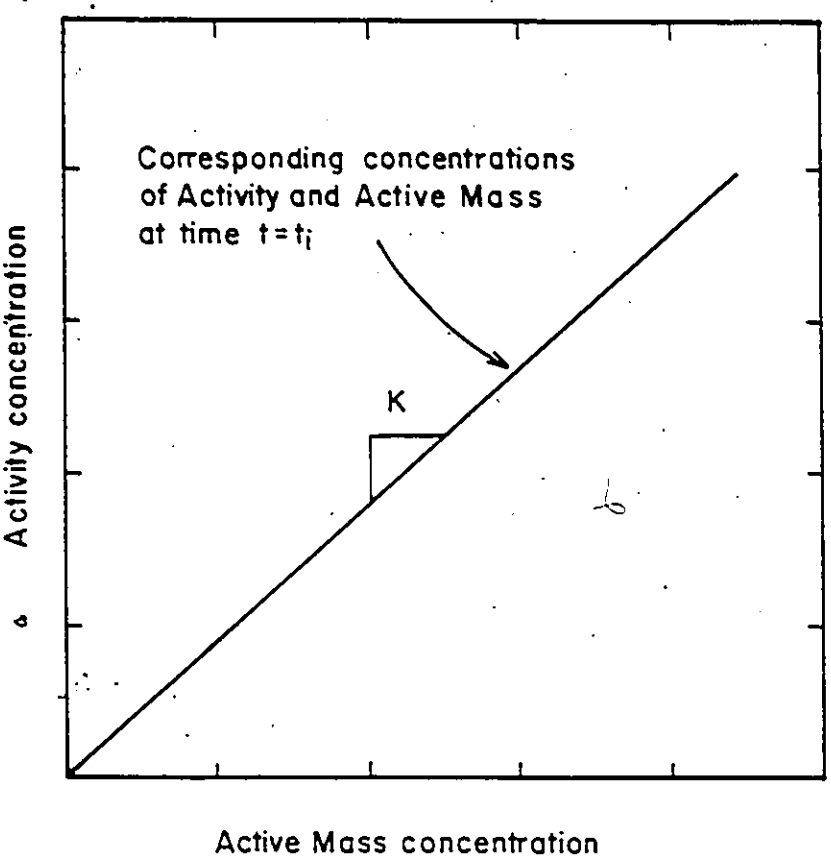

Fig. 3.1 Activity Concentration Versus Active Mass

Obviously the problem lies in the determination of active mass or at least in its variation with detention time. Since the process has been usually monitored in terms of VSS and the reduction of biodegradable cell material during digestion is usually believed to be the result of endogenous metabolism alone, it was considered that as a first approximation, the decrease in BVSS could be assumed to parallel the decrease in active mass during digestion in the batch reactors. This type of reactor was used since it is not subject to interferences from variations in feed nor accumulation of inerts nor inactive organics.

Therefore, the variations of activity parameters and corresponding BVSS concentrations were investigated. The batch reactors were run for extended periods of time to determine the non-biodegradable or residual VSS content of the sludge, and the BVSS concentration at any time was estimated by subtracting this residual from the VSS concentration in the mixed liquour at any particular time.

It was believed that this analysis would give an indication of the constancy of P_B and a verification of the common assumption that endogenous metabolism is the main process responsible for the decrease in microbial mass.

CHAPTER 4

EXPERIMENTAL PROCEDURE

4.1 Work Organization and Apparatus

4.1.1 Overall Organization

Experimental studies were conducted with both semi-continuous (fed once a day) and batch-fed laboratory scale reactors. The experiments were divided into three experimental runs of up to two months each and having corresponding mixed liquour temperatures of 10°, 20° and 30° C. For each run, three semi-continuous and one batch reactor were operated simultaneously.

The semi-continuous units were given hydraulic detention times of 10, 20, and 30 days respectively and were monitored for a minimum of three weeks after steady state conditions were reached. These units were operated without solids recycle nor decanting, and under these conditions the hydraulic detention time is equivalent to the mean cell residence time (38, 39, 41). Steady state was assumed to be reached when concentrations in VSS varied within 10 % of the average concentration for a period in excess of seven days.

The batch reactor was fed only once during the run and was monitored for extended periods of time until minimal indications of metabolic activity (as given by ATP and DOUR) were detected.

For each unit, the following tests were performed on a periodic basis : plate counts, AOE-Dehydrogenase technique, ATP, DOUR, TS,

TSS, VSS, COD and pH.

4.1.2 Set Up

All four digesters were located in a walk-in incubator. The temperature of the incubator was set to obtain the desired temperature of the reactor contents, and was maintained within ± 1°C. The reactors were built to have an operating volume of 10 l for the semi-continuous units, and for the batch unit an initial sludge volume of 20 l.

Air to supply oxygen and mixing was introduced from a circular diffuser ring located on the bottom of each reactor. These diffusers were linked to the laboratory compressed-air system. The air flowrate to each reactor was controlled through metering valves (Tri-Flat Variable-Area Flowmeters, Fisher Scientific Co.) placed in-line between the diffusers. An air flowrate of 6,500 cm^3/min was fed to the semi-continuous units whereas the rate to the batch reactor was set at 14,000 cm^3/min. These rates were sufficient to maintain at all times a dissolved oxygen concentration in the mixed liquour above 2 mg/l, and provided enough mixing to keep all solids in suspension. DO concentrations were monitored regularly, whereas complete-mix conditions were verified through preliminary dye studies and visually during the course of the study.

4.1.3 Feed Sludge

Waste activated sludge was obtained from the return sludge line of a 30,300 m^3/d (8 MGD) conventional activated sludge plant treating domestic sewage (Watt's Creek Wastewater Treatment Plant, Ottawa, Ontario).

The sludge typically contained VSS concentrations ranging between 3,000 and 4,000 mg/l. With the detention times and reactor volumes previously specified, these VSS concentrations would result in organic loadings between 0.09 to 0.4 Kg VSS/m^3/d. This range is much lower than the one encountered in the literature survey. Therefore it was deemed necessary to concentrate the sludge prior to feeding the experimental units. The preparation of the feed was done in the following manner :

Sludge was collected on a weekly basis from the treatment plant and was allowed to settle for 24 hours at 4°C. This was followed by decanting of supernatant and storage of concentrated sludge in seven individual containers, each with enough volume to feed all reactors for one day. These containers were again stored until needed at 4°C. Prior to feeding, the concentrated sludge was analysed for TSS and VSS, and was diluted with distilled water to a concentration of 7500 mg VSS/l. This value represents the highest concentration that could be achieved safely with one day settling of the raw sludge.

4.1.4 Daily Operation

Every morning, the reactors' walls were scraped clean from any sludge accumulation; the temperature and air flowrate in each tank were checked and evaporation losses made up with distilled water.

Foaming was controlled when necessary by the addition of 1 to 2 ml of antifoam agent (Dow Corning antifoam AF emulsion).

Sampling was performed immediately prior to each test, and the volume withdrawn recorded. The semi-continuous units were fed once a day, after testing for the day was completed. The remaining volume of mixed liquour necessary to maintain the corresponding detention time was withdrawn from the reactor prior to the addition of feed.

4.2 Analytical Methods

4.2.1 ATP Assay

The measurement of ATP was performed by the luciferin-luciferase enzyme reaction method. The extraction procedure for the release of ATP from sludge microorganisms followed the boiling glycine technique as outlined by Tobin et al. (61) and Afghan (2).

With this technique, ATP was extracted by :

i) Injecting 0.5 ml of sludge sample in 5 ml of boiling alkaline glycine buffer containing magnesium disodium

ethylene diamine tetra-acetate (Mg-EDTA).

ii) Allowing the mixture to boil for five minutes.

iii) Cooling the extracts to room temperature.

iv) Centrifuging the extracts to remove debris.

v) Decanting and measuring the final extract volume.

All sludge samples were processed in duplicate, always immediately after collection, and their corresponding extracts were stored just after extraction at 4°C until measurements of ATP were performed, with a maximum of three weeks storage. Extraction solution blanks were also processed with every set of samples.

A liquid scintillation counter (Beckman Instruments Co., model LS-3133P) was used for the measurement of light emission. Gain was set at 53, with a window setting of 50-1000. The spectrometer was set in a repeat count mode, with a 6-second counting interval separated by a 14-second data printout sequence. A 10 µl aliquot of final extract was injected into a standard quantity (100 µl) of buffered luciferin-luciferase reaction mixture (Dupont Reagent Kit No. 760145-902) and the light emission was measured for up to three minutes. Due to the very small volumes used in this measurement (110 µl in total) it was necessary to modify the scintillation vials, since their total volume was 7 ml (or 7,000 µl). Initial attempts to increase the reagent volumes through dilution with extraction solution resulted in very low light emissions with poor reproducibility. Therefore it was opted to modify the vials by the insertion of ploy ethylene microtestubes (Brinkmann Instruments, Inc.) in the

vials' cap as shown in Figure 4.1.

Interpretation of light emission data was closely patterned after the procedure presented by Patterson et al. (45) with only minor modifications.

The ATP luciferin-luciferase reaction is time dependent, with resulting light emission decaying rapidly with time. Therefore an accurate measurement of elapsed reaction time is necessary. Instead of attempting to reproduce the injection of the extract into the vial so that each assay entered the scintillation counting chamber in the exact same period of time, timing was started as the injection was performed and elapsed time to the end of the first counting interval recorded. Figure 4.2 illustrates results from this procedure.

Light emissions (in terms of counts per minute) were normally recorded for nine sequential counting intervals. This represented a total reaction time of about 3 minutes. Duplicate assays were run for each sample and a third was repeated if counts per minute (CPM) differed by more than 4,000 units.

It should be noted that in this figure both replicates have different median times since their times to the end of first counting interval differ. As previously stated, the counting interval lasted 6 seconds whereas the printing interval 14 seconds, with a subsequent time interval between median times of 20 seconds. Figure 4.2 represents a typical decay curve for the ATP assay.

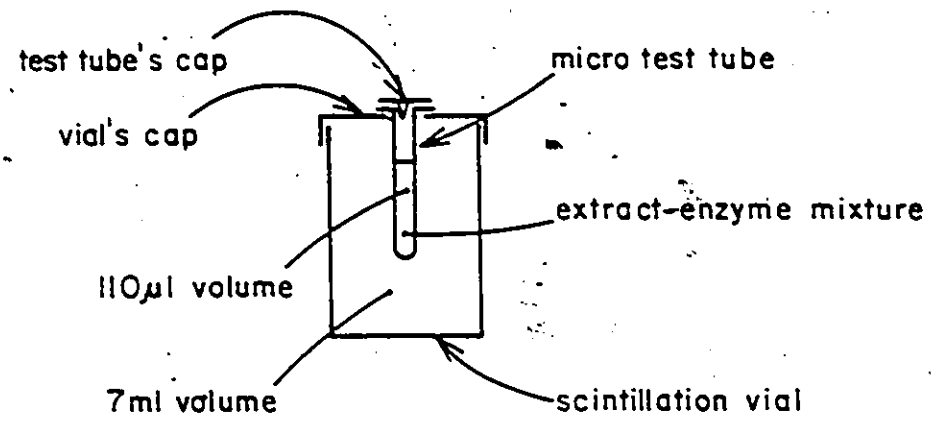

Fig. 4.1 Modifications to Scintillation Vials

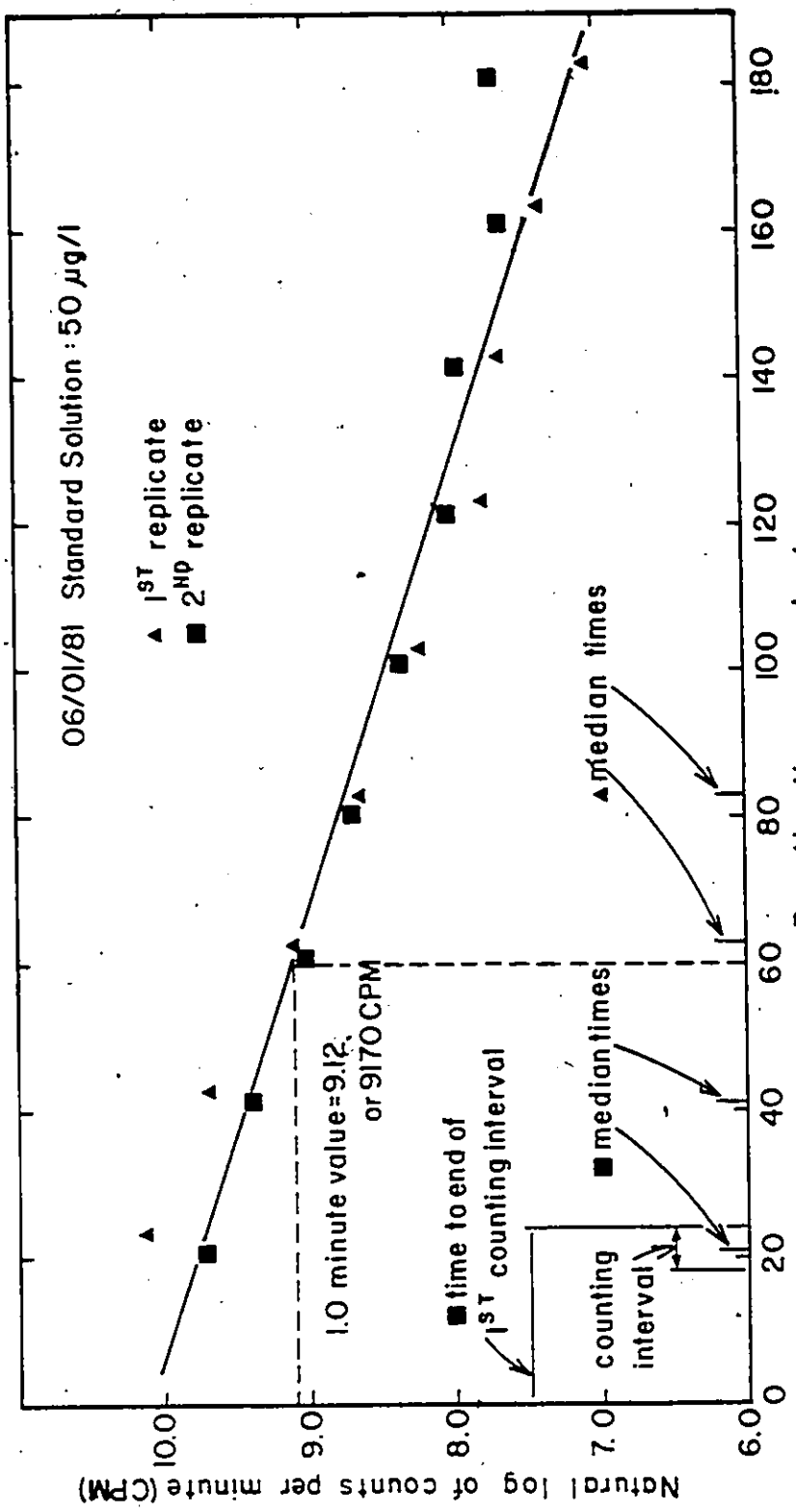

Fig. 4.2 Typical Bioluminescence Decay Curve

This curve is a linear regression (least squares method) of the natural log of CPM (6-sec counting interval) versus median reaction time. From this regression, the predicted natural log of CPM at 1.0 minutes elapsed reaction time was calculated . The selection of values at 1.0 minutes was based on the procedure of Patterson et al. (45).

Calibration curves were constructed from CPM values at 1.0 min reaction time for several ATP standard concentrations. The standards were prepared from adenosine-5'-triphosphate disodium salt (EASTMAN KODAK Co.) just prior to testing, and concentrations of 25, 50, 100, 150, 200, and 250 µg/l were used for the calibration curves. A calibration curve was prepared each time the scintillation counter was utilized. The ATP concentrations in the final extracts were estimated from these curves (see Figure 4.3), which are linear regressions (least square method) of CPM a 1.0 min reaction time versus corresponding ATP standard concentrations.

The ATP concentration in the aeration basin was estimated from the equation :

$$ATP_B = ATP_E \ (V_E / V_o) \qquad (4.1)$$

where ATP_B = ATP concentration in aeration basin, µg/l

ATP_E = ATP concentration in final extract, µg/l

V_E = final extract volume, ml

V_o = original sample volume, ml.

A more detailed description of the assay is presented in Appendix B.

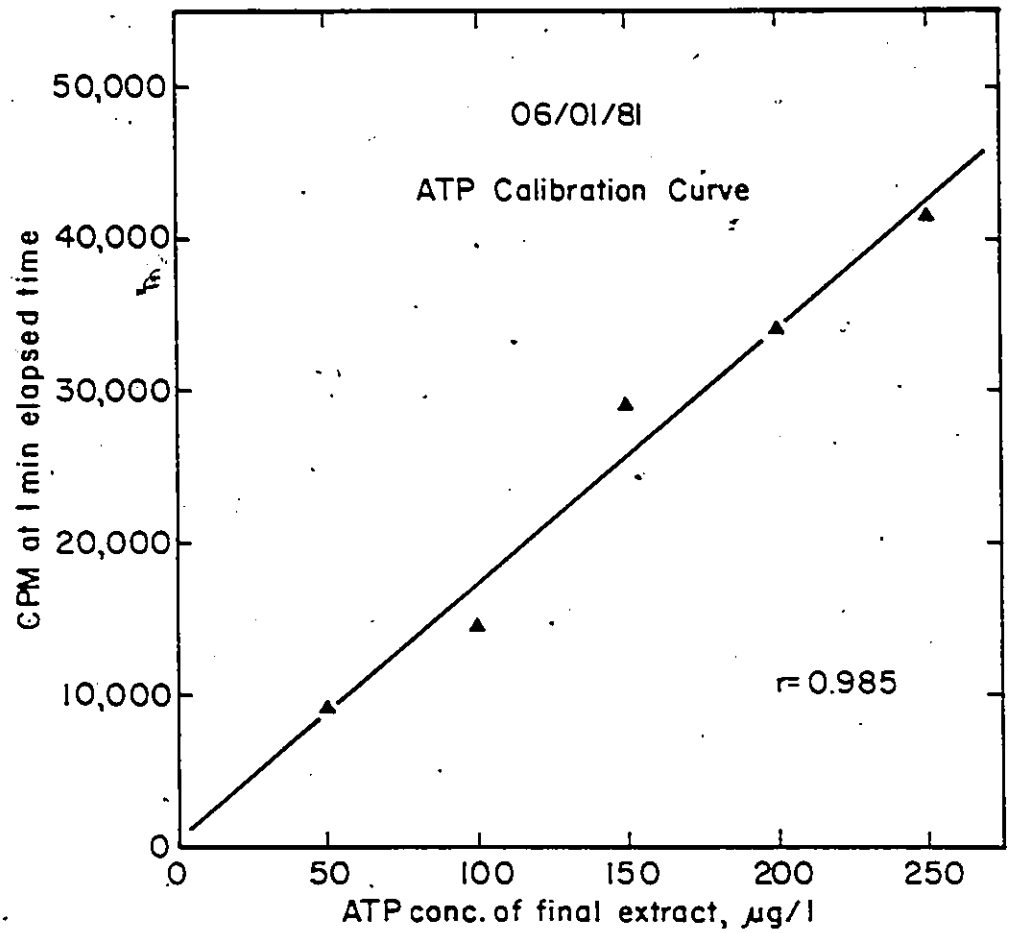

Fig. 4.3 Typical ATP Calibration Curve

4.2.2 DOUR

The oxygen uptake rate was estimated as outlined in Section 213B of Standard Methods (5). Dissolved oxygen concentrations were measured with a polarographic membrane electrode (YSI Oxygen Meter). BOD bottles of 60 ml capacity were used for the test, with no dilutions attempted since preliminary investigations on these indicated very poor reproducibility between replicate samples. The meter was calibrated always prior to testing by means of the iodometric method (azide modification or Winkler method) as outlined in Section 422B of Standard Methods (5).

Sample collection was always done just prior to testing and duplicate samples were analysed for each reactor. The monitoring of dissolved oxygen concentration in the sample was done by means of a recorder (Perkin-Elmer, Colman 165) for a period of 5 to 10 minutes, until the DO concentration in the BOD bottle became limiting (at about 1 mg/l). The temperature of the mixed liquour was recorded at the begining and end of the testing period, and usually varied within 2 or 3 °C. VSS were also determined on the DOUR sample and the DOUR values were expressed in terms of mg O_2/g VSS-hr. The detailed testing procedure is presented in Appendix B.

4.2.3 Plate Count

The plating procedure was as outlined in Section 907 of Standard Methods (5) with some modifications :

Diluted and dispersed suspensions of mixed liquour were plated on the surface of the culture media with a bent glass rod. Dispersion of biological flocs was performed with a Waring blender. Plating was done on TGE agar and the samples were incubated for up to 7 days at 20±1°C. The samples were collected just prior to processing and controls were always run.

4.2.4 AOE-Dehydrogenase Activity

Dehydrogenase enzyme activity was analysed by the method presented by Zimmermann et al. (68) with the following modifications :

i) The floc forming characteristics of biological sludges make necessary the dispersion of these flocs in order for a microscopic examination to be possible. Therefore dispersion was performed on every sample by means of a Waring blender.

ii) High dilutions were also necessary to achieve an adequate distribution of organisms for microscopic examination (40-60 microorganisms per field). These were carried out as recommended in Section 907 of Standard Methods (5).

iii) The microorganisms in processed samples were collected for microscopic examination on 0.1 μm pore size polycarbonate filters, N type (Nucleopore Corp.). The filters were supported during filtration by drain discs (Nucleopore Corp.) instead of silver membranes as recommended by Zimmermann et al. (68). The use of these supports resulted in uniform distribution of particles on the filter.

All samples were processed immediately after collection and stored at 4°C for a maximum of 10 days. The microscopic preparation (filtration and staining) of the processed samples was performed always prior to microscopic examinations.

A detailed description of the procedure is presented in Appendix B.

4.2.5 Overall Monitoring of the Units

The determination of solids concentrations, including total solids (TS), total suspended solids (TSS), and volatile suspended solids (VSS) were performed by the gravimetric techniques given in section 208 of Standard Methods (5). TSS and VSS were determined by the glass fiber filter technique. A drying temperature of 103 to 105°C was used for the TS and TSS tests, whereas VSS samples were ignited in a muffle furnace at 550°C for 15 min. All tests were carried out immediately after sample collection to prevent deterioration.

Measurements of pH were made in accordance with Section 424 of Standard Methods (5). A Fisher Acumet electronic pH meter (model 210) equipped with temperature compensation adjustment and combination electrode was utilized. The apparatus was calibrated always prior to testing with commercially available buffers of known pH.

Chemical Oxygen Demand (COD) was measured with the dichromate reflux method as outlined in section 508 of Standard Methods (5).

CHAPTER 5

EXPERIMENTAL RESULTS

5.1 Batch Reactors

5.1.1 Solids Variations

TS, TSS, and VSS were monitored in all reactors throughout the study. Figures 5.1 to 5.3 summarize the variations obtained for the batch runs.

Two important observations can be made from these figures :

i) The difference between TS and TSS concentrations increased with detention time for the 20 and 30°C runs. This increase indicates an accumulation of total dissolved solids (TDS) in the reactors as digestion proceeds. This is especially true at 20°C where variation of TDS from 500 to 2400 mg/l occurred within 30 days of digestion. For the 30°C run, a gradual increase from 0 to 1400 mg/l was observed during the 55 days of detention whereas no net increase occured at 10°C.

ii) As previously stated, the non-biodegradable portion of the sludge (NBVSS) was estimated as the residual VSS content in the mixed liquour after extended periods of aeration. Values of 4420, 2600, and 2730 mg/l were obtained from these figures at 10, 20 and 30°C respectively. NBVSS values were assumed to remain constant with detention time for each particular temperature and set of operating conditions.

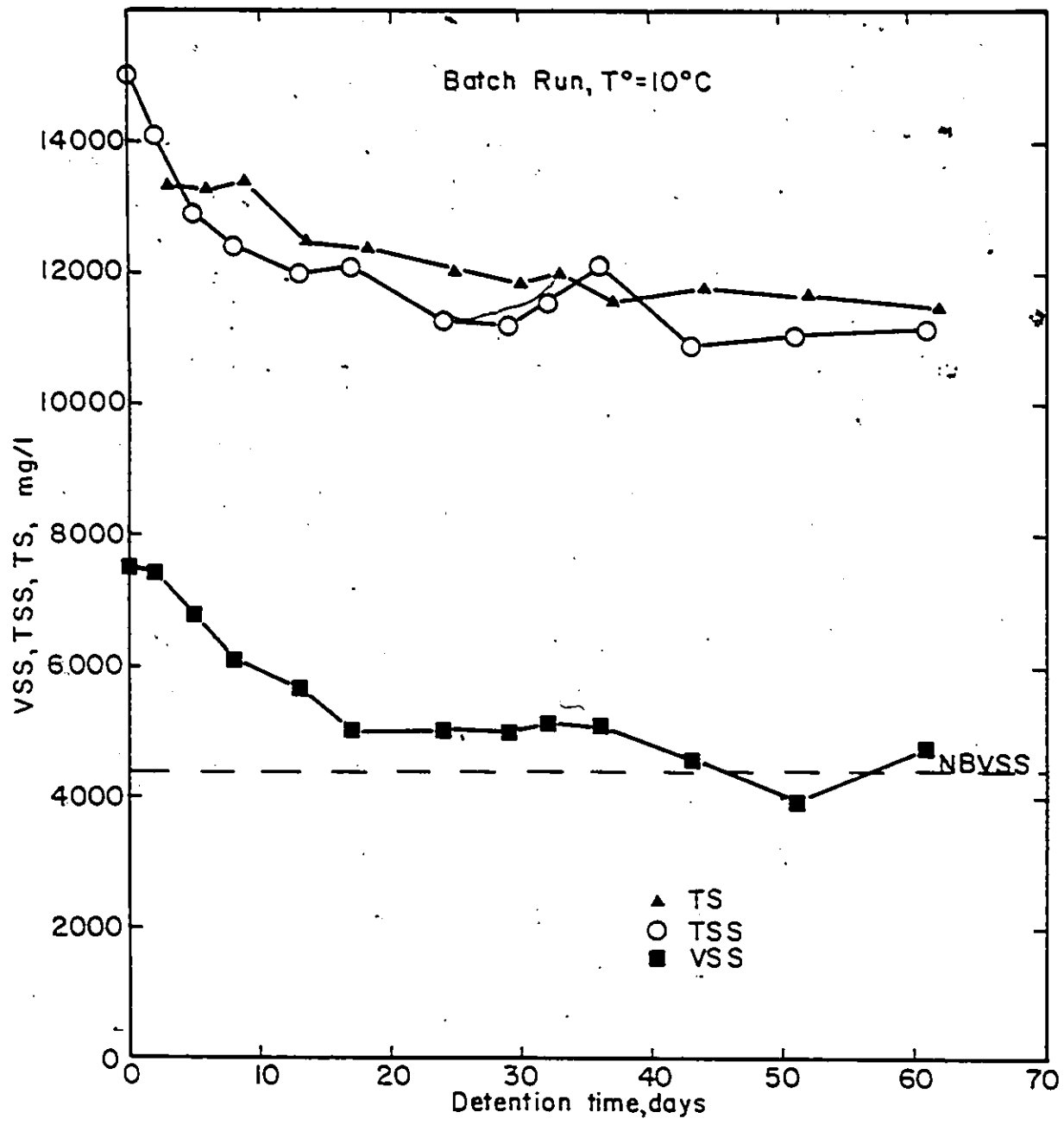

Fig. 5.1 Solids Variations in 10°C Batch Run

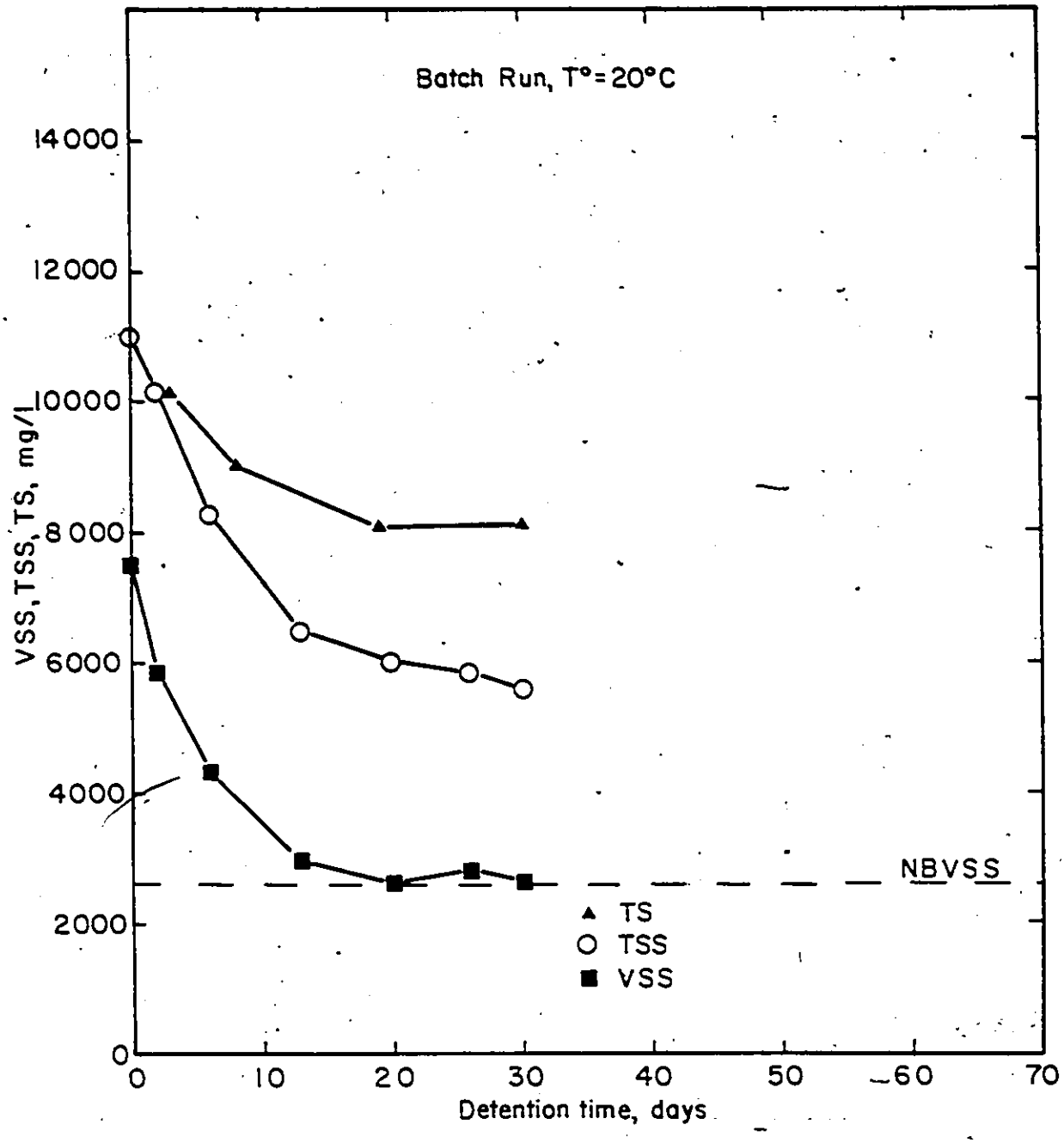

Fig. 5.2 Solids Variations in 20°C Batch Run

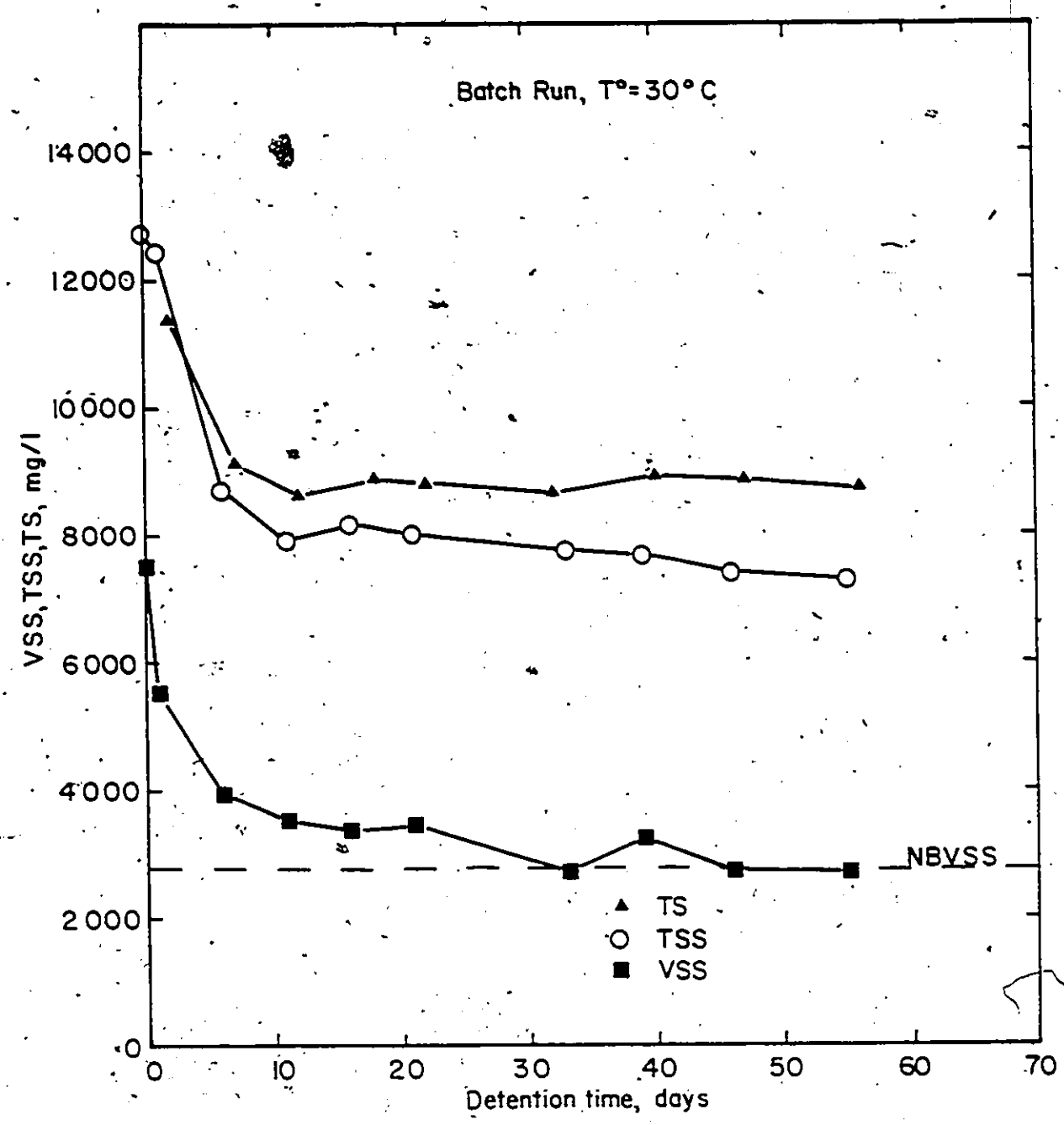

Fig. 5.3 Solids Variations in 30° C Batch Run

5.1.2 Plate Counts

No significant decrease in the number of colonies per mg mixed liquour VSS (MLVSS) was observed for any of the temperatures studied (Figs. 5.4-5.6). For temperatures of 10°C and 30°C, the varations in specific viability (plate counts/MLVSS) were erratic with only mild downward trends as opposed to the sharp decreases observed for ATP and DOUR. The range of values obtained were 0.25-2.40 $x10^8$ cells/mg VSS for 10°C and 0.26-3.45 $x10^8$ cells/mg VSS for 30°C. The specific viability remained constant for the 20°C reactor at a mean value of 0.77 $x10^8$ cells/mg VSS.

5.1.3 Dehydrogenase Enzyme Activity

The technique employed in this study allows the determination of the total and active (cells with formazan deposits) number of bacteria from the same sample which enables one to make a direct measure of the active fraction of the population. This fraction was termed % Activity and its varation with digestion time is shown in Figures 5.4 through 5.6. The technique has formerly been restricted to the enumeration of active microorganisms in sea and lake waters. For these environments it has the great advantage of being closer to <u>in situ</u> conditions than other techniques requiring incubation with labeled compounds (68). Early in the study several limitations for its application to the wastewater treatment field became evident.

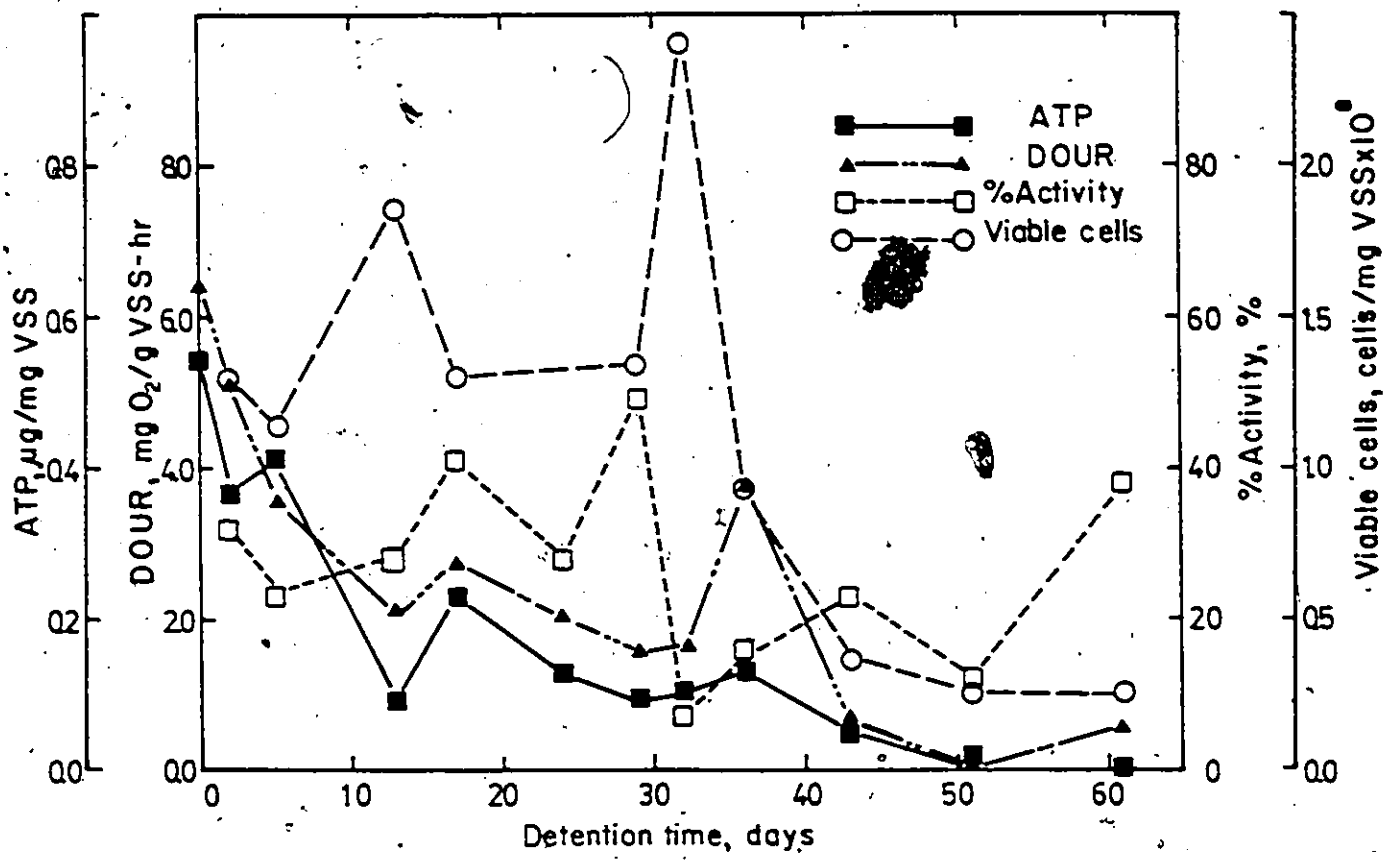

Fig. 5.4 Activity Data for Batch Run at 10° C

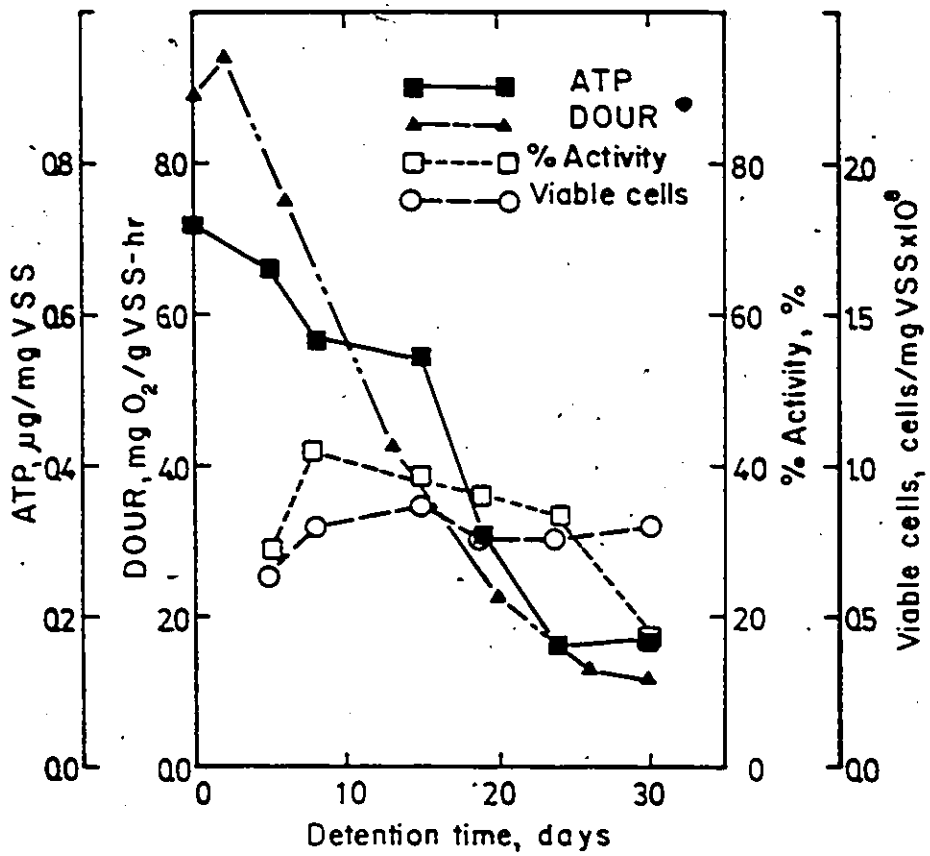

Fig. 5.5 Activity Data for Batch Run at 20°C

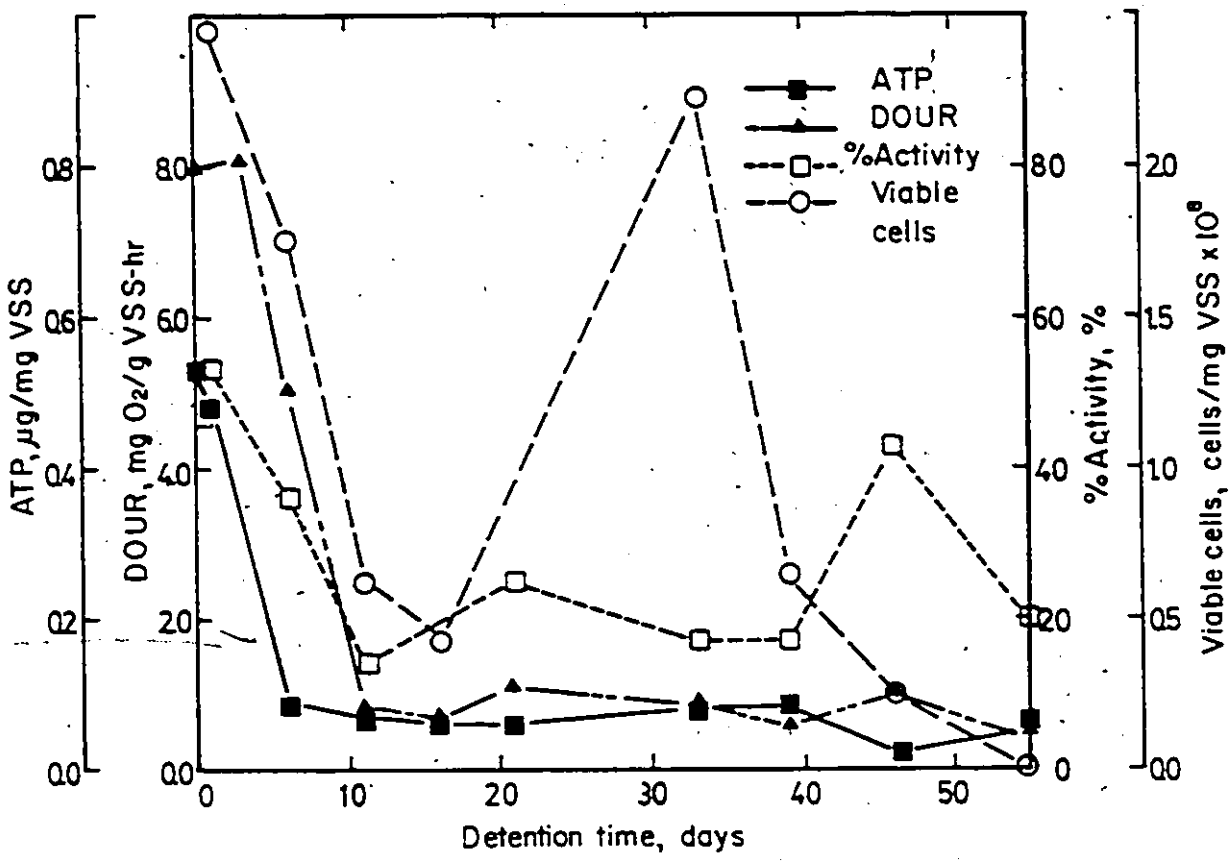

Fig. 5.6 Activity Data for Batch Run at 30°C

As with plate counts, dispersion of biological flocs and serial dilutions are required. These drastic changes in environment can cause shocks in the population and thus influence the rate of dehydrogenase activity during testing.

Another limitation is the inability to distinguish formazan deposits in small bacteria due to interference from the structure (pore openings) of the filter on which the microorganisms were collected. It is to be noted that a large number of "small" bacteria was observed throughout the study and thus the values reported here are considered to be conservative estimates. In spite of these problems which seem to account for the random variation in the % Activity parameter (Figures 5.4-5.6), the test was continued througout the study to obtain useful information regarding conflicting statements from plate counts. The principal one is the ability of viable cells to represent the active number of microorganisms in the culture (63, 65). Therefore testing for dehydrogenase activity was always performed with samples taken from the same serial dilutions used for plating. Results are summarized in Fig. 5.7. From this table it can be seen that viable counts (plate counts) were significantly lower than active and total counts, with viable counts ranging between 0.1 % to 30 % of active counts and between 0.0 % to 5.1 % of total counts. The values presented in this table represent only the minimum and maximum estimates obtained for each operating condition. A complete tabulation of these results is given in Appendix D.

Fig. 5.7 [1]Viability and Dehydrogenase Enzyme Activity Data for Batch Reactors.

Temp. °C	Viable Cells (cells/ml)x10^{-8}	Active Cells (cells/ml)x10^{-10}	Total Cells (cells/ml)x10^{-10}	[2]V/A (%)	[3]V/T (%)
10	1.0 - 12.3	0.6 - 21.0	1.9 - 57.1	0.1-16.0	0.0-5.1
20	2.0 - 3.2	0.4 - 0.8	1.1 - 2.8	2.6- 5.3	0.9-1.9
30	0.7 - 19.1	0.2 - 7.0	0.7 - 13.2	1.2-30.0	0.2-5.0

[1]Above results represent minimum and maximum values obtained for each operating condition. They do not necessarily reflect their order of occurrence.

[2]V/A = Viable to Active cell ratio.

[3]V/T = Viable to Total cell ratio.

5.1.4 ATP and DOUR

A striking similarity between the decrease in µg ATP/mg VSS and mg O_2/g VSS-hr was observed for all three temperatures (Fig. 5.4-5.6). Reductions in specific ATP content from 0.54 to 0.0 µg ATP/mg VSS after 50 days of digestion, 0.72 to 0.17 µg ATP/mg VSS after 20 days and 0.53 to 0.06 µg ATP/mg VSS [redacted]10 days of digestion were observed at 10°, 20° and 30°C respectively. For the same digestion periods, the DOUR varied from 6.4 to 0.0 mg O_2/g VSS-hr at 10°C, 9.4 to 1.3 mg O_2/g VSS-hr at 20°C and 8.1 to 0.8 mg O_2/g VSS-hr at 30°C.

Comparison of the above results with those of previous researchers is difficult since most studies have been concerned with the activity of the microorganisms in the acivated sludge process. Nevertheless for small growth-rates, Weddle and Jenkins (65) reported DOUR values of about 8 mg O_2/g VSS-hr at net growth rates of 0.026 d^{-1} and corresponding specific ATP contents of 0.34 µg ATP/mg VSS. Upadhyaya and Eckenfelder (62) report values of 5.5 mg O_2/g VSS-hr and 0.53 µg ATP/mg VSS for net growth rates of 0.025 d^{-1}. Both sets of data are in general agreement with the values obtained at the begining of digestion in this study.

5.2 Semi-continuous Reactors

5.2.1 Solids Variations

It was remarked in the previous chapter that VSS was used as the basis for expression of the activity parameters and that steady state was assumed to be reached as the concentrations in VSS varied within 10 % of the average concentration. The steady state values obtained for these solids are presented in Figures 5.8 through 5.10. These values are averages estimated for over one month of operation for each reactor operating at 10, 20 and 30 days detention time. The individual concentrations are presented in Appendix D. The values plotted at 0 days represent the start-up concentrations of the sludge.

Particular variations in TSS and TS resulted from the adjustment of the feed concentrations to a constant value of 7500 mg VSS/l. The values for TS and TSS shown in the figures above represent steady state concentrations corrected for these variations. The corrections were made by means of a mass balance around the reactor with the assumption that mg/l of TSS and TS in excess of the startup concentrations consisted of inert solids. The detailed procedure is presented in Appendix E.

As for the batch reactors, a sharp increase in the difference between TS and TSS was observed at 20 °C, indicating high accumulations of dissolved solids. This phenomenon was less apparent for the other two temperatures.

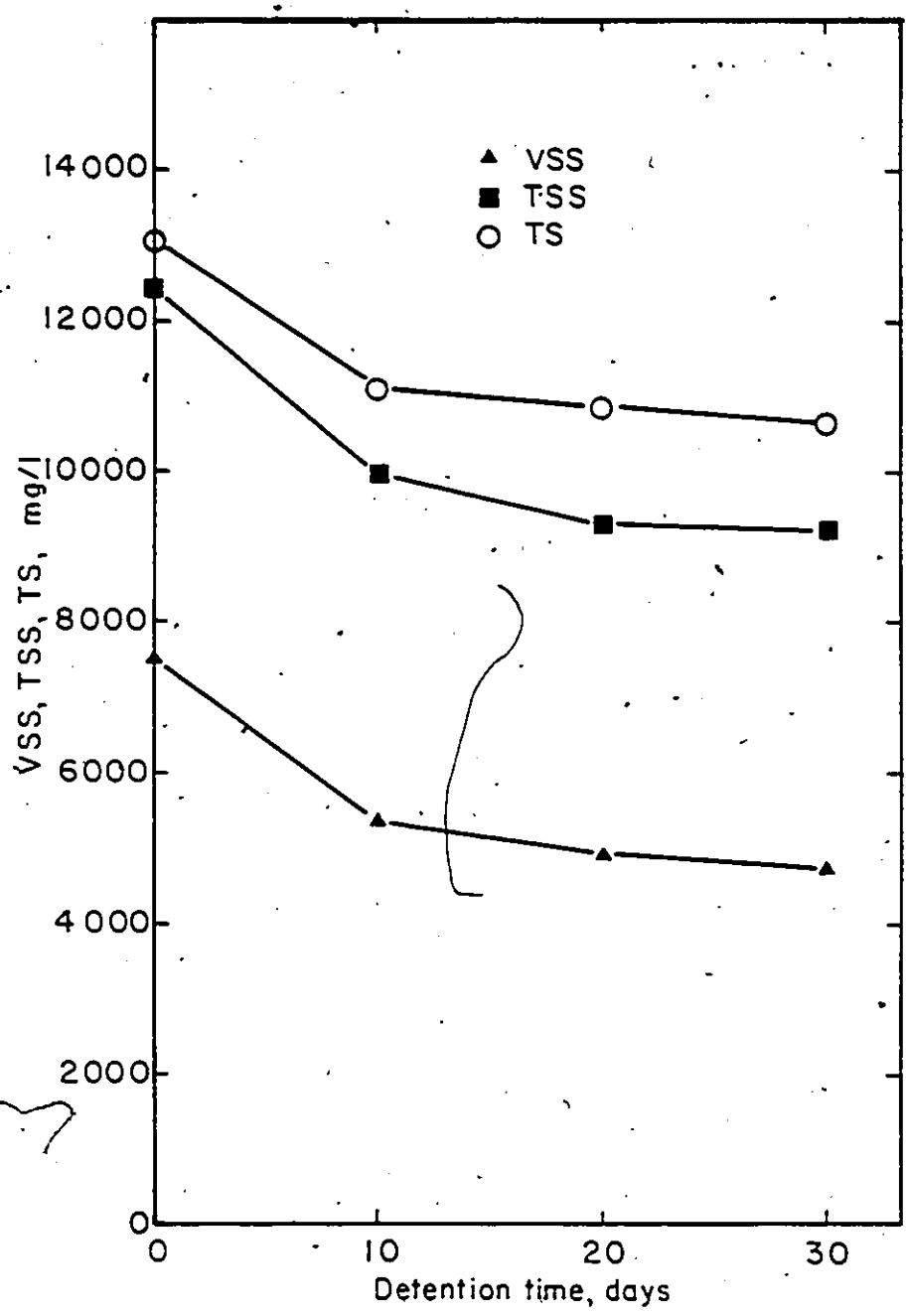

Fig. 5.8 Solids Concentrations for Semi-Continuous Units at 10°C

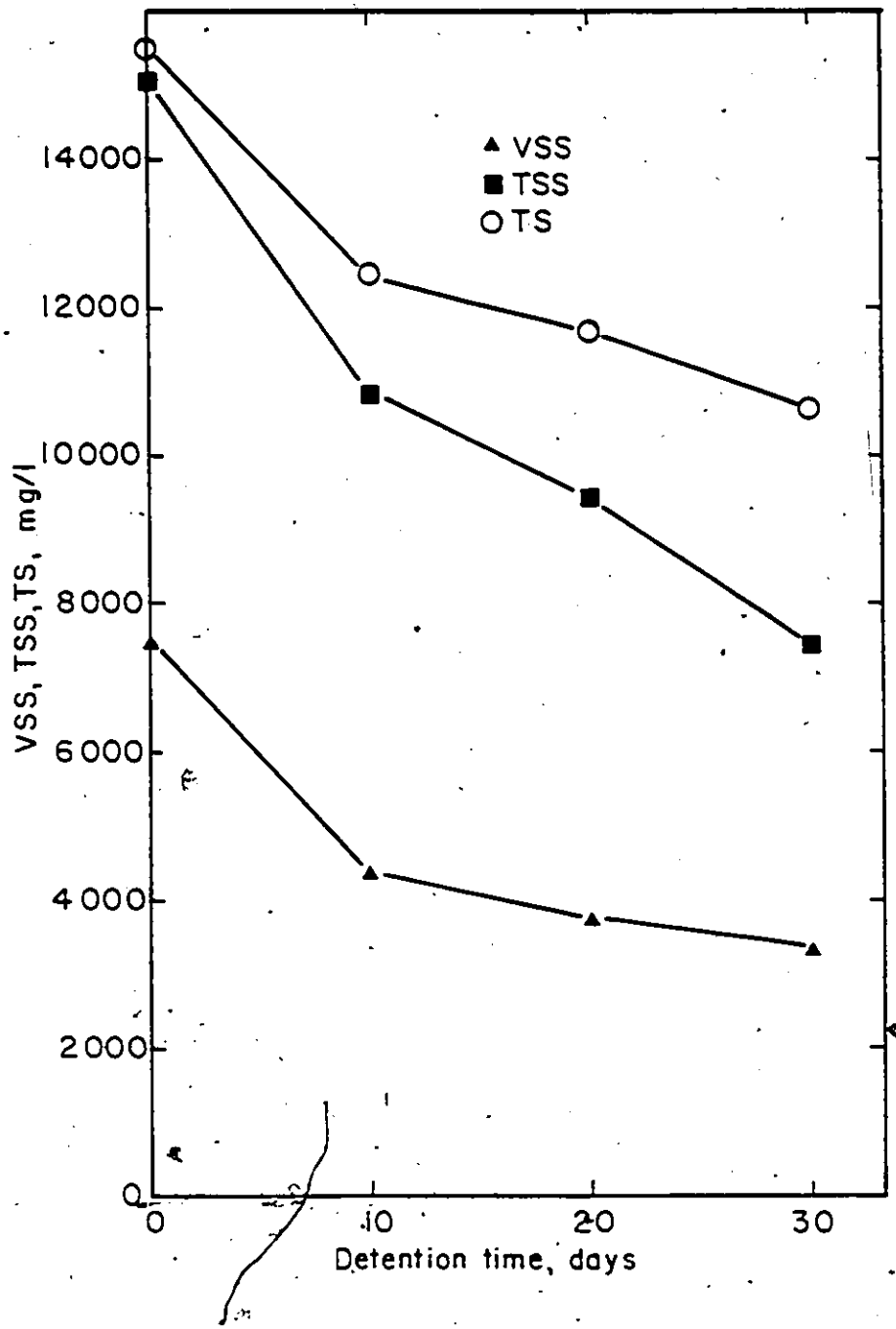

Fig. 5.9 Solids Concentrations for Semi-Continuous Units at 20° C

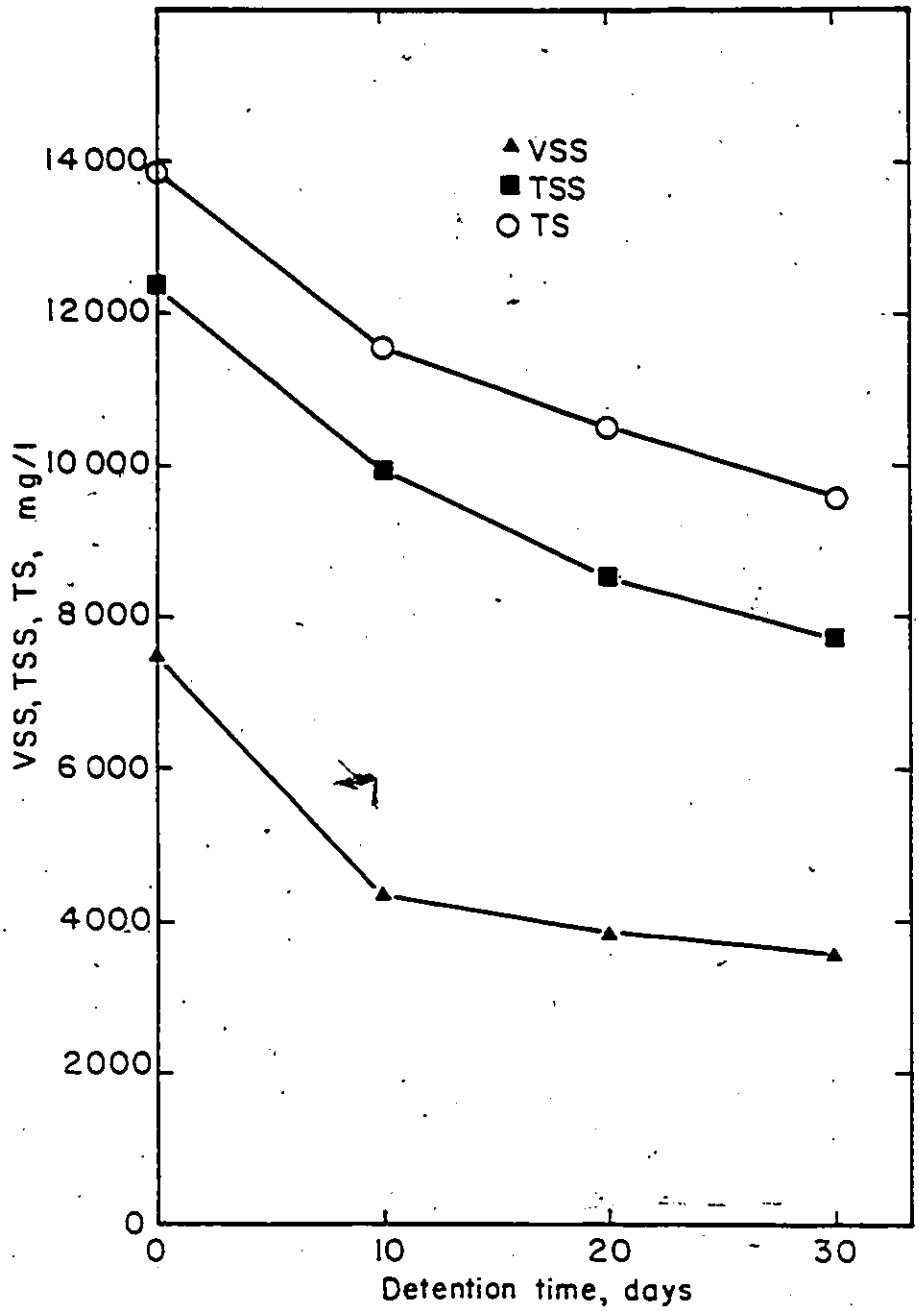

Fig. 5.10 Solids Concentrations for Semi-Continuous Units at 30° C

5.2.2 Activity Measures

Figures 5.11 through 5.13 summarize the specific activity data obtained from semi-continuous units.

Specific viability and % Activity remained relatively constant with increasing detention times. Similar to the batch reactors, viable cell counts were consistently lower than active and total cell counts (Fig. 5.11). Viable to active cell ratios varied from 0.4 % to 7.2 % and viable to total cell ratios from 0.1 % to 2.6 %. A complete tabulation of these data are given in Appendix D. Both specific values for ATP and DOUR were much higher than in the batch reactors even after 30 days of detention time. In particular, µg ATP/mg VSS remained close to the initial values recorded in the batch digesters, and were observed to decrease only slightly with sludge age. DOUR's did vary significantly with detention time but remained considerably higher than their corresponding batch digestion values. The effect of temperature on the activity parameters is complex, seeming to affect the rate of decrease of the specific values.

It was remarked in the literature review that the ATP content per cell mass, rather than cell number, was a more meaningful way of expressing the ATP pool. Nevertheless ATP concentrations in terms of viable, active and total cell counts were calculated for purposes of comparison with those from other authors. Figure 5.14 gives a tabulation of these values, while Figure 5.15 summarizes the concentrations reported in the literature.

Fig. 5.11 [1]Viability and Dehydrogenase Enzyme Activity Data for Semi-Continuous Reactors.

[2]T (°C)	Viable Cells (cells/ml) $\times 10^{-8}$			Active Cells (cells/ml) $\times 10^{-10}$			Total Cells (cells/ml) $\times 10^{-10}$			[3]V/A (%)			[4]V/T (%)		
	Sludge Age (d)			Sludge Age (d)			Sludge Age (d)			Sludge Age (d)			Sludge Age (d)		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
10	5.1	4.5	3.8	11.4	10.8	9.3	39.9	37.6	35.7	0.5	0.4	0.5	0.1	0.1	0.1
20	6.5	2.8	3.8	0.9	0.7	0.9	2.5	2.0	3.0	7.2	4.0	4.2	2.6	1.4	1.3
30	1.9	1.5	1.2	0.5	0.5	0.5	1.6	1.7	1.5	3.8	3.0	2.4	1.2	0.9	0.8

[1]Steady state values averaged over one month of operation.

[2]T = Temperature.

[3]V/A = Viable to Active cell ratio.

[4]V/T = Viable to Total cell ratio.

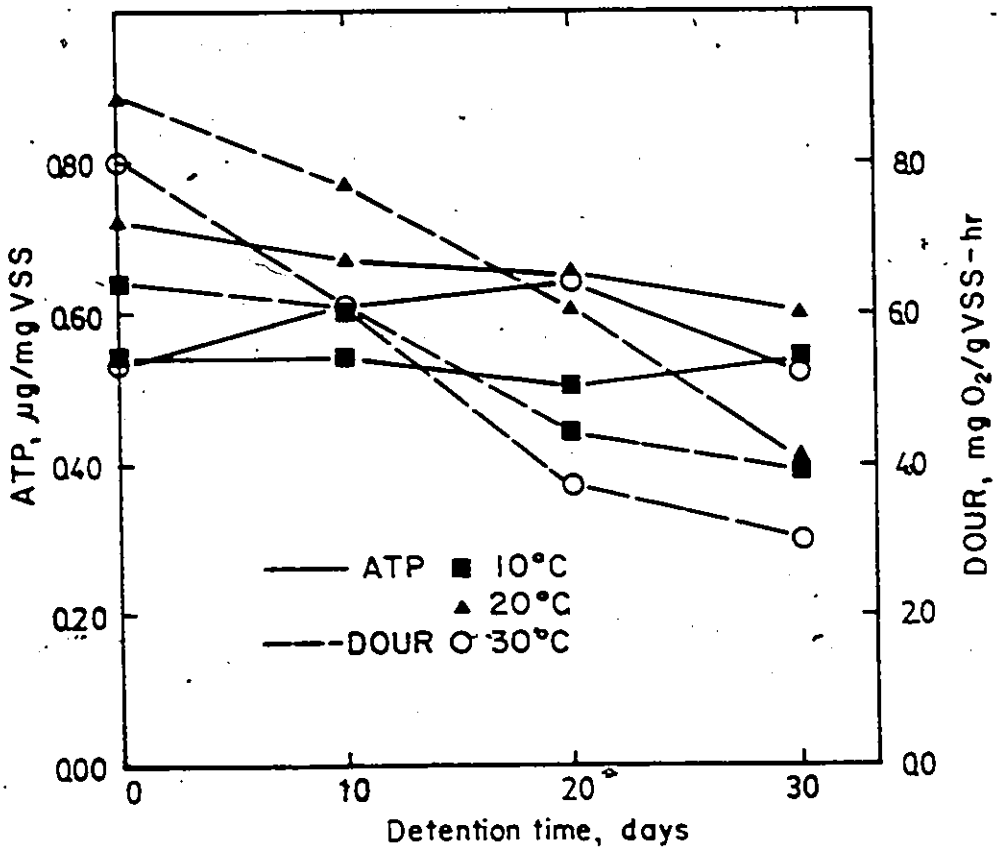

Fig. 5.12 ATP and DOUR Variations in Semi-Continuous Units

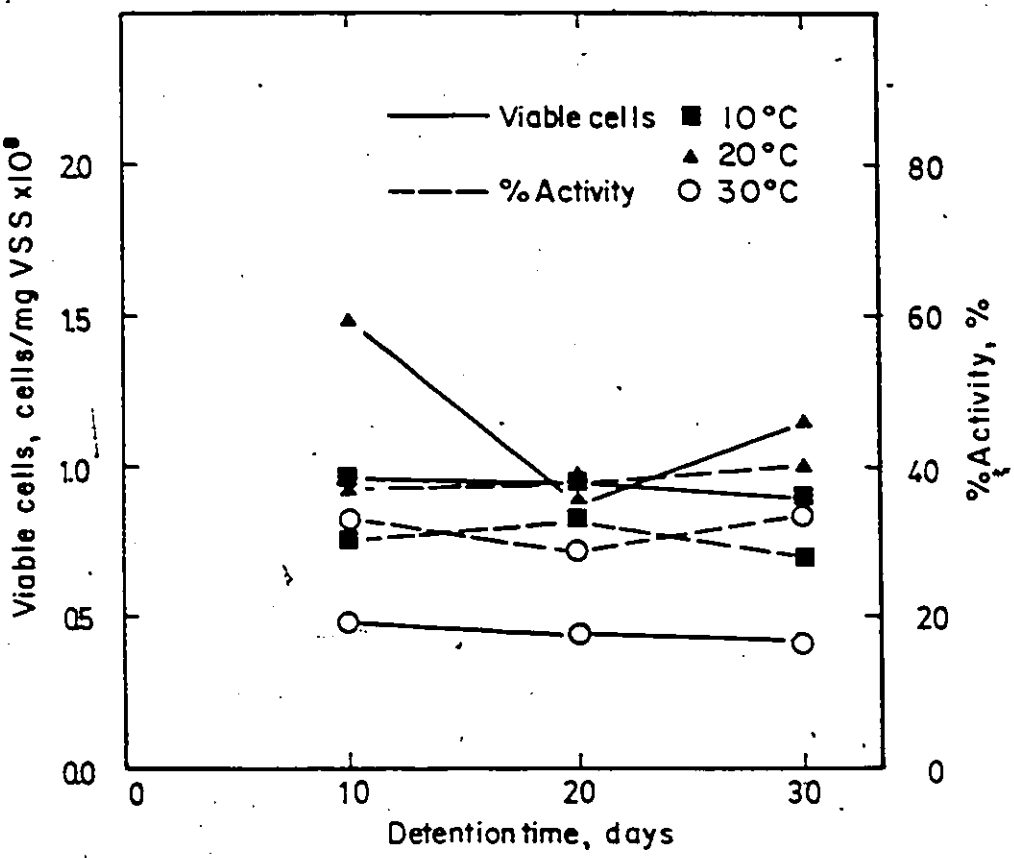

Fig. 5.13 Viable Cells and % Activity in Semi-Continuous Units

Fig. 5.14 ATP Content per Cell in Semi-Continuous Units.

Temp. (°C)	Detention Time (days)	ATP/Viable Cell ($\mu g/cell \times 10^{10}$)	ATP/Active Cell ($\mu g/cell \times 10^{10}$)	ATP/Total Cell ($\mu g/cell \times 10^{10}$)
10	10	56.9	0.25	0.07
	20	54.7	0.23	0.07
	30	68.1	0.31	0.07
20	10	44.9	3.24	1.17
	20	86.8	3.47	1.22
	30	53.4	2.26	0.68
30	10	139.0	5.30	1.66
	20	165.0	4.96	1.46
	30	152.0	3.66	1.22

Fig. 5.15 ATP Content per Cell Reported in the Literature.

ATP pool ($\mu g/cell \times 10^{10}$)	Comments	Reference
range = 2.2 - 10.3 mean = 4.7	13 bacterial species (gram negative and positive)	D'Eustachio and Johnson (17)
mean = 1.97	19 bacterial species in stationary growth phase	Chapelle and Levin (14)
range = 2 - 78 mean = 15 range = 2 - 5	Seven species of marine bacteria Pure cultures under endogenous metabolism	Hamilton and Holm-Hansen(25)
mean = 1.5	Three marine species	Holm-Hansen and Booth (29)
mean = 3	Recommended average	Picciolo et al (46)

The values obtained in this study in terms of active cells differ by a factor of 10 to 100 from those in terms of viable cells (i.e., plate counts), but agree in general with the values reported in the literature. Also, concentrations per viable cell from this study differ by an order of magnitude from those of other authors (Fig. 5.15).

These observations appear at first to be in contradiction since the values in Figure 5.15 are all in terms of viable cells. It should be noted however that the concentrations in the table above are for pure cultures of microorganisms. For these conditions, plate counts offer a better representation of the active microbial population in the media tested.

The only ATP pools encountered in terms of viable cells for mixed cultures were reported by Weddle and Jenkins (65). They estimated concentrations ranging from 10 to 100 $x10^{-10}$ µg ATP/cell in activated sludge cultures. These values compare very well with the range of 45 to 165 $x10^{-10}$ µg ATP/cell obtained in this study.

CHAPTER 6

DISCUSSION

6.1 Activity Parameters

6.1.1 AOE-Dehydrogenase Activity

Percent activity as determined from epifluorescence microscopy and dehydrogenase enzymes does not reflect varations in activity or active biomass in the mixed liquour. When compared with other activity measures, this parameter varied randomly with time during batch digestion studies, as opposed to significant decreases observed in the specific measures of ATP and DOUR (Fig. 5.4-5.6). The lack of accuracy of the test can be explained by the adverse conditions created in the bacterial environment from the required blending, high dilution factors and filter interference in the microscopic enumeration of small bacteria, the latter being the most significant. Nevertheless, important inferences can be made from these test results regarding plate counts. As previously stated, sampling for the test was done from the same serial dilutions used in plating. This allowed a direct comparison between viable (plate counts), active (cells with formazan deposits) and total cell counts.

6.1.2 Plate Counts

Viability has been used by many researchers to be synonymous to

activity, implying that only reproducing cells are responsible for substrate removal or endogenous metabolism. Although existence of non-reproducing bacteria which retain their enzymatic activity has long been recognized (31, 66), their importance has been neglected due to the inability of determining their number and activity rate. The only study encountered by the author is the work of Walker and Davies (63), in which non-reproducing bacteria were shown to be responsible for up to 80 % of the total respiration in an activated sludge unit at low growth rates.

Further evidence on the metabolic importance of these type of cells has been accumulating in recent years. Extensive research by DEARBORN ENVIRONMENTAL CONSULTING SERVICES on chlorination of treatment plant effluents and corresponding ATP variations have indicated that chlorinated cells incapable of reproduction in plating media contained high levels of ATP (13), and thus a high level of metabolic activity.

Picciolo et al. (46), in their study of environmental applications of the ATP assay, compared bacterial concentrations determined from ATP and plate counts for pure cultures of E. coli. The bacteria/ml calculated from ATP measurements was reported as 1.76 times the bacteria/ml calculated from plate counts. The departure from unity was explained in part by the existence of cells that cannot reproduce in the plating media but that are otherwise intact.

Results in the present study show viable cells to account for only 0.1 % to 30 % of actively respiring cells, and percent viabilities (viable to total cell ratio) of only 0.0 % to 5.1 %

for all temperatures and flow regimes analysed (see Fig. 5.7 and 5.11). These low values demonstrate the inadequacy of plate counts as a measure of activity and corroborate the existence of nonviable bacteria with significant metabolic activity, as proposed above.

However a further clarification is in order. Nonviable bacteria refer to bacteria that were not able to reproduce in the plating media under specific experimental conditions. That these microorganisms are not able to reproduce in the mixed liquour, with no abrupt changes in their environment, has yet to be shown.

6.1.3 ATP and DOUR

A close relation between specific DOUR and ATP was observed in the batch units for all three temperatures studied. The suitability of both parameters as measures of activity has been recognized by several authors (8, 43, 50, 65), and the similarity in their variation with digestion time would seem to confirm the above. However, examination of the semi-continuous data reveals an inconsistency between the two measures. As shown in Figure 5.12, DOUR decreased significantly with detention time whereas ATP remained relatively constant. Similar discrepancies have been reported by Eikum et al. (21) and Upadhyaya and Eckenfelder (62). The difference in behaviour has been attributed to inherent errors in the DOUR technique and/or variations in oxygen penetration through the biological floc from variations in the floc size with operating conditions. These arguments alone

would not be sufficient to explain the differences obtained in the present study due to the close relation observed between the two activity parameters in the batch reactors, for all three temperatures considered.

The major requirement for oxygen during aerobic metabolism is for the generation of energy. Oxygen is used as the terminal electron acceptor in the electron transport chain (ETC). In eucaryotic cells, the electron transport chains are neatly arranged within a specialized organelle, the mitochondrion, and the components of the chain are similar among different types of organisms. Electron transport and ATP synthesis from oxidative phosphorylation are tightly coupled in this organelle, and thus the number of molecules of ATP synthesized for each atom of oxygen reduced can be predicted (23). In procaryotes however, the electron transport system with associated enzymes and ATP synthesizing apparatus are embedded in the cytoplasmic membrane. Uniformity of components among different genera is nonexistent. Branching in the electron transport chains is common, with electrons entering the chain at different levels and with more than one electron carrier capable of transferring electrons to oxygen. The situation is further complicated by the ability of many bacteria to use alternate pathways of electron transport with switching from one pathway to another depending on environmental conditions such as changing DO concentrations in the medium or variations in substrate composition.

It seems possible then that changes in environmental conditions and/or changes in predominant species could result in different

amounts of ATP synthesized per electron pair transferred to oxygen.

Partial proof of this can be seen in the situation where respiration (utilization of oxygen) proceeds as rapidly in cells that are unable to grow because of the abscence of an essential nutrient (such as nitrogen) as it does in growing cells. In such a situation, uncoupling between cell growth and respiration has been accounted for by production of reserve carbon and/or energy sources, use of alternate electron transport pathways with different sites of oxidative phosphorylation and possibly waste of energy through ATP hydrolysis (23).

The question then arises as to which parameter, DOUR or ATP is a better indicator of activity or active biomass. To address this question an examination of the relation between ATP, DOUR, and cell synthesis is required.

ATP is essential to the metabolism of the cell, without its generation no biosynthetic process can function. The mass of cell material that can be synthesized from a substrate depends upon two factors : the efficiency of energy generation (i.e., the amount of ATP formed from available substrate) and the efficiency of energy utilization (i.e., the amount of cell material synthesized from available energy). It has been shown by many researchers that a large number of microorganisms produce 10.5 grams of cell material per mole of ATP generated. Consequently, this value has been considered to be a "universal constant" by many. For this to be true, it would be necessary for the efficiency of cell production to be the same for all organisms

and all substrates. However, the energy required for synthesis is influenced by the type and oxidation state of the energy substrate as well as the complexity of the growing medium. Therefore it is likely that the constant efficiencies of cell production obtained are due to the narrow range of substrate and media employed in microbiological research, and it is doubtful that this constancy would be observed with the broad range of conditions encountered in wastewater treatment (24). Nevertheless the close relation between ATP production and cell synthesis is evident.

This is not the case with DOUR. As previously mentioned, bacteria have the ability of uncoupling cell synthesis and respiration in part through the use of alternate electron transport pathways with different sites of oxidative phosphorylation and through ATP hydrolysis. Furthermore, the uptake rate is obtained by the measurement of the dissolved oxygen concentration in the mixed liquour, an external element to the cell, and thus is subject to interferences from the medium and dependent on the efficiency of oxygen penetration into the biological floc.

In summary, it is believed that ATP offers the best estimate of activity for a mixed bacterial population. The discrepancy between ATP and DOUR in the semi-continuous units could be explained as a result of the development of predominant species having a more "efficient" ATP yield per oxygen atom reduced.

6.2 Active Fraction Determination

Extensive research has been done with regard to the use of ATP as a measure of biomass. The general belief is that ATP is maintained at fairly constant levels in the cell through biochemical regulation. On this basis it has been proposed to estimate active mass by means of an average ATP content per cell or cell mass. However several factors have been recognized to affect the ATP concentration in the cell. These include :

i) growth rate of culture,

ii) substrate composition and concentration,

iii) predominance of species in mixed cultures,

iv) environmental stresses.

Assessing the extent of variation from these factors is difficult due to the different ways in which the ATP pool has been expressed (i.e., in terms of cell mass or cell number), therefore the use of an universal average seems presently inconclusive.

These problems have led several researchers to estimate the ATP content per active cell mass (P_B) for a particular type of substrate and environmental conditions, and to determine active mass or active fraction of suspended solids from the relationship presented in Section 3.2 (Equation 3.1).

An attempt was made in this study to estimate the base value P_B in the equation above and to verify its constancy with detention time during aerobic digestion. Therefore, with the assumption that the decrease in VSS in the batch reactors (no

interference from variations in feed) represents the decrease of active mass in the mixed liquour, ATP and BVSS concentrations were plotted versus digestion time (Figures 6.1-6.3). The underlying consideration was that in order for P_B to be constant, the rate of decrease in ATP concentration in the tank should be proportional to the rate of decrease in BVSS. The biodegradable VSS remaining at time t in the aeration tank was obtained by subtracting the VSS residual at the end of the run from the VSS concentration in the mixed liquour at that particular time.

Examination of these plots revealed that for the 20°C run and the latter part of the 30°C run, the rate of decrease in BVSS was much larger than the ATP rate of decrease. Under the assumptions considered, this would imply that larger amounts of ATP were being generated from lesser amounts of active mass available. It is unlikely for such a situation to occur. A more plausible explanation would be that processes other than endogenous metabolism were being responsible for the reduction in suspended solids.

Indeed, for these two runs, a large increase in total dissolved solids (TDS) and a corresponding decrease in fixed suspended solids (FSS) were observed (Fig. 6.4). These variations revealed that a large portion of the cellular solids had been solubilized and not metabolized. The reduction of VSS through solubilization would account for the higher rate of decrease in BVSS as compared to the ATP rate.

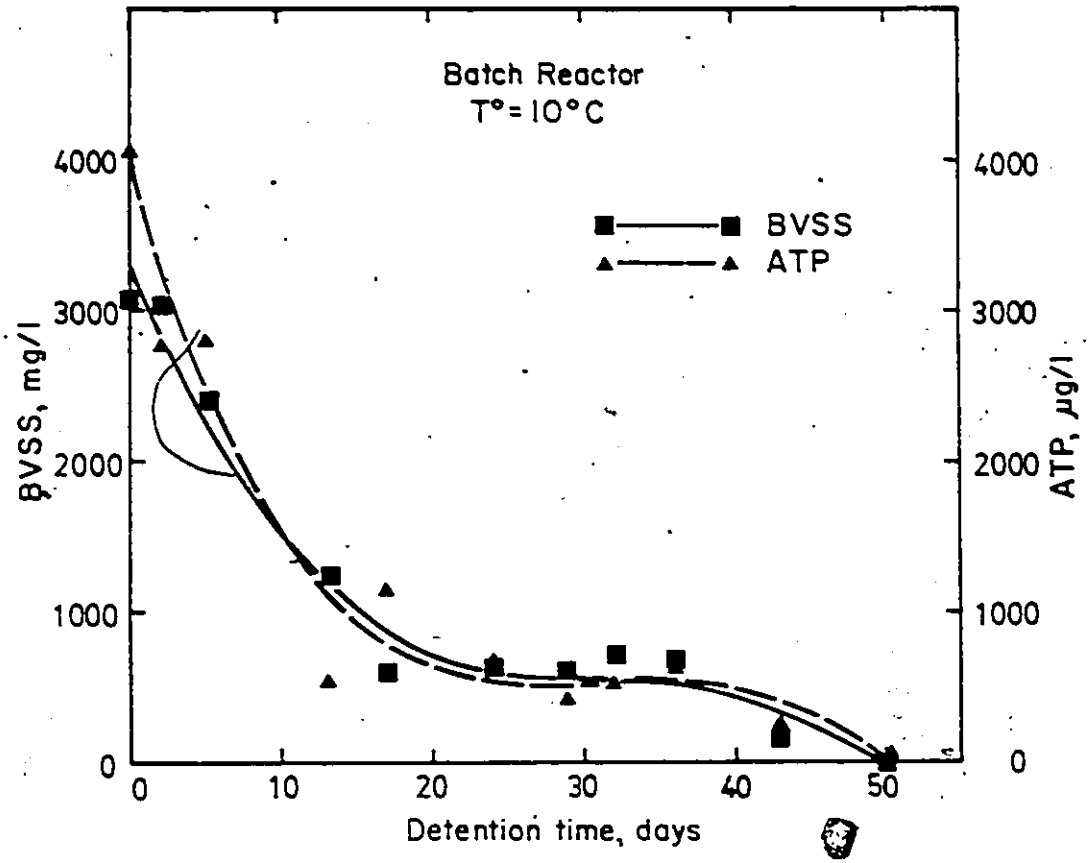

Fig. 6.1 BVSS and ATP vs. Time, for 10°C Run

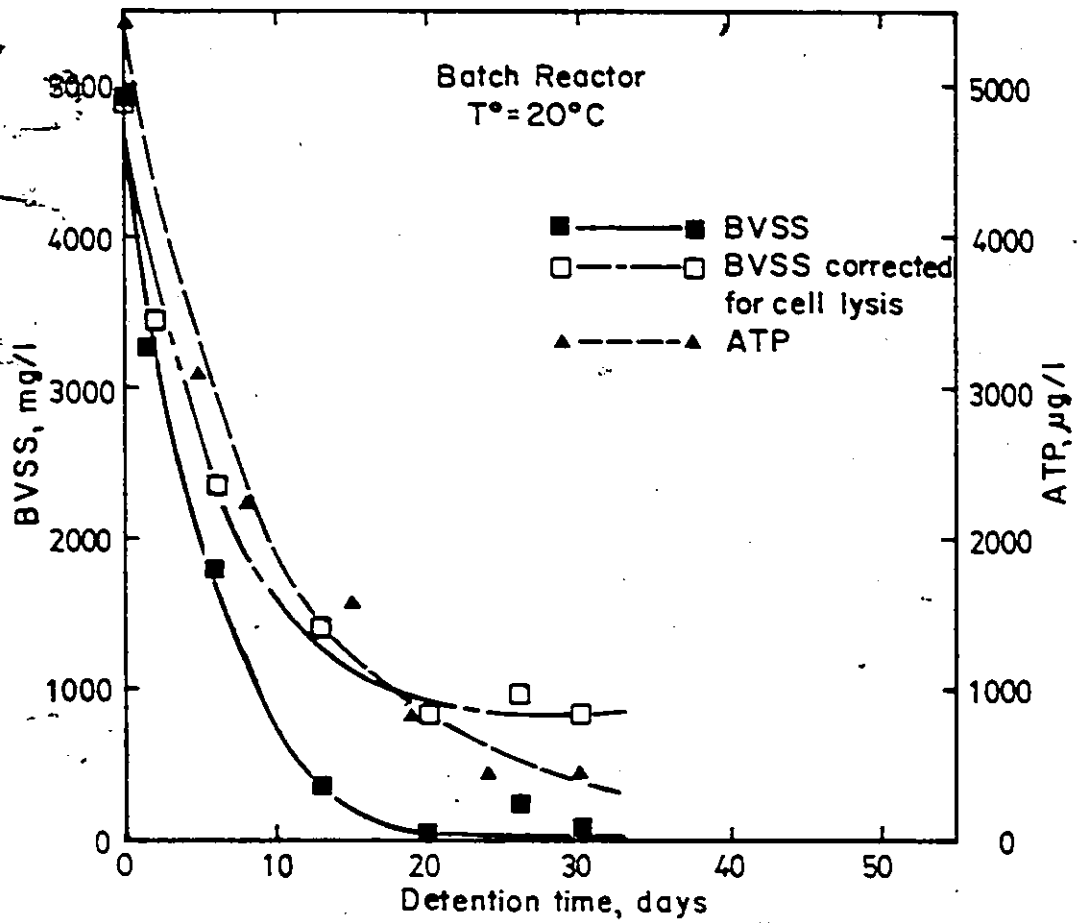

Fig. 6.2 BVSS and ATP vs. Time, for 20° C Run

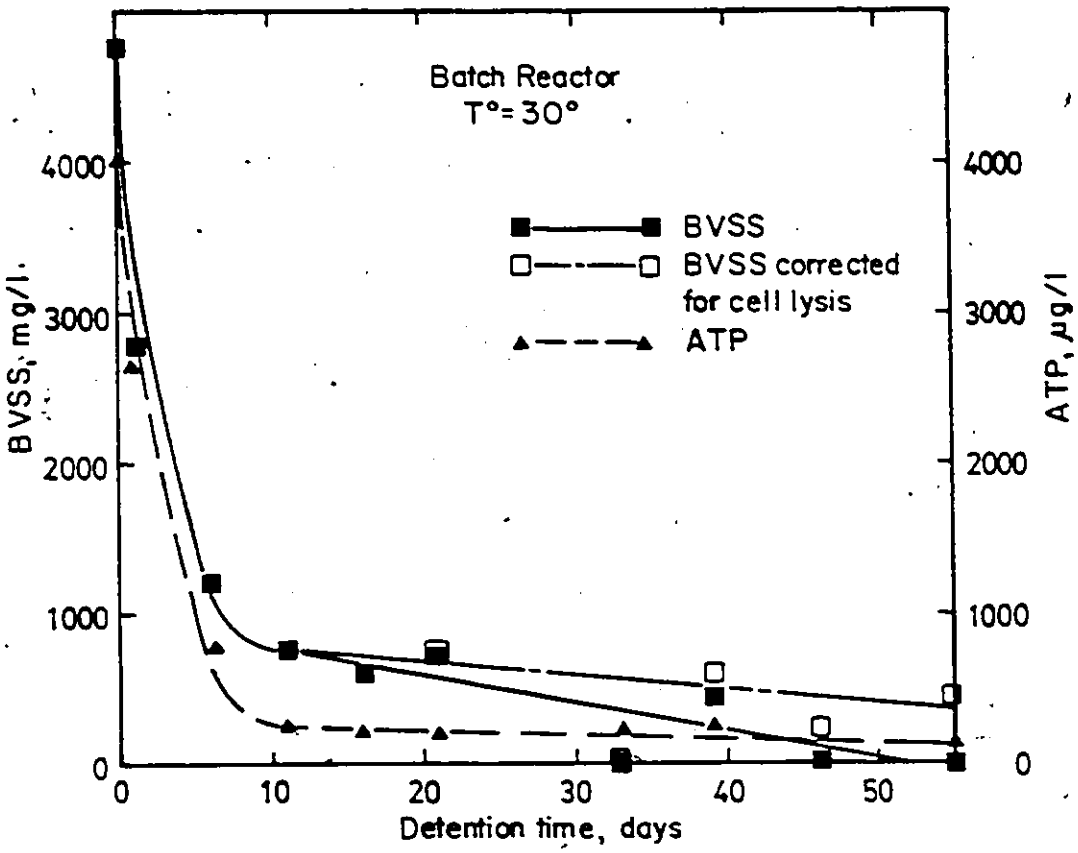

Fig. 6.3 BVSS and ATP vs. Time, for 30°C Run

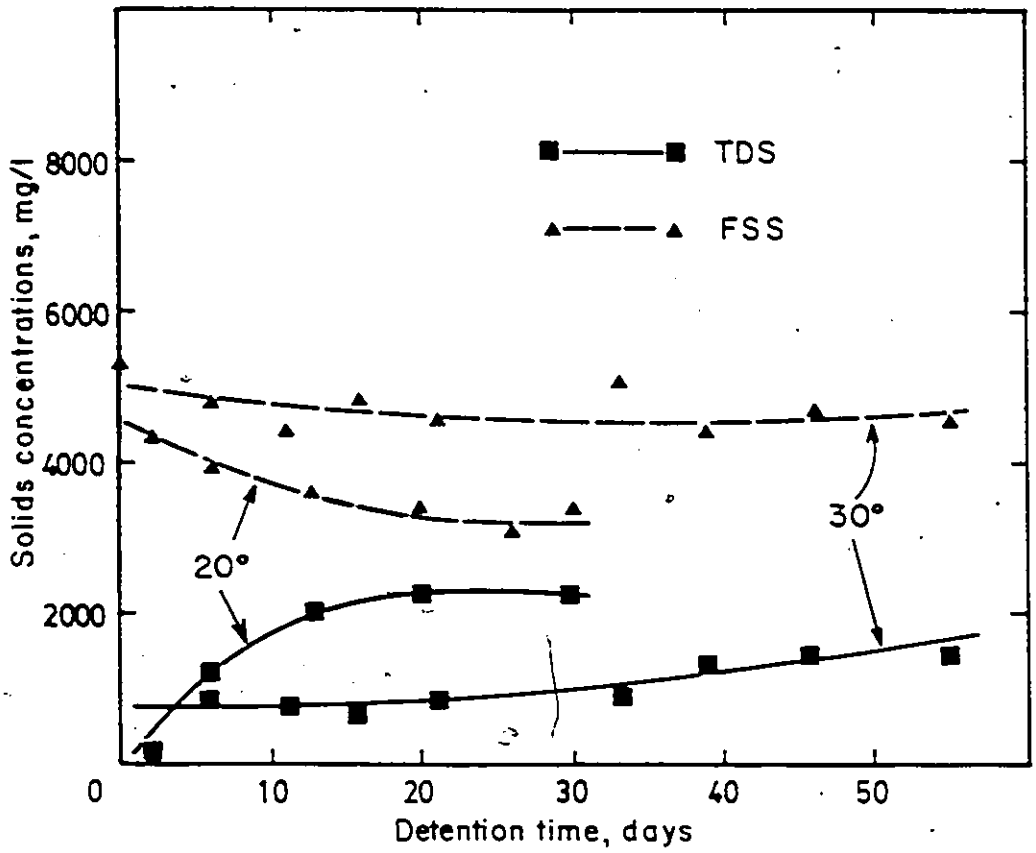

Fig. 6.4 Solubilization of Organic Solids in Batch Reactors

Similar situations in which high rates of solubilization occur with no further utilization of the soluble material by the remaining organisms have been reported in the literature (49). The solubilization of fixed and volatile solids was attributed to cell lysis.

For further analysis between the rates of decrease of ATP and BVSS, an attempt was made to correct for this reduction in solids. This was done by assuming that for a specific digestion time, the amount of VSS solubilized could be approximated by the difference between TDS increase and FSS decrease from their initial respective value. Sample calculations are shown in Appendix F.

It is recognized that such an approach is rather crude, nevertheless it gives an indication of the endogenous mass present in the reactors. Plots of the "corrected" BVSS values are presented in Figures 6.2 and 6.3. It should be noted that no correction was made for the 10°C reactor since no significant variations of either TDS or FSS were observed. From these plots it can be seen that the rate of decrease in BVSS from the adjusted solids concentrations does follow relatively closely the rate of decrease of ATP. The latter point is better illustrated in Figures 6.5 through 6.7 which are plots of µg/l ATP versus mg/l of "corrected" BVSS at corresponding detention times. All values plotted were derived from fitted values on curves from Fig. 6.1-6.3. The linear regressions were performed by the least squares method and the correlation coefficients (r) obtained are indicated in each figure. All regression analysis are shown in Appendix C.

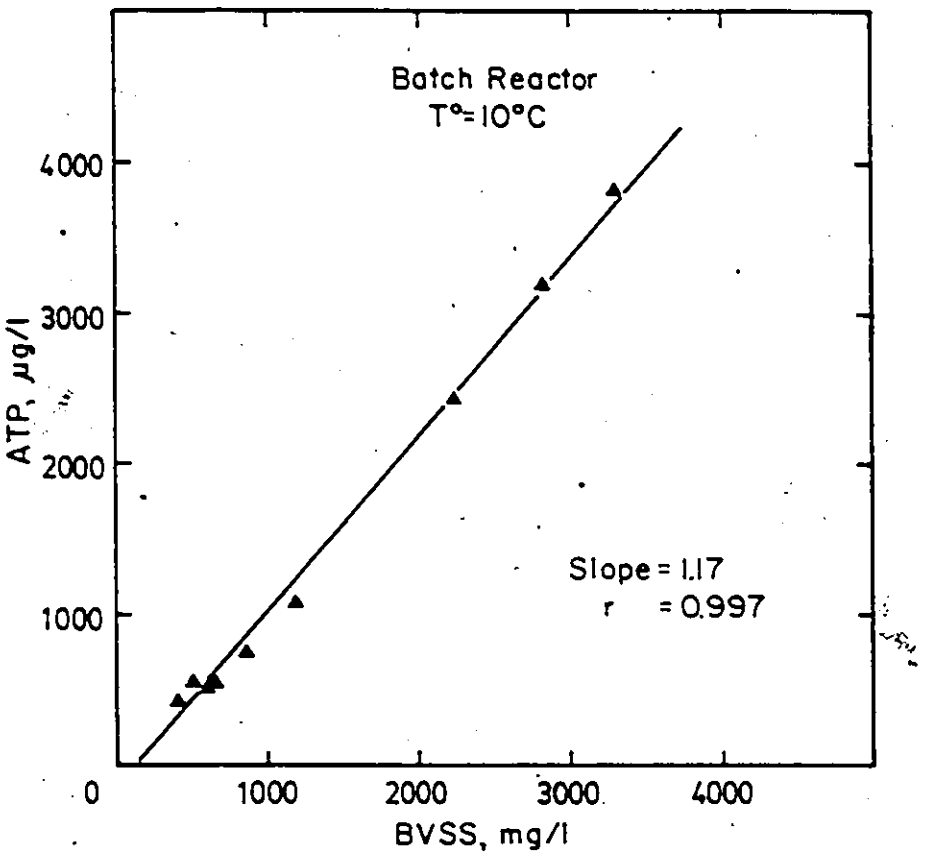

Fig. 6.5 ATP vs. BVSS, 10° C Run

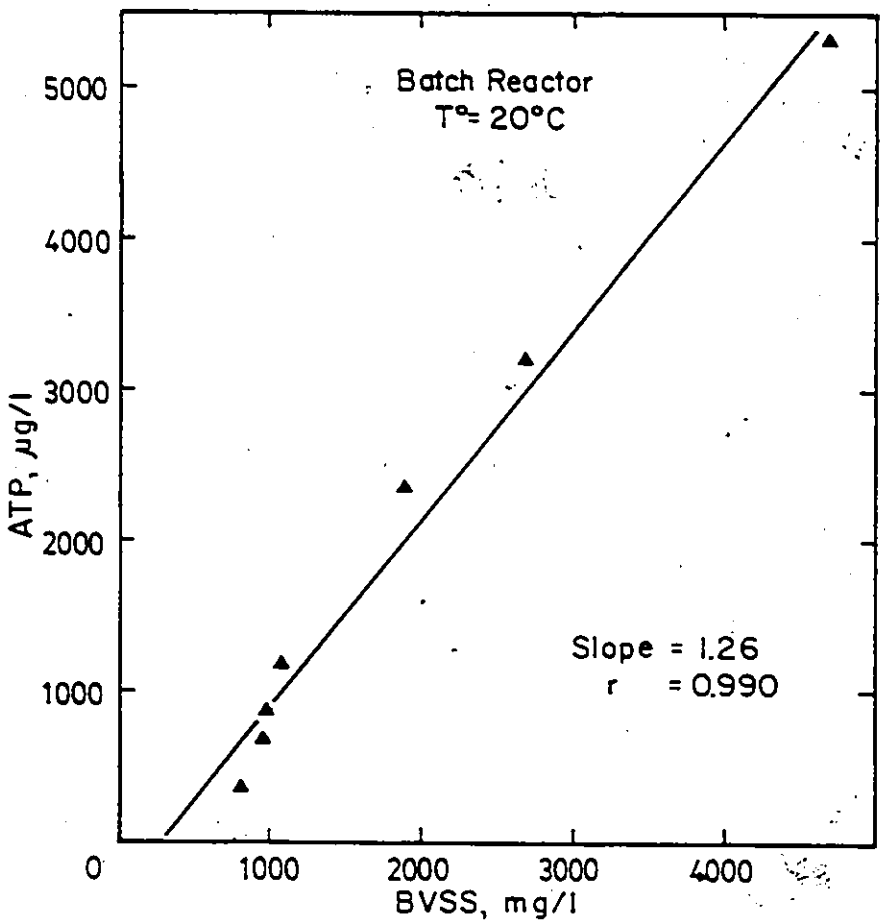

Fig. 6.6 ATP vs. BVSS, 20°C Run

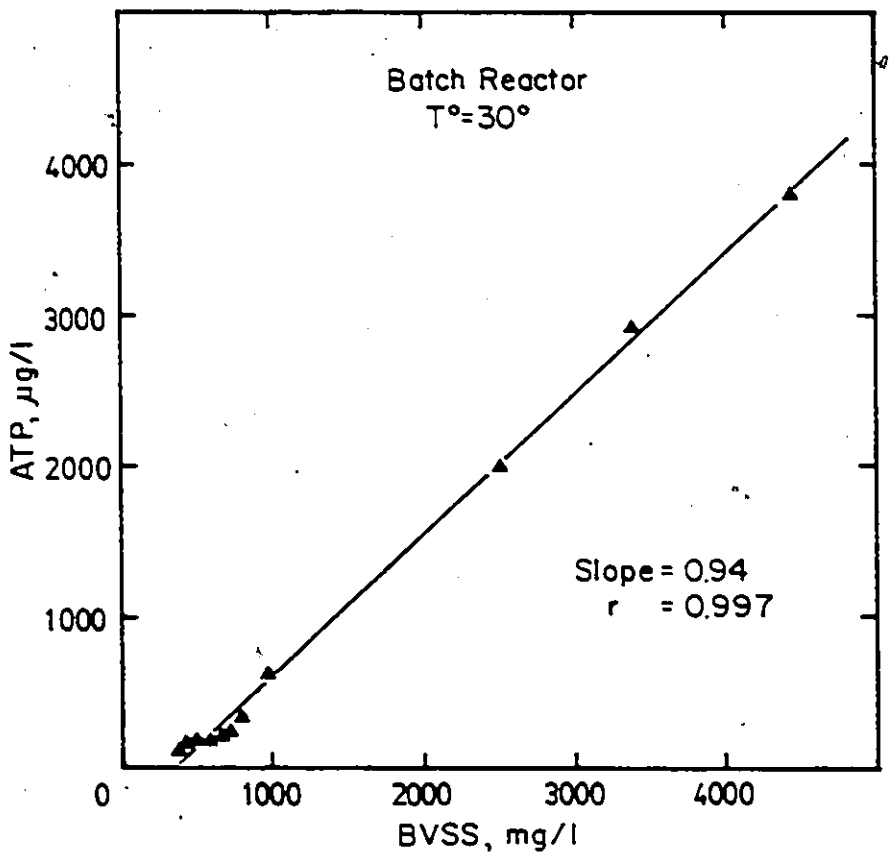

Fig. 6.7 ATP vs. BVSS, 30°C Run

For all three temperatures, the correlations were found to be significant at the 1 % level. The above results indicate that both rates of decrease are indeed proportional and that the ATP pool in terms of BVSS is constant with detention time.

Furthermore, with the previous assumption that variations in active mass parallel variations in "corrected" BVSS, the ATP content per active cell mass (or factor P_B) may be approximated by the slopes of the above linear regressions, yielding the values of 1.2, 1.3 and 0.9 µg ATP/mg active cell mass at 10, 20 and 30°C respectively. These values compare very well to the value of 1.1 µg ATP/mg active MLVSS given by Upadhyaya and Eckenfelder (62), but are somewhat smaller than those reported by Patterson et al. (45) of 2.0 µg ATP/mg of cell material, and Nelson and Lawrence (43) of 2.5 µg ATP/mg VSS at 100 % activity.

The determination of the ATP content per active cell mass (P_B) allows the calculation of the active fraction of the sludge (F_A) from Equation 3.1.

A comment on the assumptions used in the previous sections is in order. From the definition in this study of BVSS and by correcting for solubilized organics that accumulated in the batch reactors, the value of the adjusted volatile solids represents the amount of VSS that remains to be degraded through metabolic activity at any time t. The assumption that the decrease in these solids parallels the decrease in active mass during batch digestion implies that the reduction in mass from the digestion of inactive organic matter is negligible. This is in agreement with the usual overall simplification of the process, for

secondary sludges, in which autooxidation of microbial cell mass is assumed to be the main process occuring. In defence of this, it can be argued that for it to be otherwise, ATP would be expected to decrease less rapidly than BVSS if at all, since the inactive organics would act as exogenous sources of food to the remaining organisms. Therefore the close interrelation between the rates of decrease of ATP and BVSS ,as shown in Figures 6.5 through 6.7, seem to justify the assumption .

Benefield and Randall (8) in their proposed model for aerobic digestion made similar assumptions by considering the inactive fraction of influent TSS to be non-degradable and by assuming that only degradable active TSS decreased during digestion.

6.3 Active fraction variations

Plots of active fraction versus digestion time indicate that very significant reductions in activity occurred in the batch runs (Figure 6.8). Percent reductions in F_A greater than 80 % of their initial value were observed for all three temperatures after 30 days of digestion. The curves in Figure 6.8 were fitted by the least square method assuming a first order rate of decrease (i.e., $d(F_A)/dt = - K(F_A)$).

The corresponding values of the rate constants and correlation coefficients of the curves in their linearized form are tabulated in Figure 6.9. All regressions were significant at the 1 % level.

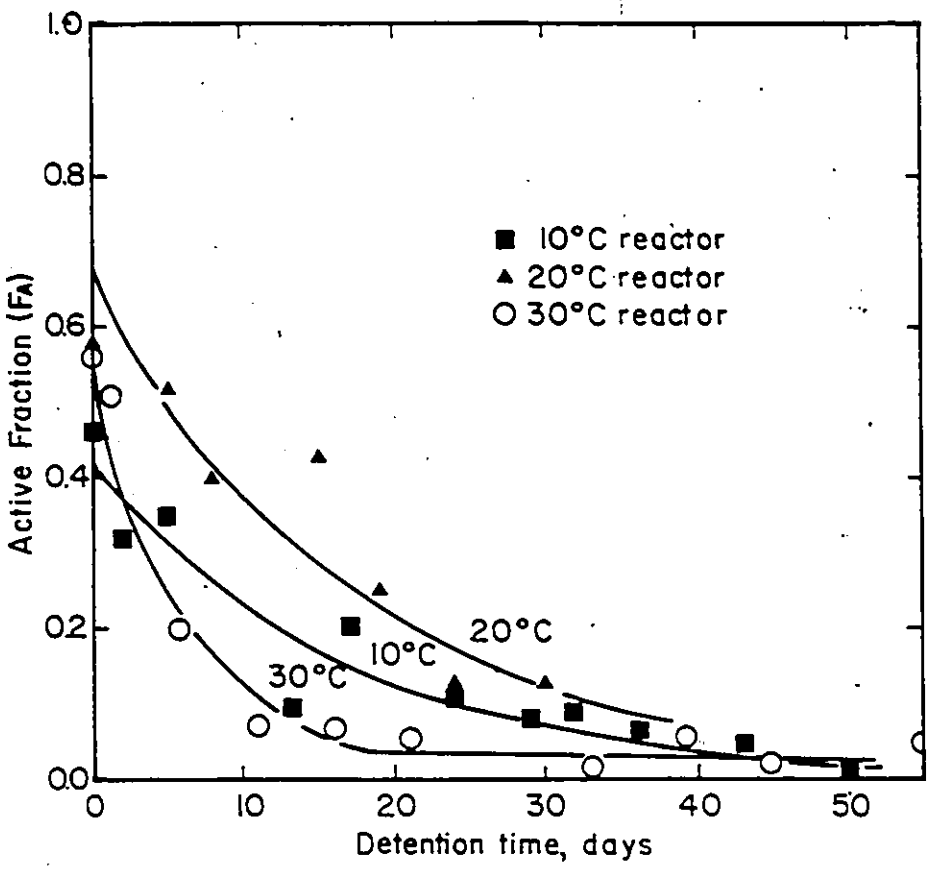

Fig. 6.8 Active Fraction Variations in Batch Units

Fig. 6.9 Active Fraction Regression Analysis in Batch Units.

Temp. (°C)	Initial Value F_o	Rate Constant K (d^{-1})	Correlation Coefficient r
10	0.41	0.058	0.892
20	0.67	0.057	0.940
30	0.53	0.151	0.977

Above coefficients refer to equations of the form :

$$F_A = F_o e^{-Kt}$$

Surprisingly, such magnitudes of reduction in F_A were not observed in the semi-continuous units. On the contrary, the active fraction of the sludge remained more or less constant with digestion time for all three temperatures (Figure 6.10). The average values obtained were 0.45, 0.53 and 0.61 at 10°, 20° and 30°C respectively. For these reactors, the active fraction was determined from Equation 3.1 with the extrapolation of base value P_B from the batch data at the corresponding temperature.

The difference in variation of F_A indicates that essentially different metabolic states of the mixed liquor occurred beteween the two flow patterns. The high active fractions observed in the semi-continuous units would seem to suggest a better adaptation of microorganisms to the environmental conditions at which they were subjected, although decreases in volatile solids still occurred in these units in similar magnitudes as in the batch reactors (Figures 5.8 through 5.10). The above observations have direct implications in process design, and will be discussed in the following sections.

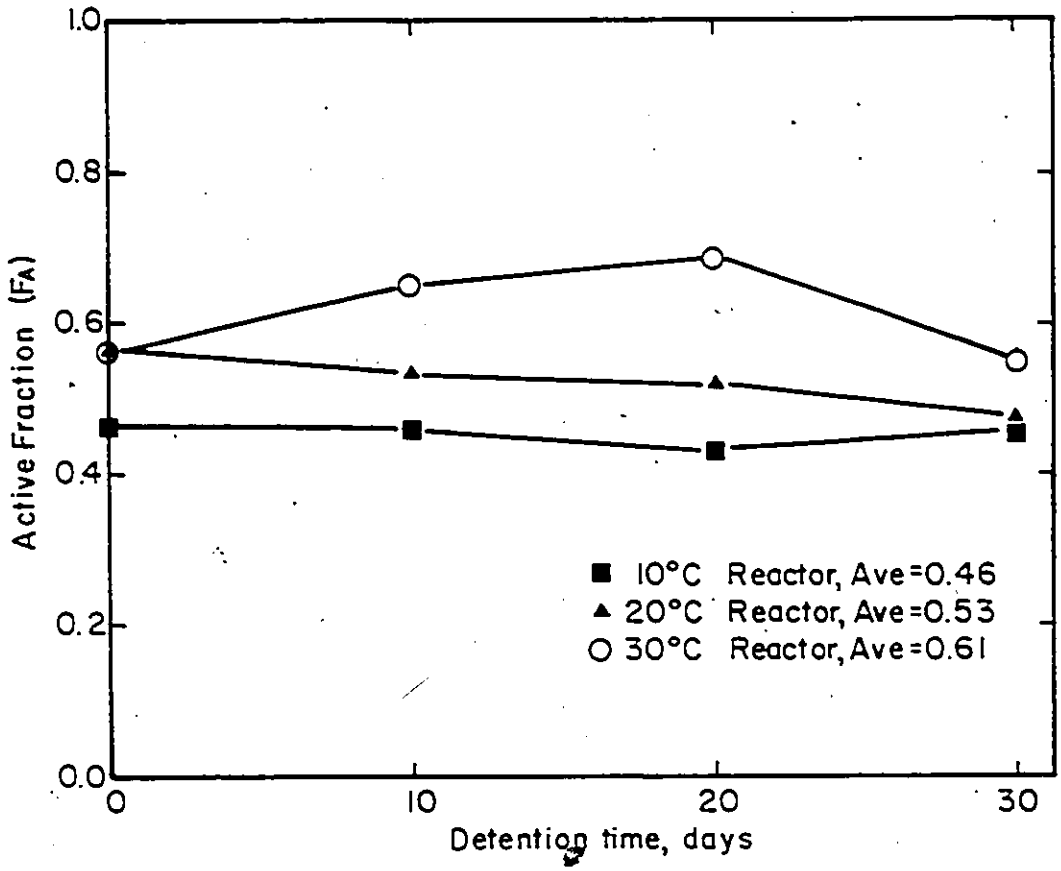

Fig. 6.10 Active Fraction Variations in Semi-Continuous Units

6.4 Design Considerations

6.4.1 Solubilization of Volatile Organics

It was observed in Section 6.2 that a large portion of cellular solids was solubilized and not metabolized during batch digestion at 20°and 30°C. Randall et al. (49) have reported similar solubilization of volatile solids in batch reactors. They found this phenomenon to be most pronounced at high temperatures (above 40°C) although significant solubilization at a temperature of 25°C was also reported.

This type of solids reduction can have significant influence on the magnitude of the endogenous decay coefficient when estimated in terms of volatile organic solids. Rate coefficients calculated from the model of Adams et al. (1), for the 20°C batch run, resulted in values of $K = 0.151\ d^{-1}$ with no corrections for cell lysis and $K = 0.057\ d^{-1}$ with corrections. It is doubtful for the uncorrected value to be representative of endogenous digestion as the loss in suspended mass from solubilization is both random and independent of cellular metabolism.

The effect of solubilization on the decay coefficient was minimal at 30°C. This coefficient was determined from the first 16 days of digestion since no significant decrease in solids was apparent after that time. During this initial period no solubilization was detected (refer to Appendix F).

The assessment of this phenomenon in semi-continuous units is complicated since variations in feed composition and detention

time from the previous process affect the concentrations of the different solids in the reactors. The problem can nevertheless be eliminated by the estimation of decay coefficients from more direct measures of active mass.

6.4.2 Extrapolation of decay coefficients

Another important point that developed from this research is the significant difference that exists in the metabolic activity of the sludge between batch and semi-continuous flow regimes. This has direct implications in the determination of the endogenous coefficients since it is usual practice to determine these coefficients from batch studies and to extrapolate them to semi-continuous or continuous flow regimes (1, 24). The difference between the two metabolic states would caution against such an extrapolation. Indeed, analysis of the experimental data revealed large differences in rate coefficients between the two flow patterns. These coefficients are tabulated in Figure 6.11. They were determined from the model presented by Adams et al. (1). Detailed calculations are presented in Appendix C (Sections C.6 and C.7). Also indicated in the figure are the correlation coefficients of the linear regressions. All correlations were found to be significant at the 1 % level.

The table below shows that the decay coefficients from batch reactors were consistently lower than those obtained from

Fig 6.11 Comparison Between Batch and Semi-Continuous Decay Coefficients

Temp. (°C)	Batch Reactors ^1K (d^{-1})	^2r	Semi-Continuous Reactors ^1K (d^{-1})	^2r
10	0.056	0.925	0.267	0.995
20[3]	0.151 0.057	0.894 0.944	0.187	0.996
30	0.120	0.959	0.158	0.998

^1K = Decay rate coefficient.

^2r = Correlation coefficient.

[3]Lower values correspond to BVSS corrected for cell lysis.

semi-continuous reactors. This is particularly true at 10°C where the decay coefficient from the semi-continuous unit is up to 5 times larger than that from the batch unit. Similarly the corrected value for the 20°C coefficient is much smaller than its corresponding semi-continuous one.

These results are in contradiction to the claim by Adams et al. (1) that the endogenous decay coefficient from batch data is interchangeable with the one from semi-continuous studies, a claim that has been widely accepted (24). Their conclusion was made with data from a single batch reactor at a single unspecified temperature. It is believed that the results presented here offer enough evidence to prove the above wrong and imply the need of designing digestion processes from bench scale studies with similar flow regimes.

6.4.3 Temperature effects

It is well recognized that temperature has a significant effect on the aerobic digestion of activated sludge. However there is considerable doubt on how to represent this effect. As pointed out in the literature review, the preferred approach has been to quantify variations in decay rate coefficients by means of an Arrhenius relationship (Equation 2.2).

Some authors however, warn against the accuracy of this approach, especially for temperature variations from mesophilic to thermophilic ranges (24).

Inspection of data from the present study reveals that the

decay coefficients from the semi-continuous units decrease with increasing temperature. This is in direct contradiction to the formulation above (Eq. 2.2). The trend observed can only be explained by the important effect of temperature on the estimation of the non-degradable portion of volatile solids (NBVSS). These values are tabulated in Figure 6.12 along with the ultimate biodegradability of the sludge at each temperature. A sharp difference in NBVSS exists between the 10°C and the other two units.

The dependence of decay rate coefficients on NBVSS is best illustrated with the equation :

$$\frac{VSS_o - VSS_e}{VSS_e - NBVSS} = Kt \qquad (6.1)$$

where all variables are as defined previously.

This equation is the linearized form of Equation A.9 with the substitution of NBVSS from Equation A.5 (see Appendix A). The above relationship indicates that a significant increase in NBVSS would result in a corresponding increase in decay rate coefficients, as can be verified in Figure 6.12. These coefficients are based on how rapidly BVSS are destroyed rather than on the magnitude of solids reductions.

Therefore it is important to note that Equation 2.2 cannot be used to predict the effect of temperature variations when determining decay coefficients in terms of BVSS for semi-continuous units. An experimental estimation of the temperature influence is recommended.

Fig 6.12 Temperature Effects on Decay Rate Coefficients.

Temp.	NBVSS	[1]Ultimate Biodegradability	[2]Decay Rate Coefficient in Semi-Continuous Reactors
(°C)	(mg/l)	(mg/l)	(d^{-1})
10	4420	3080	0.267
20	2600	4900	0.187
30	2700	4800	0.158

[1] Ultimate Biodegradability = Feed VSS - NBVSS.

[2] Decay rate coefficients, estimated in terms of BVSS.

6.5 Active mass as process variable

The main objectives of this study were to determine the best measure of microbial activity and its relation to the active mass content of the sludge. In the previous sections, the expression of activity as active fraction of the organic content of the sludge (F_A) was established. This factor allows the determination of active mass (M_A) by means of the equation :

$$M_A = F_A \ (VSS) \qquad (6.2)$$

where M_A = active mass in mixed liquour at time, t

F_A = active fraction of mixed liquour VSS at time, t

VSS = volatile suspended solids concentration at time,t

The use of active mass as a process variable offers many advantages over the traditional use of VSS in that active mass responds more rapidly to loading variations, shifts in metabolic activity and/or toxic conditions, allowing a better control of the process. In terms of design, activity studies have already been proven useful in this research, as they indicated: i) the possibility of solubilization of large amounts of organic matter with no subsequent degradation by the remaining organisms, and ii) the difference in metabolic activity between batch and flow-through patterns; both points have direct implications in design.

Unfortunately, due to the extent of the topic, not enough data was gathered for a formal development and presentation of a mathematical model of the process. Further research is required for such a development. Nevertheless, several observations are

presented below and may prove to be useful in future investigations.

Examination of active mass variations in the batch units indicates that the decrease in M_A may be approximated by first order kinetics as follows :

$$\frac{dM_A}{dt} = -K M_A \qquad (6.3)$$

which from a mass balance around a batch reactor gives :

$$\frac{M_{Ae}}{M_{A\,o}} = e^{-Kt} \qquad (6.4)$$

where M_{Ae} = active mass in reactor at time, t (mg/l)

M_{Ao} = initial active mass concentration at time, t (mg/l)

K = batch reactor rate coefficient for active mass, d^{-1}.

Figure 6.13 shows the variation in active mass with time for the three temperatures investigated. Correlation coefficients and decay rate constants are tabulated in Figure 6.14. All three correlations were found to be significant at the 1 % level. Active mass at any time of digestion was determined from Equation 6.2.

In the semi-continuous units however , the use of first order kinetics did not give significant correlations at the 1 % level. The decay and correlation coefficients obtained are tabulated in Figure 6.15. For the reactors run at 20°C and 30°C, the correlations were significant at the 5 % level, but this is not the case for the 10°C run. Comparison of percent mass reductions illustrate the poor fits obtained, particularly for the first 10 days of detention time.

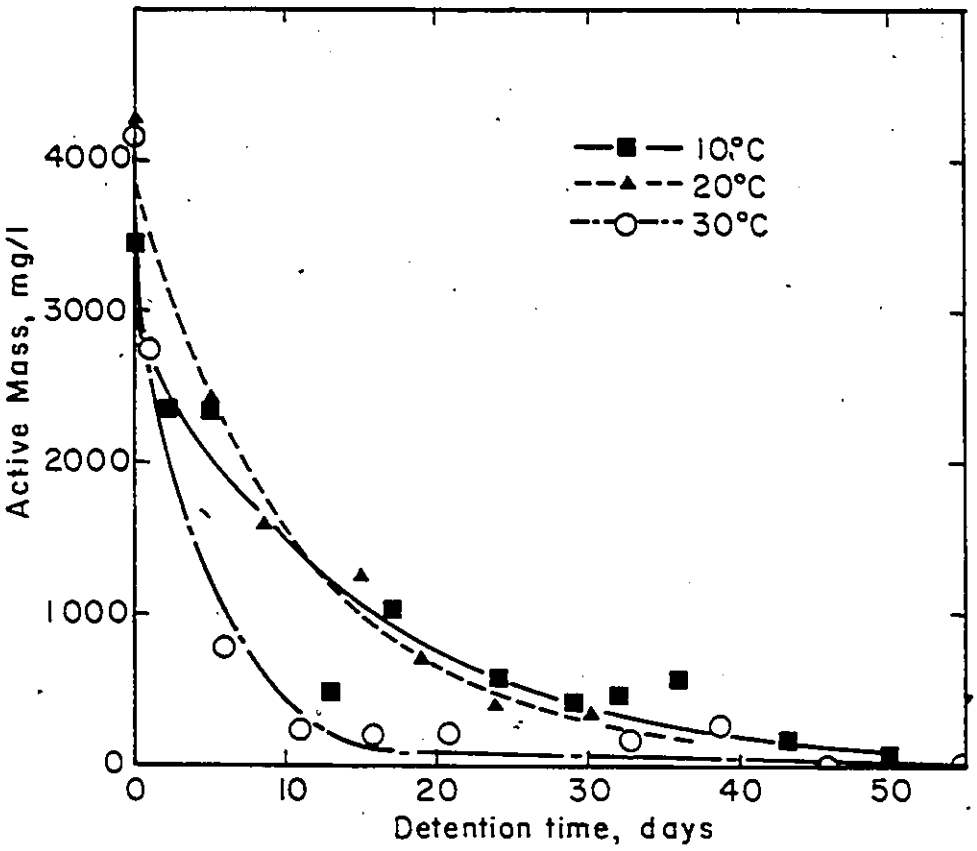

Fig. 6.13 Active Mass Variations in Batch Units

[1]Fig. 6.14 Active Mass Regression Analysis in Batch Units.

Temp. (°C)	Initial Value (M_o) (mg/l)	Rate Constant K (d^{-1})	Correlation Coefficient r
10	2,900	0.069	0.917
20	3,780	0.087	0.981
30	3,310	0.197	0.973

[1]Detailed calculations are shown in Appendix C.

[1]Fig. 6.15 Active Mass Regression Analysis in Semi-Continuous Units.

Temp. (°C)	Rate Constant K (d^{-1})	Correlation Coefficient	Detention Time (days)	Mass Reduction Observed (%)	Mass Reduction Calculated (%)
			0	0	0
10	0.0195	0.887	10	28	16
			20	39	28
			30	36	36
			0	0	0
20	0.0537	0.981	10	46	35
			20	55	52
			30	62	62
			0	0	0
30	0.0357	0.969	10	33	26
			20	37	42
			30	54	52

[1]Detailed calculations are shown in Appendix C.

Investigation of higher order kinetics or variations in decay rate with time of digestion is not justified at present due to the limited amount of data gathered. Such an investigation would require the monitoring of the digesters at more detention times, in particular during the first 15 days of digestion since most of the solids reduction occurs in this time period.

A last point to consider is the difference in active mass variations between the two flow patterns. This difference raises important questions regarding the portion of the organic volatile solids that can be biodegraded and the degree of stabilization achieved in the reactors.

In this study, the maximum amount of biodegradable material at each operating temperature was estimated as the difference between the initial VSS content and the residual volatile mass in the batch reactors at the end of extended periods of digestion (all in excess of 30 days). This is the usual procedure recommended in the literature (1, 62). At these stages the active mass content of the mixed liquour was minimal (Figure 6.13) and thus no further biodegradation was possible. On the other hand, high concentrations of ATP and consequently of active mass were observed in the semi-continuous units even at thirty days detention time; and as previously remarked, reductions in volatile solids in these units were very similar to those of the batch units, and were approaching the maximum biodegradable content of the sludge. This may be illustrated in Figure 6.16 which compares activity and organic solids reductions after 30 days of digestion for batch and semi-continuous units.

Fig. 6.16 Batch and Semi-Continuous Reactor Performance at 30 days Detention Time.

Temp.	Ultimate Biodegradable Mass	Batch Units		Semi-Continuous Units	
		Active Mass Reduction	VSS Reduction	Active Mass Reduction	VSS Reduction
(°C)	(%)	(%)	(%)	(%)	(%)
10	41	88	33	36	28
20	65	92	65	62	56
30	64	95	60	54	53

The smaller reductions of active mass in the semi-continuous reactors indicate a priori, that further biodegradation of organic matter could be achieved if the reactors' effluent was batch digested once steady state was reached. This point requires experimental verification, and its clarification may prove to be useful in the overall understanding of the process, since it would indicate whether or not microbial populations in flow-through units become better adapted to their environment and consequently are able to maintain higher levels of activity. Also, the possibility of further degradation of organics and the high active mass content observed after long detention times have important implications in the definitions of non-biodegradable content and degree of stabilization of a sludge under specific operating conditions.

CHAPTER 7

CONCLUSIONS

Based on specific objectives and analysis of the data collected, the following conclusions can be made :

(1) The floc nature of the sludge, high cell concentrations and background filter interference for small bacteria, restrict the use of the epifluorescence microscopy technique in the monitoring of activity of activated sludges.

(2) Plate counts do not offer a reliable estimation of activity in a mixed microbial population. Viable cells were found to account for only a small percentage of either respiring or total cell counts for all conditions tested.

(3) Changes in environmental conditions and/or changes in predominant species of the mixed liquour can result in different yields of ATP per oxygen atom reduced in the bacterial population, explaining then the different variations of specific ATP and DOUR measures.

(4) The close interrelation between ATP generation and cell synthesis makes ATP a better index of activity among the four measures examined, in a mixed microbial population.

(5) Cell lysis can play a significant role in the reduction of organic volatile solids during aerobic digestion, and therefore its possible interference in the determination of kinetic coefficients should be recognized.

(6) The ATP content per active biodegradable cell mass was

found to be constant with digestion time for a specific waste and set of operating conditions during aerobic batch digestion. Temperature did not seem to have a significant effect on this ATP pool. Values of 0.9 to 1.3 µg ATP/mg active cell mass were obtained for temperatures between 10 and 30°C. These facts permit the estimation of the active fraction of the sludge (F_A) by the ratio of the specific ATP content to the content per active cell mass.

(7) Studies on the variation of F_A with detention time revealed essentially different metabolic states between batch and semi-continuous flow regimes.

(8) Decay rate coefficients determined in terms of BVSS from batch experiments cannot be used to accurately predict percent solids reductions in semi-continuous or continuous units. This is particularly true at low temperatures of operation, where batch coefficients were found to be up to 5 times smaller than the semi-continuous ones.

(9) Decay rate coefficients determined in terms of BVSS from semi-continuous data were observed to decrease with increasing temperature, in contradiction to common expectations. This behaviour can be explained by the high variations in non-degradable organic solids with temperature changes. An experimental estimation of temperature effects is recommended for design purposes.

(10) Active mass, as determined in this study, is considered to be a useful parameter for the monitoring and design of aerobic sludge digestion. Its use in the determination of the kinetic

constants would overcome the problems associated with solubilization of volatile organics and/or accumulation of non-degradable solids in flow-through reactors. However further research is required to better define its behaviour in both batch and flow-through processes.

CHAPTER 8

SUGGESTIONS FOR FUTURE WORK

Further research is required before a comprehensive mathematical formulation of the process in terms of active mass or metabolic activity is possible. From experimental observations and the experience gained from this study, several suggestions may be put forward which will help in future investigations on the topic.

Specifically :

(1) Laboratory scale batch and semi-continuous digestion experiments should be conducted to further assess the importance of various design variables such as solids detention time and organic solids loading rate.

With respect to detention time, semi-continuous units at several detention times between 0 and 15 days should be monitored since the major part of solids reductions was observed during this time interval.

Organic solids loading rate is a process variable that can significantly influence the performance of the process. Unfortunately, time and economic constraints did not allow its investigation in this study, nonetheless its consideration is deemed essential in the development of a model. Variations in loading rate from 0.4 to 4.5 Kg VSS per m^3 digester volume per day are recommended, this being the range of values encountered in the literature.

(2) Use of a laboratory scale activated sludge unit for the production of the feed sludge to the digesters is recommended. The unit should be run at the temperature of operation of the aerobic digesters. This would result in : i) more consistent characteristics of the feed sludge in terms of metabolic activity and dissolved or suspended solids concentrations, and ii) microbial populations acclimatized to the temperatures under consideration, with consequent elimination of possible adverse conditions created from abrupt temperature changes.

(3) A close monitoring of the soluble organic content of the sludge, through tests for soluble COD and TOC, is recommended. The importance of solubilization of organic solids with no further biological degradation was highlighted in this study.

(4) Examination and comparison of the non-biodegradable portion of the mixed liquour between batch and semi-continuous units is suggested. This may be carried out by batch digesting the mixed liquour content of the semi-continuous reactors once steady state conditions have been achieved, and monitoring solids variations until no microbial activity is detected. Information obtained from such experiments would be of considerable help in determining the efficiency of stabilization of the reactors and also would provide a better understanding of the overall process kinetics.

REFERENCES

1. Adams, C. E., Eckenfelder, W. W., Jr. and Stein, R. M., "Modifications to Aerobic Digestor Design", Water Research, Vol.8, 1974, pp. 213-224.

2. Afghan, B.K., " Proposed Test Method for the Extraction and Measurement of Adenosine Triphosphate (ATP) from Microorganisms in Environmental Samples",Internal Draft No 3, Canada Center for Inland Waters, Unpublished Report, 1980.

3. Ahlberg, N.R., and Boyko, B.I., " Evaluation and Design of Aerobic Digesters", Jour. Water Poll. Control Fed., Vol. 44, 1972, pp. 634-646.

4. Allen, L.A., " The Bacteriology of Activated Sludge", Jour. Hyg., Camb., Vol. 43, 1944, pp. 424.

5. American Public Health Association, " Standard Methods for the Examination of Water and Wastewater", 14th Edition, Washington, D. C., 1975.

6. Barnhart, E.L., " Application of Aerobic Digestion to Industrial Waste Treatment", Proc. 16th Ind. Waste Conf., Purdue University, Ext. Ser. 109, 1961, pp. 612-626.

7. Benefield, L.D., Lawrence, D. and Randall, C., " The Effect of Sludge Viability on Biokinetic Coefficient Evaluation", Jour. Water Poll. Control Fed., Vol. 51, 1979, pp. 187-194.

8. Benefield, L.D., and Randall, C. W., " Biological Process Design for Wastewater Treatment", Prentice-Hall, Inc., Englewood Cliffs, N.Y., 1980.

9. Benges, H. H., Jr., " Discussion ", Jour. Envir. Eng. Div.,

Proc. of Am. Soc. of Civil Eng., Vol. 102, EE2, 1976, pp. 500.

10. Brezonik, P.L., and Patterson, J.W., " Activated Sludge ATP: Effects of Environmental Stress", Jour. Sanit. Engr. Div., Am. Soc. of Civil Eng., Vol. 97, SA6, 1971, pp. 813-824.

11. Brunker, R., " Autoinjector for the Determination of Picomolar Quantities of ATP with a Liquid Scintillation Counter", Anal. Biochem., Vol. 63, 1975, pp. 418-422.

12. Bucherer, V.H., " Zurfrage der Differential Farbung Lebender und Toter Mikroorganismen mit Acridineorange nach Strugger", Z. Biol., Vol. 115, 1966, pp. 175-184.

13. Carlisle, D. B., " The Use of the ATP Assay for Rapid Assessment of Water Quality", Envir. Canada, Inland Waters Directorate, Technical Bulletin, No 109, 1979.

14. Chapelle, E.W., and Levin, G.V.,"Use of Firefly Bioluminescent Reaction for Rapid Detection and Counting of Bacteria", Biochem. Med., Vol. 2, 1968, pp. 41.

15. Chiu, S.Y., Kao, I.C., Erickson, L.E., and Fan, L.T., " ATP Pools in Activated Sludge", Jour. Wat. Poll. Control Fed., Vol. 45, No 8, 1973, pp. 1746-1758.

16. Cohen, D.B., and Fullerton, D.G., " Aerobic Stabilization on Waste Activated Sludge", U.S. Environmental Protection Agency Report, EPA-600/2-7-035, PB-246-593, 1975.

17. D'Eustachio, A.J., and Johnson, D.R., " Adenosine Triphosphate Content of Bacteria", Fed. Proc., Vol. 27, 1968, pp. 761.

18. Dias, F.F., and Bhat, J.U., " Microbial Ecology of Activated Sludge", Appl. Microbiol., Vol. 12, 1964, pp. 412.

19. Drier, D.E., " Aerobic Digestion of Solids ", Proc. 18th Ind. Waste Conf., Purdue University, Ext. Ser. 115, 1963, pp. 123.

20. Donald, E.F., Mah, R.A., and Robin, A.C., " Acridine Orange Epifluorescence Technique for Counting Bacteria in Natural Waters", Trans. Amer. Micros. Soc., Vol. 92, No 3, 1973, pp. 416-421.

21. Eikum, A. S., Carlson, D. A., and Paulsrud, B., " Aerobic Stabilization of Primary and Mixed-Primary-Chemical (Alum) Sludge", Water Research, Vol. 8, 1974, pp. 927-935.

22. Ford, D.L., Eckenfelder, W.W., and Yang, T., " Dehydrogenase Enzyme as a Parameter of Activated Sludge Activity", Proc. 21st Industrial Waste Conference, Purdue University, 1966.

23. Gaudy, A.F., and Gaudy, E.T., " Microbiology for Environmental Scientists and Engineers", McGraw-Hill Book Co., 1980.

24. Grady, C.P.L., and Lim, H.C.,"Biological Wastewater Treatment, Theory and Applications ", Pollution Engineering and Technology/12, Marcel Dekker, New York, 1980.

25. Hamilton, R.D., and Holm-Hansen, O., "Adenosine Triphosphate Content of Marine Bacteria", Limnol. and Oceanog., Vol. 12, 1967, pp. 319.

26. Hammerstedt, R. H., " An Automated Method for ATP Analysis Utilizing the Luciferin Luciferase Reaction", Anal. Biochem., Vol. 52, 1973, pp. 449-455.

27. Hegg, B.A., and Rakness, K.L., " Design Considerations for Aerobic Digesters", U.S. Environmental Protection Agency Report, SA/TSB-8, PB-255 577, 1973.

28. Heukelekian, H., and Gurbaxani, M., " Effect of Certain Physical and Chemical Agents on the Bacteria and Protozoa of Activated Sludge", Sewage Works Jour., Vol. 21, 1949, pp. 811.

29. Holm-Hansen, O., and Booth, C. R., " The Measurement of Adenosine Triphosphate in the Ocean and its Ecological Significance", Limnol. and Oceanogr., Vol. 11, 1966, pp. 510.

30. Jaworski, N., et al., " Aerobic Sludge Digestion", In Advances of Biological Waste Treatment, Manhattan College, N.Y., 1980.

31. Jones, G.L., " Bacterial Growth Kinetics : Measurement and Significance in the Activated Sludge Process", Water Research, Vol. 7, 1973, pp. 1475-1492.

32. Kao, I.C., Chiu, S.Y., Fan, L.T., and Erickson,L.E.,"ATP Pools in Pure and Mixed Cultures", Jour. Wat. Poll. Control Fed., Vol. 45, No 5, 1973, pp. 926-931.

33. Korgaonkar, K.S., and Ranade, S.S., " Evaluation of Acridine Orange Fluorescence Test in Viability Studies on Escherichia Coli ", Canad. Jour. Microbiol., Vol. 12, 1966, pp. 185-190.

34. Kountz, R.R., and Forney, C., Jr., " Metabolic Energy Balances in a Total Oxidation Activated Sludge System", Sewage and Industrial Wastes, Vol. 31, 1959, pp. 819.

35. Lawton, G.W., and Norman, J.D., " Aerobic Sludge Digestion Studies", Jour. Wat. Poll. Control Fed., Vol. 36, 1964, pp. 495.

36. Levin, G.V., Schort, J.R., and Hess, W.C., " Methodology for

Application of Adenosine Triphosphate Determination in Wastewater Treatment", Envir. Sci. Tech., Vol. 9, 1975, pp. 961-965.

37. Loehr, R.C., " Aerobic Digestion : Factors Affecting Design ", Water and Sewage Works, R-169, 112, 1965.

38. Mavinic, D.S., and Koers, D.A., "Aerobic Sludge Digestion at Cold Temperatures", Can. Jour. of Civil Eng., Vol. 4, Dec. 1977, pp. 445-454.

39. Mavinic, D.S., and Koers, D.A., "Aerobic Sludge Digestion - a suggested design procedure", Can. Jour. of Civil Eng., Vol. 8, March 1981, pp. 9-15.

40. McElroy, W.D., " The Energy Source for Bioluminescence in an Isolated System", Proc. Nat. Acad. Sci. U.S., Vol. 33, 1947, pp. 342.

41. Metcalf and Eddy, Inc., " Wastewater Engineering, Treatment / Disposal / Reuse ", McGraw-Hill Book Co., New York, 1979.

42. Morand, J. M., " Physical Factors Affecting the Size of Bacteria Floc", PhD. Thesis, University of Wisconsin, 1964.

43. Nelson, P. O., and Lawrence, A. W., " Microbial Viability Measurements and Activated Sludge Kinetics", Water Research, Vol. 14, 1980, pp. 217-225.

44. Patterson, J.W., Brezonik, P.L., and Putnam, H.D., " Sludge Activity Parameters and their Application to Toxicity Measurements in Activated Sludge", Proc. 24th Ind. Waste Conf., Purdue University, Ext. Ser. 135, 1969, pp. 127.

45. Patterson, J.W., Brezonik, P.L., and Putnam, H.D.,"Measurement and Significance of Adenosine Triphosphate in Activated

Sludge", Environmental Science and Technology, ESTHA, Vol. 4, 1970, pp. 569-575.

46. Picciolo, G.L., Thomas, K.R., Deming, J.W. and Chapelle, E.W., " Environmental Applications of the Firefly Luciferase ATP Assay: Flow Techniques for Monitoring of Wastewater Effluent and Drinking Water Supplies", In Proc. of 2nd Bi-Annual ATP Methodology Symposium, San Diego, Calif., 1977, pp. 597-567.

47. Prakasam, T.B.S., and Dondero, N.C., " Aerobic Heterotrophic Bacterial Population of Sewage and Activated Sludge - 1. Enumeration", Appl. Microbiol., Vol. 15, 1967, pp. 461.

48. Randall, C. W., Saunders, F. M., and King, P. H., " Aerobic Digestion of Trickling Filter Humus", Proc. 4th Annual Environmental Eng. and Science Conf., University of Louisville, Ky., 1974.

49. Randall, C.W., Gardner, R.A., and King, P.H., " The Aerobic Digestion of Activated Sludge at Elevated Temperatures", Fifth Annual Environmental Engineering and Science Conference, University of Louisville, Louisville, Kentucky, 1975.

50. Randall, C.W., Richards, J. B., and King, P. H., " Temperature Effects on Aerobic Digestion Kinetics", Jour. of the Environ. Engr. Div., ASCE, EE5, Oct. 1975, pp. 795-811.

51. Reynolds, T.D., "Aerobic Digestion of Waste Activated Sludge", Water and Sewage Works, Vol. 114, No 2, 1967, pp. 37.

52. Ritter, L., " Design and Operation Experiences Using Diffused Aeration for Sludge Digestion", Jour. Wat. Poll.

Control Fed., Vol. 42, 1970, pp. 1782.

53. Roe, P.C., " ATP as a Biokinetic and Settling Parameter in the Operation of an Activated Sludge Process", M.S. Thesis, Washington State University, USA, 1979.

54. Schram, E., "Use of Scintillation Counters for Bioluminescence Assay of Adenosine Triphosphate (ATP)", In : The Current Status of Liquid Scintillation Counting, Edited by E.D. Bransome Jr., Gruve and Straton, N.Y., 1970, pp. 129-133.

55. Seitz, W. R., and, Neary, M. P., " Chemiluminescence and Bioluminescence in Chemical Analysis", Anal. Chem., Vol. 46, 1974, pp. 118A-202A.

56. Stanier, R.Y., Doudoroff, M., and Adelberg,E.A.,"The Microbial World", Prentice Hall, New Jersey, 1963.

57. Stanley, P. E., and Williams, S. G., " Use of the Liquid Scintillation Spectrometer for Determining Adenosine Triphosphate by the Luciferase Enzyme", Anal. Biochem., Vol.29, 1969, pp. 381.

58. Stein, R.M., et al., " A Study of Aerobic Sludge Digestion Comparing Pure Oxygen and Air", Proc. 27th Ind. Waste Conf., Purdue University, Ext. Ser. 141, 1972, pp. 492.

59. Strugger, S., " Fluorescence Microscope Examination of Bacteria in Soil", Canad. Jour. Res., Vol. 26, 1948, pp. 188-193.

60. Tal., E., Dickstein, S., Shuman, F.G., "ATP Determination with the Tricrab Scintillation Counter", Experientia, Vol. 20, 1964, pp. 652.

61. Tobin, R.S., Ryan, J.F., and Afghan, B.K., "An Improved Method for the Determination of Adenosine Triphosphate in Environmental Samples", Water Research, Vol. 12, 1978, pp. 783-792.

62. Upadhyaya, A., and Eckenfelder, W.W., "Biodegradable Fraction as an Activity Parameter of Activated Sludge", Water Research, Vol. 9, 1975, pp. 691-694.

63. Walker, I., and Davies, M., " The Relationship between Viability and Respiration Rate in the Activated Sludge Process", Water Research, Vol. 11, pp. 575-578.

64. Washington, D.R., " Activity of High Solids Mixed Cultures ", Proc. 152nd National Meeting Amer. Chemical Soc., Division Microbiol. Chem. and Technol., N.Y., 1966.

65. Weddle, C.L., and Jenkins, D., "The Viability and Activity of Activated Sludge", Water Research, Vol. 5, 1971, pp. 621-640.

66. Wooldrige, W. K., and Standfast, A. F. B., " The Biochemical Oxygen Demand of Sewage", Biochem. Jour., Vol. 27, 1933, pp. 183.

67. WPCF-ASCE, " Wastewater Treatment Plant Design ", Manual of Practice/8, Lancaster press, Inc., Lancaster, Pa., 1977.

68. Zimmermann, R.,Iturriaga, R.,and Becker-Brik, J.,"Simultaneous Determination of the Total Number of Aquatic Bacteria and the Number Thereof Involved in Respiration ", Appl. and Env. Microbiol., Vol. 36, No 8, 1978, pp. 926-935.

APPENDIX A

KINETIC MODEL DERIVATIONS

A.1 Adams et al. model

Refer to figure below :

Fig. A.1 Complete-Mix Aeration Basin

where Q = Volumetric flow rate, (L^3T^{-1})

V = Reactor volume, (L^3)

$BVSS_o$ = Biodegradable suspended solids concentration in influent, $(M\ L^{-3})$

$BVSS_e$ = Biodegradable suspended solids concentration in effluent, $(M\ L^{-3})$.

i) In the case of a batch reactor, the rate of biomass loss through biological activity is assumed to be first order :

$$d(BVSS) / dt = -K(BVSS) \qquad (A.1)$$

where K = Reaction rate coefficient, (T^{-1})

t = Detention time, (T^{-1}).

Integration of above equation between limits gives :

$$\int_{BVSS_o}^{BVSS_e} d(BVSS)/(BVSS) = -K \int_0^t t\, dt \qquad (A.2)$$

$$\ln (BVSS_e / BVSS_o) = -K\, t \qquad (A.3)$$

or

$$BVSS_e / BVSS_o = e^{-Kt} \qquad (A.4)$$

The biodegradable fraction of VSS is determined from the equation:

$$BVSS_i = VSS_i - NBVSS \qquad (A.5)$$

where VSS = total volatile suspended solids in aeration basin at any time, t=i, ($M\ L^3$)

NBVSS = non-biodegradable VSS content of sludge; value is equated to the residual VSS obtained in batch reactors at end of run, and assumed constant throughout the digestion process, ($M\ L^3$).

Substituting the above equation into Equation A.4 and solving for VSS_e gives :

$$VSS_e = e^{-Kt} + (1-e^{-Kt})\ NBVSS \qquad (A.6)$$

ii) In the case of flow-through reactors with no recycle, a mass balance arround the reactor gives :

IN - OUT + GENERATION = ACCUMULATION

or in terms of the variables presented in figure A.1 :

$$Q(BVSS_o) - Q(BVSS_e) - V(dBVSS/dt)_R = V(dBVSS/dt) \qquad (A.7)$$

where (dBVSS/dt) = Rate of net biomass loss with time

$(dBVSS/dt)_R$ = Rate of biomass loss through biological activity.

With a first order rate assumption for $(dBVSS/dt)_R$, and under steady state conditions equation (A.7) becomes :

$$Q(BVSS_o) - Q(BVSS_e) - K(BVSS_e)V = 0 \qquad (A.8)$$

Letting $t = V/Q$ and solving for $BVSS_e/BVSS_o$ gives :

$$\frac{BVSS_e}{BVSS_o} = \frac{1}{1 + Kt} \qquad (A.9)$$

or from equation (A.5) :

$$VSS_e = \frac{VSS_o + Kt(NBVSS)}{1 + Kt} \qquad (A.10)$$

A.2 Benefield and Randall's Model

Assumptions :

i) Total suspended solids are composed of an active and an inactive fraction.

ii) Inactive fraction of influent TSS is non-degradable (i.e., not oxidized nor solubilized).

iii) Active fraction of influent TSS is composed of non-degradable and degradable fractions, where degradable describes material that can be oxidized or solubilized through microbial activity.

iv) Only the degradable active fraction of TSS decreases during digestion.

TSS breakdown and changes during the process are presented in Figure A.2.

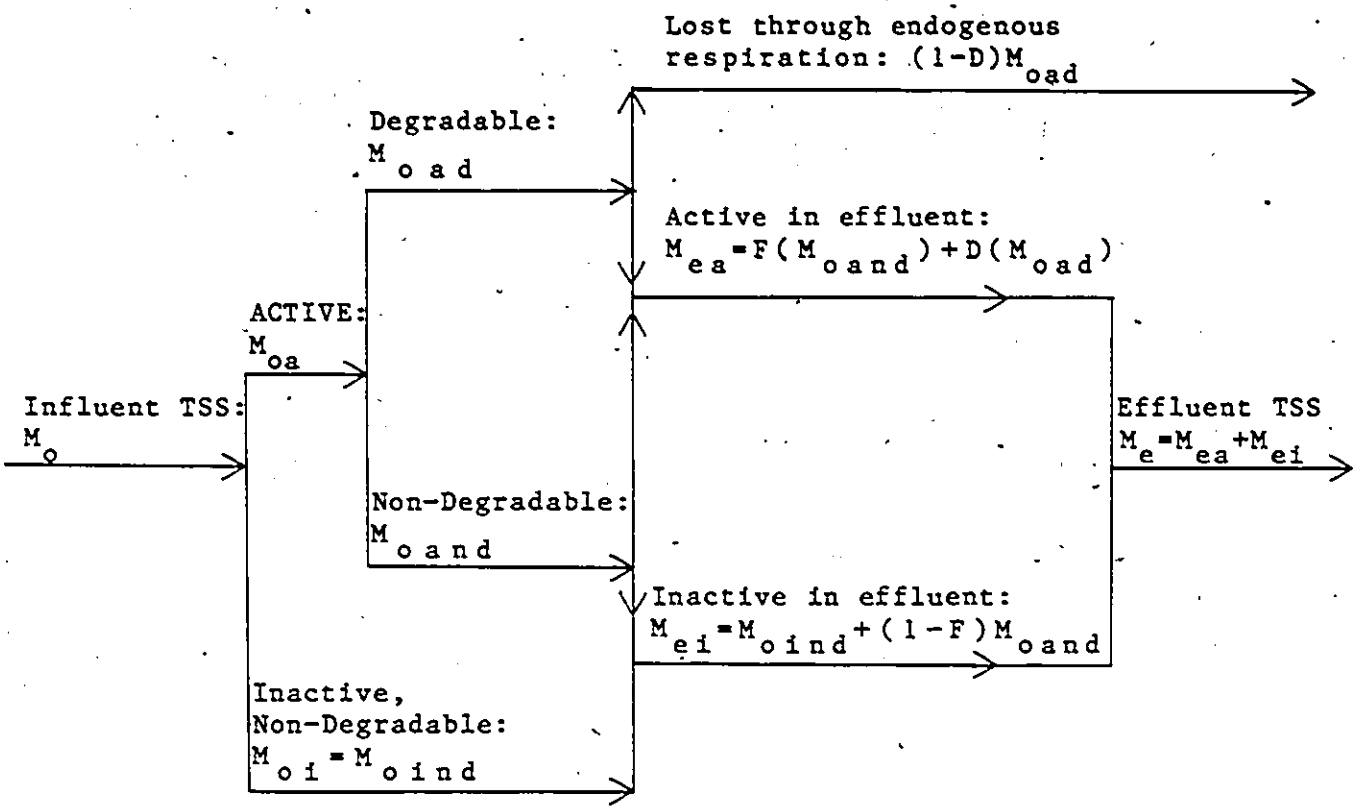

Figure A.2 Changes in TSS during aerobic digestion

The constants in the figure above are defined as follows :

F = fraction of non-degradable active mass that remains active

1-F = fraction of non-degradable active mass converted to inactive mass

D = fraction of degradable active mass in influent that appears in effluent

1-D = fraction of degradable active mass that is lost through endogenous respiration.

The model is based on the assumption that the loss of active degradable mass through endogenous respiration follows a first order relation :

$$(dM/dt)_R = -K_e(D\ M_{oad}) \qquad (A.11)$$

where K_e = decay rate of degradable active biomass approximated from the decrease in TSS, d^{-1}

$D\ M_{oad}$ = degradable active biomass in effluent or reactor, mg/l.

A mass balance arround a flow-through complete-mix reactor with no recycle gives :

IN - OUT + GENERATION = ACCUMULATION

In terms of active degradable biomass :

$$Q(M_{oad}) - Q(D\ M_{oad}) + V(dM/dt)_R = V(dM/dt) \qquad (A.12)$$

For steady state conditions and substituting Equation A.11 into A.12 :

$$Q(M_{oad}) - Q(D\ M_{oad}) - K_e(D\ M_{oad})V = 0$$

Dividing by Q and letting $t_d = V/Q$ gives :

$$M_{oad} - (D\ M_{oad}) - K_e t_d(D\ M_{oad}) = 0$$

or in terms of t_d :

$$t_d = \frac{(1-D)}{D} \frac{1}{K_e} \qquad (A.13)$$

In order to obtain a relation between mass and retention time, the following steps are necessary :

From Figure A.3 it can be seen that :

$$M_e = M_{ea} + M_{ei} \qquad (A.14)$$

$$M_{ea} = F(M_{oand}) + D(M_{oad}) \qquad (A.15)$$

$$M_{ei} = M_{oind} + (1-F)M_{oand} \qquad (A.16)$$

Therefore,

$$M_e = (F\ M_{oand} + D\ M_{oad}) + (M_{oind} + (1-F)M_{oand})$$

$$M_e = D(M_{oad}) + M_{oind} + M_{oand} \qquad (A.17)$$

Also from the figure :

$$M_{oa} = M_{oad} + M_{oand} \qquad (A.18)$$

Solving for M_{oand} and substituting into equation A.17 :

$$M_e = D(M_{oad}) + M_{oind} + (M_{oa}-M_{oad}) \qquad (A.19)$$

From the figure :

$$M_o = M_{oa} + M_{oind} \qquad (A.20)$$

Therefore substituting into Equation A.19 gives :

$$M_e = D(M_{oad}) + M_o - M_{oad} \qquad (A.21)$$

Factoring M_{oad} gives :

$$M_e = M_o + (D-1)M_{oad}$$

Solving for (1-D) :

$$1-D = (M_o-M_e)/M_{oad}$$

Substituting into equation (A.13) gives finally :

$$t_d = \frac{M_o-M_e}{D(M_{oad})K_e} \qquad (A.22)$$

From the definition of D, the value of $D(M_{oad})$ represents

the degradable active mass in the effluent, or:

$$M_{ead} = D(M_{oad}) \qquad (A.23)$$

Therefore :

$$D = M_{ead}/M_{oad} \qquad (A.24)$$

Substituting D into equation (A.13) and solving for M_{ead}/M_{oad} gives :

$$1-D = K_e t_d D$$

or,

$$D = \frac{1}{1 + K_e t_d}$$

or,

$$\frac{M_{ead}}{M_{oad}} = \frac{1}{1 + K_e t_d} \qquad (A.25)$$

APPENDIX B

SELECTED LABORATORY PROCEDURES

B.1 ATP Extraction Procedure

Boiling Glycine Technique :

1. Transfer 5 ml of extraction solution (glycine-Mg EDTA solution) to a clean vial and loosely secure the cap.
2. Prior to the introduction of the sample, preheat the extractant for 15 minutes in a heating block, maintained at 110-115°C.
3. Into the preheated extraction solution, pipette 0.5 ml of sludge sample.
4. Quickly replace the cap loosely on the vial.
5. Begin timing of extraction. Swirl vials occasionally to facilitate mixing.
6. Heat for 5 minutes, maintaining the block temperature between 110° and 115°C.
7. Remove vial from heating block and cool the extract to room temperature by placing vial at 4°C for 10-20 minutes.
8. Transfer extract to centrifuge tube and centrifuge at 1500 to 2000 xg for 10 min.
9. Carefully decant the supernatant into a suitable size stoppered tube and record volume.
10. Store extract at 4°C until measurements of ATP are performed.

B.2 ATP Measurement Procedure :

Use of Liquid Scintillation Counter :

1. Prepare standard solutions for scintillation counter

   i) Prepare stock ATP solution (1.0 mg/ml) from Adenosine-5'-Triphosphate disodium salt.

   ii) Prepare standard solutions of ATP at concentrations of 25, 50, 100, 150, 200, and 250 µg/l by dilution of stock ATP solution with extraction solution(glycine-Mg EDTA solution).

2. Prepare Luciferin-Luciferase Enzyme reagent half an hour prior to testing.

   i) Dissolve one buffer salt tablet from reagent kit (Dupont No. 760 145-902) in 3 ml of freshly deionized distilled water.

   ii) Add entire contents of one vial of enzyme powder and mix gently (do not shake).

   iii) Allow to stand 15 to 20 minutes at ambient temperature.

3. Prepare liquid scintillation counter (Beckman, model LS-3133P) for measurements:

   i) Set gain at 53.

   ii) For channel C, set window setting at 50-1000.

   iii) Set preset error to 0.2 %.

   iv) Switch to 6 sec repeat count mode.

   v) Activate channel C.

4. Perform ATP measurements as follows :

   i) Pipette 100 µl of buffered luciferin-luciferase reaction mixture into modified scintillation vial with a micropipetting sampler system (Oxford P-7000 Adjustable Sampler).

   ii) Inject 10 µl of sample into vial contents by means of a micropipetting sampler system (Oxford P-7000 10 µl Sampler), and concurrently start stopwatch.

   iii) Place scintillation vial in sample position over the elevator of scintillation counter. Lower elevator and start automatic counting sequence.

   iv) Monitor stopwatch and record elapsed time until end of first

6 sec counting interval as indicated by optical counting timer in scintillation counter.

v) Allow at least nine data printout sequences before raising sample elevator and removing vial from scintillation counter.

vi) Run duplicates for standards, blanks and reactor samples.

B.3 DOUR

1. Calibrate DO probe by means of Winkler method (part 422B of "Standard Methods") prior to testing.

2. Calibrate recorder (Perkin-Elmer, Colman 165) :

   i) Adjust DO scale to 1 cm per mg/l dissolved oxygen with a variable resistor placed in series between oxygen meter and recorder.

   ii) Set paper feed rate to 10 mm/min and verify rate with stopwatch.

   iii) Put recorder in stand by mode.

3. Fill 60 ml bottle with sludge to be analysed, insert BOD probe as quickly as possible, switch on stirrer, switch meter to temperature mode and record temperature.

4. Switch meter to DO measurement mode, switch recorder to chart mode and record DO variations until concentrations reach 1 mg/l or time exceeds 10 minutes.

5. Switch chart to stand by mode, switch meter to temperature mode and record temperature.

6. Rinse DO probe thouroughly with distilled water.

7. Perform duplicate measurements for each reactor.

8. Determine VSS concentration in reactor by gravimetric procedure.

9. Estimate DOUR in mg O_2/l/min from slope of line obtained with recorder.

10. Express DOUR in terms of mg O_2/g/hr with the following equation :

$$\text{Specific DOUR}(\text{mg } O_2/\text{g/hr}) = \frac{\text{Slope of Curve}(\text{mg}O_2/\text{l/min}) \times 60(\text{min/hr})}{\text{VSS}(\text{mg/l}) \times 10^{-3}(\text{mg/g})}$$

B.4 AOE-Dehydrogenase Activity Procedure

1. Reagents :

Phosphate buffer (6.6 mM, pH=6.7): Dissolve required amount of KH_2PO_4 in double deionized distilled water and set to pH 6.7 with 2.5 M NaOH.

Acridine Orange Solution (1:10 000): Dissolve required amount of Acridine Orange (BDH Chemicals Ltd.) in phosphate buffer, filter through 0.22 µm pore size filters (Millipore Corp.) and store refigerated at 4°C.

0.2 % INT aquous solution : Dissolve required amount of p-IODONITROTETRAZOLIUM VIOLET (Sigma Chemical Co.) in double deionized distilled water, filter through 0.22 µm pore size filters and store refrigerated at 4°C.

Sudan Black B (1:15 000) : Dissolve required amount of Sudan Black B (Harleco div.) in absolute ethanol, dilute to required concentration with double deionized distilled water, filter through 0.22 µm pore size filters and store refrigerated at 4°C.

Dilution Water for plating (refer to section 907 of "Standard Methods").

37 % Formaldehyde solution.

2. Processing of Samples

i) Obtain 10^{-6} dilution from serial dilutions used in plating.

ii) Pour 10 ml of diluted sample into small sterilized test tubes.

iii) Add 1 or 2 drops of 5 % Na_2SO_3 to drive off excess oxygen.

iv) Add 1 ml of 0.2 % INT solution into test tube and mix carefully.

v) Store for 30 minutes in the dark at in situ temperature.

vi) Stop reaction by adding 0.1 ml of 37 % Formaldehyde and mixing.

vii) Store at 4°C until microscopic examination is performed.

3. Microscopic preparation

i) Stain 0.1 µm polycarbonate filters (Nucleopore Corp.) by immersing in Sudan Black B solution for 24 hours. Prior to

utilization, rinse filters thouroughly with distilled water.

ii) Filter 2-5 ml of processed samples (depending on total concentrations of microorganisms in samples) through stained filters. Filters should be supported by drain discs (Nucleopore Corp.) and care should be taken into avoiding gas bubbles.

iii) After filtration, pipette 1 ml of Acridine Orange solution on the filter and leave for 2 minutes contact time.

iv) Remove Acridine Orange solution by filtration.

v) Remove filters from filtration apparatus and perform microscopic examination.

4. Microscopic Examination

Apparatus :

Microscope : Zeiss Universal
Lamp : HBO 200 W
Exitation filters : KP490 AND KP500
Dichroic beam-splitting filter 510
Barrier filter : LP 520
Adsorption filter : Zeiss VG9 (for bright field illumination)
Oil immersion objective : Planchromat 100x

Examination

i) Mount each filter with a drop of immersion oil (Cargille, Type A) on a microscopic slide and cover with cover slip. Do not store embeded filter wedges for more than 2 hrs.

ii) Examine slides by simultaneous (mixed light) or by alternative epifluorescence and transmitted bright field illumination. For the latter case mask diaphragms of bright field and incident light paths alternatively with small object.

iii) Estimate respiring and total cell concentrations in reactor from equation :

$$(\text{Counts/ml of mixed liquour}) = ACF \times AR \times DR \times VR \quad (B.2)$$

where ACF = Average Count per Field

AR = (filter area)/(field area)

DR = (dilution factor)/(filtered volume)

$$VR = \frac{11\ \text{ml(volume of sample + INT solution)}}{10\ \text{ml(sample volume)}}$$

v) Estimate percent of active cells in sample from the equation :

$$\% \text{ Activity } (\%) = \frac{\text{respiring cell count (counts/ml)}}{\text{total cell count (counts/ml)}} \times 100$$

where respiring cell counts and total cell counts are as estimated from Equation B.1.

APPENDIX C

REGRESSION ANALYSIS

C.1 Fitting of ATP and BVSS vs Time curves (Fig. 6.1-6.3)

Least squares approximation with polynomial of 3th degree :

$$Y = aX^3 + bX^2 + cX + d \qquad (C.1)$$

where X = time, days

Y = ATP, µg/l ; or BVSS, mg/l.

Fig. C.1 [1] ATP, BVSS vs Time Curves, Regression Parameters for Batch Reactors.

Temp. (°C)	Para.	a	b	c	d	[2] C.C.	[3] D.C.	[4] S.E.
10°C	ATP	-0.1171	10.955	-333.243	3824.	0.972	0.946	369.
	BVSS	-0.0844	7.942	-253.217	3294.	0.989	0.978	198.
20°C	ATP	-0.2872	20.282	-515.360	5320.	0.993	0.988	279.
	BVSS	-0.3601	23.616	-512.950	4679.	0.993	0.986	261.
30°C	ATP	-2.459	84.287	-944.289	3786.	0.994	0.988	370.
	BVSS	-3.607	112.991	-1121.368	4397.	0.979	0.959	710.

[1] Curves fitted for first 16 days of digestion since only small variations occurred after this time.

[2] C.C. = Correlation Coefficient.

[3] D.C. = Determination Coefficient.

[4] S.E. = Standard Error, µg/l.

NOTE : S.E. in this appendix refers to the standard deviation of the correlation, i.e. 68 % of estimated values will lie within ± one standard error of the regression curve. S.E. has therefore the same units as the ordinate of the regression equation.

Fig. C.2 ATP, BVSS vs Time Curves, Regression Data for Batch Reactors.

Temp. (°C)	Detention Time (d)	ATP Conc. in Reactor (µg/l)	BVSS Conc. Corrected for lysis (mg/l)
10	0	4050	3080
10	2	2750	3000
10	5	2800	2350
10	13	520	1240
10	17	1140	580
10	24	640	610
10	29	430	570
10	32	530	710
10	36	670	660
10	43	250	170
10	50	0	0
20	0	5410	4900
20	2	--	3430
20	5	3070	—
20	6	--	2385
20	8	2230	—
20	13	--	1405
20	15	1550	—
20	19	820	--
20	20	--	850
20	24	450	--
20	26	--	985
20	30	440	845
30	0	3980	4740
30	1	2650	2860
30	6	760	1220
30	11	250	790
30	16	200	640
30	21	200	720
30	33	220	15
30	39	270	610
30	46	60	240
30	55	140	440

C.2 Fitting of ATP vs BVSS curves (Fig. 6.5-6.7)

Least squares approximation with polynomial of first degree :

$$Y = aX + b \qquad (C.2)$$

where X = BVSS, mg/l

Y = ATP, µg/l

Fig. C.3 ATP vs BVSS curves, Regression Parameters for Batch Reactors.

Temp. (°C)	a	b	[1]C.C.	[2]D.C.	[3]S.E.	[4]V	[5]R_c
10	1.17	-133.1	0.997	0.994	100.	9	0.735
20	1.26	-362.1	0.990	0.980	274.	5	0.874
30	0.93	-325.7	0.998	0.996	93.	9	0.765

[1] C.C. = Correlation Coefficient.

[2] D.C. = Determination Coefficient.

[3] S.E. = Standard Error, µg/l.

[4] V = Degrees of freedom.

[5] R_c = Critical Correlation Coefficient at 1 % level of significance.

Notes :

1. If C.C. $> R_c$, then it can be concluded that correlation exists and that the level of significance represents the probability of having drawn the wrong conclusion.

2. Critical correlation coefficients were obtained from table A.11 of J.B. Kennedy and A.M. Neville, "Basic Statistical Methods for Engineers and Scientists", 2nd edition, Harper and Row, N.Y., 1976.

Fig. C.4 ATP vs BVSS Curves, Regression Data for Batch Reactors.

Temp. (°C)	Detention Time	ATP (µg/l)	BVSS (mg/l)
10	0	3820	3290
10	2	3200	2820
10	5	2420	2220
10	13	1090	1170
10	17	750	880
10	24	520	620
10	29	520	560
10	32	540	550
10	36	540	530
10	43	440	400
10	50	0	0
20	0	5320	4680
20	5	3210	2660
20	8	2350	1900
20	15	1180	1080
20	19	880	990
20	24	660	990
20	30	360	820
30	0	3790	4440
30	1	2920	3390
30	2.5	2000	2500
30	6	620	960
30	11	320	800
30	16	230	715
30	21	220	670
30	33	190	560
30	39	170	500
30	46	160	440
30	55	130	360

Note : Above values were obtained from lines of best fit in section C.1.

C.3 Fitting of F_A vs Time Curves (Fig. 6.8)

Least squares method, linearized equation of the form :

$$- \ln (F_A/F_{Ao}) = Kt + b \qquad (C.3)$$

where F_A = Active fraction of sludge at any time, t

F_{Ao} = Initial active fraction

t = Detention time, d

K = Decay rate coefficient, d^{-1}.

Fig. C.5 F_A vs Time Curves, Regression Parameters for Batch Reactors.

Temp. (°C)	K (d^{-1})	b	[1] F_o	[2] C.C.	[3] D.C.	[4] S.E.	[5] V	[6] R_c
10	0.058	0.110	0.41	0.892	0.794	0.521	9	0.735
20	0.057	-0.166	0.67	0.940	0.882	0.239	5	0.874
[7] 30	0.151	0.048	0.53	0.977	0.955	0.257	3	0.959

[1] Equation C.3 can be converted to :

$$F_A = F_o\, e^{-Kt} \quad \text{with} \quad F_o = F_{Ao}\, e^{-b}.$$

[2] C.C. = Correlation Coefficient.

[3] D.C. = Determination Coefficient.

[4] S.E. = Standard Error.

[5] V = Degrees of freedom.

[6] R_c = Critical correlation coefficient at 1 % level of significance (refer to notes in Fig. C.3).

[7] Values at 30°C were obtained through fitting for first 16 days only as no significant variations occurred after this day.

Fig. C.6 F_A vs Time Curve, Regression Data for Batch Reactors

Temp. (°C)	Detention time (d)	P_B	ATP/VSS	^1F_A	$-\ln F_A/F_{Ao}$
10	0	1.17	0.54	0.46	--
10	2	1.17	0.37	0.32	0.38
10	5	1.17	0.41	0.35	0.27
10	13	1.17	0.09	0.08	1.71
10	17	1.17	0.23	0.20	0.87
10	24	1.17	0.13	0.11	1.43
10	29	1.17	0.09	0.08	1.83
10	32	1.17	0.10	0.09	1.61
10	36	1.17	0.13	0.11	1.43
10	43	1.17	0.05	0.04	2.30
10	50	1.17	0.01	0.01	3.91
20	0	1.26	0.72	0.57	0.0
20	5	1.26	0.66	0.52	0.08
20	8	1.26	0.50	0.40	0.27
20	15	1.26	0.54	0.43	0.30
20	19	1.26	0.31	0.25	0.85
20	24	1.26	0.16	0.13	1.51
20	30	1.26	0.17	0.13	1.45
30	0	0.94	0.53	0.56	0.0
30	1	0.94	0.48	0.51	0.10
30	6	0.94	0.19	0.20	1.01
30	11	0.94	0.07	0.07	2.06
30	16	0.94	0.06	0.06	2.22
30	21	0.94	0.06	0.06	2.22
30	33	0.94	0.08	0.03	2.51
30	39	0.94	0.08	0.06	2.22
30	46	0.94	0.02	0.02	2.60
30	55	0.94	0.05	0.05	2.48

$$^1F_A = \frac{(ATP/VSS)}{P_B}$$

C.4 Fitting of M_A vs Time for Batch Reactors (Fig. 6.12)

Least square method, linearized equation of the form :

$$-\ln (M_A/M_{Ao}) = Kt + b \qquad (C.4)$$

where M_A = Active mass in mixed liquour, mg/l

M_{Ao} = Initial active mass, mg/l

t = Detention time, d

K = Decay rate coefficient, d^{-1}.

Fig. C.7 M_A vs Time Curves, Regression Parameters for Batch Reactors

Temp. (°C)	K (d^{-1})	b	[1] M_o (mg/l)	[2] C.C.	[3] D.C.	[4] S.E.	[5] V	[6] R_c
10	0.069	0.174	2900	0.917	0.841	0.536	9	0.735
20	0.087	0.124	3780	0.981	0.963	0.199	5	0.874
[7] 30	0.197	0.237	3310	0.973	0.947	0.197	3	0.959

[1] Equation C.4 can be converted to :

$$M_A = M_o\, e^{-Kt} \quad \text{with} \quad M_o = M_{Ao}\, e^{-b}.$$

[2] C.C. = Correlation Coefficient.

[3] D.C. = Determination Coefficient.

[4] S.E. = Standard Error.

[5] V = Degrees of freedom.

[6] R_c = Critical correlation coefficient at 1 % level of significance (refer to notes in Fig. C.3).

[7] Values at 30°C were obtained through fitting for first 16 days only as no significant variations occurred after this day.

Fig. C.8 M_A vs Time Curve, Regression Data for Batch Reactors.

Temp. (°C)	Detention Time (d)	[1] M_A (mg/l)	$-\ln(M_A/M_{Ao})$
10	0	3450	0.0
10	2	2370	0.38
10	5	2370	0.38
10	13	450	2.04
10	17	1000	1.24
10	24	550	1.84
10	29	400	2.15
10	32	460	2.01
10	36	560	1.82
10	43	180	2.95
10	50	40	4.46
20	0	4275	0.0
20	5	2410	0.57
20	8	1600	0.48
20	15	1230	1.25
20	19	670	1.84
20	24	360	2.47
20	30	350	2.50
30	0	4200	0.0
30	1	2820	0.40
30	6	790	1.67
30	11	250	2.82
30	16	200	3.04
30	21	210	3.00
30	33	250	2.82
30	39	290	2.67
30	46	55	4.34
30	55	140	3.40

[1] $M_A = F_A \times VSS$

C.5 Fitting of M_A vs Time for Semi-Continuous Reactors

Least square method, equation of the form :

$$M_{Ae}/M_{Ao} = 1/(1 + Kt) \qquad (C.5)$$

or in linearized form :

$$(M_{Ao} - M_{Ae})/M_{Ae} = Kt \qquad (C.6)$$

where M_{Ae} = Active mass in effluent or reactor at time t, mg/l

M_{Ao} = Active mass in influent, mg/l

t = Detention time, d

K = Decay rate coefficient, d^{-1}.

Fig. C.9 M_A vs Time Curves, Regression Parameters for Semi-Continuous Reactors.

Temp. (°C)	K (d^{-1})	[1]C.C.	[2]D.C.	[3]S.E.	[4]V	[5]$R_{c1\%}$	[6]$R_{c5\%}$
10	0.0195	0.887	0.786	0.161	2	0.990	0.950
20	0.0537	0.981	0.963	0.164	2	0.990	0.950
30	0.0357	0.969	0.940	0.142	2	0.990	0.950

[1] C.C. = Correlation Coefficient.

[2] D.C. = Determination Coefficient.

[3] S.E. = Standard Error.

[4] V = Degrees of freedom.

[5] $R_{c1\%}$ = Critical correlation coefficient at 1 % level of significance.

[6] $R_{c5\%}$ = Critical correlation coefficient at 5 % level of significance.

Fig. C.10 M_A vs Time Curves, Regression Data for Semi-Continuous Reactors.

Temp. (°C)	Detention Time (d)	M_{Ae} (mg/l)	$\frac{M_{Ao}-M_{Ae}}{M_{Ae}}$
10	0	3450	0.0
10	10	2480	0.39
10	20	2120	0.63
10	30	2220	0.57
20	0	4290	0.0
20	10	2330	0.84
20	20	1920	1.23
20	30	1610	1.66
30	0	4230	0.0
30	10	2820	0.50
30	20	2660	0.59
30	30	1960	1.16

C.6 Fitting of BVSS vs Time for Batch Reactors

Least squares method, linearized equation of the form :

$$-\ln (BVSS_e/BVSS_o) = Kt \qquad (C.7)$$

where $BVSS_e$ = Biodegradable volatile suspended solids in effluent, mg/l

$BVSS_o$ = Biodegradable volatile suspended solids in influent, mg/l

K = Endogenous decay rate coefficient, d^{-1}

t = Detention time, d.

Fig. C.11 BVSS vs Time, Regression Parameters for Batch Reactors.

Temp. (°C)	K (d^{-1})	$BVSS_o$ (mg/l)	[1]C.C.	[2]D.C.	[3]S.E.	[4]V	[5]R_c
10	0.056	3080	0.925	0.854	0.368	9	0.735
[6]20	0.151	4900	0.894	0.799	0.984	5	0.874
	0.057	4900	0.944	0.891	0.257	5	0.874
[7]30	0.120	4770	0.959	0.901	0.311	3	0.959

[1] C.C. = Correlation Coefficient.

[2] D.C. = Determination Coefficient.

[3] S.E. = Standard Error.

[4] V = Degrees of freedom.

[5] R_c = Critical correlation coefficient at 1 % level of significance.

[6] Lower values correspond to corrected BVSS concentrations.

[7] Values at 30°C were obtained through fitting for first 16 days only since no significant variations occurred after that time.

Fig. C.12 BVSS vs Time, Regression Data for Batch Reactors.

Temp. (°C)	Detention Time (d)	BVSS (mg/l)		$-\ln(BVSS_e/BVSS_o)$	
10	0	3080		0.0	
10	2	3000		0.026	
10	5	2350		0.271	
10	13	1240		0.910	
10	17	580		1.670	
10	24	610		1.619	
10	29	570		1.687	
10	32	710		1.467	
10	36	660		1.590	
10	43	170		2.897	
10	51	0		--	
			*		*
20	0	4900	4900	0.0	0.0
20	2	3260	3430	0.41	0.36
20	6	1730	2385	1.04	0.72
20	13	370	1405	2.58	1.25
20	20	30	850	5.10	1.75
20	26	230	985	3.06	1.60
20	30	50	845	4.58	1.76
30	0	4770		0.0	
30	1	2860		0.51	
30	6	1220		1.36	
30	11	790		1.80	
30	16	640		2.01	
30	21	720		1.89	
30	33	0		--	
30	39	510		2.24	
30	46	10		6.17	
30	55	1		8.47	

*Corrected values for cell lysis.

C.7 Fitting of BVSS vs Time for Semi-Continuous Reactors

Least squares method, equation of the form :

$$(BVSS_e/BVSS_o) = 1/(1+Kt) \qquad (C.8)$$

or in linearized form :

$$(BVSS_o-BVSS_e)/BVSS_e = Kt \qquad (C.9)$$

where $BVSS_e$ = Biodegradable volatile suspended solids in effluent, mg/l

$BVSS_o$ = Biodegradable volatile suspended solids in influent, mg/l

K = Endogenous decay rate coefficient, d^{-1}

t = Detention time, d.

Fig. C.13 BVSS vs Time, Regression Parameters for Semi-Continuous Reactors.

Temp. (°C)	K (d^{-1})	[1] C.C.	[2] D.C.	[3] S.E	[4] V	[5] R_c
10	0.267	0.995	0.991	0.413	2	0.990
20	0.187	0.996	0.993	0.253	2	0.990
30	0.158	0.998	0.994	0.200	2	0.990

[1] C.C. = Correlation Coefficient.

[2] D.C. = Determination Coefficient.

[3] S.E. = Standard Error.

[4] V = Degrees of freedom.

[5] R_c = Critical correlation coefficient at 1 % level of significance.

Fig C.14 BVSS vs Time, Regression Data for Semi-Continuous Reactors.

Temp. (°C)	Detention Time (d)	$BVSS_e$ (mg/l)	$\frac{BVSS_o - BVSS_e}{BVSS_e}$
10	0	3080	0.0
10	10	955	2.23
10	20	540	4.70
10	30	340	8.06
20	0	4900	0.0
20	10	1775	1.76
20	20	1125	3.36
20	30	730	5.71
30	0	4770	0.0
30	10	1610	1.96
30	20	1110	3.30
30	30	820	4.82

APPENDIX D

EXPERIMENTAL DATA

Fig. D.1 Solids Concentrations in Batch Reactor at 10°C.

Sludge Age (d)	TSS (mg/l)	VSS (mg/l)	FSS (mg/l)	TS (mg/l)	TDS (mg/l)
0	15030	7500	7530	—	—
2	14110	7420	6690	—	—
3	—	—	—	13320	—
5	12900	6770	6130	—	380
6	—	—	—	13260	—
13	11990	5660	6330	—	590
14	—	—	—	12480	—
17	12100	5000	7100	—	320
18	—	—	—	12400	—
24	11280	5030	6250	—	880
25	—	—	—	12120	—
29	11220	4990	6230	—	700
30	—	—	—	11870	—
32	11570	5130	6440	—	390
33	—	—	—	12000	—
36	11120	5080	6040	—	—
37	—	—	—	11570	—
43	10900	4590	6310	—	860
44	—	—	—	11790	—
51	11070	3940	7130	—	620
52	—	—	—	11670	—
61	11160	4740	6420	—	350
62	—	—	—	11440	—

NOTE : All tabulated values in this section represent averages of duplicate measurements.

Fig. D.2 Solids Concentrations in Batch Reactor at 20°C.

Sludge Age (d)	TSS (mg/l)	VSS (mg/l)	FSS (mg/l)	TS (mg/l)	TDS (mg/l)
0	11010	7500	3510	—	—
2	10190	5860	4320	—	175
3	—	—	—	10140	—
6	8290	4330	3960	—	1180
8	—	—	—	9030	—
13	6500	2970	3530	—	2100
19	—	—	—	8080	—
20	6030	2630	3400	—	2020
26	5860	2830	3030	—	—
30	6000	2650	3350	8200	2200

Fig. D.3 Solids Concentrations in Batch Reactor at 30°C.

Sludge Age (d)	TSS (mg/l)	VSS (mg/l)	FSS (mg/l)	TS (mg/l)	TDS (mg/l)
0	12730	7500	5230	—	—
1	12450	5530	6920	—	—
2	—	—	—	11370	—
6	8720	3950	4770	—	850
7	—	—	—	9120	—
11	7910	3520	4390	—	810
12	—	—	—	8620	—
16	8170	3370	4800	—	660
18	—	—	—	8870	—
21	8000	3450	4550	—	825
22	—	—	—	8810	—
32	—	—	—	8650	—
33	7750	2730	5020	—	930
39	7680	3240	4440	—	1210
40	—	—	—	8920	—
46	7410	2740	4670	—	1460
47	—	—	—	8860	—
55	7290	2730	4530	—	1450
56	—	—	—	8730	—

Fig. D.4 Activity Concentrations in Batch Reactor at 10°C.

Sludge Age (d)	ATP (µg/l)	DOUR (mg/l/hr)	Viable Count (cells/mlx10^{-8})
0	4050	48.0	—
2	2750	37.8	9.6
5	2800	24.0	7.7
13	520	11.4	10.5
17	1140	13.8	6.5
24	640	10.2	56.0
29	430	7.8	6.7
32	530	8.4	12.3
36	670	18.8	4.0
43	250	3.0	1.7
51	0	0.0	1.0
61	0	2.4	1.2

Fig. D.5 Activity Concentrations in Batch Reactor at 20°C.

Sludge Age (d)	ATP (µg/l)	DOUR (mg/l/hr)	Viable Count (cells/mlx10^{-8})
0	5410	67.0	—
2	—	55.0	—
5	3070	—	2.9
6	—	32.4	—
8	2230	—	3.2
13	—	12.6	—
15	1550	—	2.5
19	820	—	2.0
20	—	6.0	—
24	450	—	2.1
26	—	3.6	—
30	440	3.0	2.1

Fig. D.6 Activity Concentrations in Batch Reactor at 30°C.

Sludge Age (d)	ATP (µg/l)	DOUR (mg/l/hr)	Viable Count (cells/mlx10^{-8})
0	3980	60.0	—
1	2650	—	19.1
3	—	39.6	—
6	760	19.5	7.0
11	250	3.0	2.2
16	200	2.4	1.4
21	200	3.9	—
33	220	2.4	6.0
39	270	1.8	2.1
46	60	2.7	0.7
55	140	1.1	—

Fig. D.7 Specific Activities for Batch Reactor at 10°C.

Sludge Age (d)	Specifc ATP (µg/mg VSS)	Specific DOUR (mg O_2/g VSS/hr)	Specific Plate Counts (cells/mgVSSx10^{-8})
0	0.54	6.40	—
2	0.37	5.05	1.29
5	0.41	3.55	1.14
13	0.09	2.04	1.86
17	0.23	2.76	1.30
24	0.13	2.03	11.13
29	0.09	1.56	1.34
32	0.10	1.64	2.40
36	0.13	3.70	0.79
43	0.05	0.65	0.37
51	0.00	0.00	0.25
61	0.00	0.51	0.25

Fig. D.8 Specific Activities for Batch Reactor at 20°C.

Sludge Age (d)	Specifc ATP (µg/mg VSS)	Specific DOUR (mg O_2/g VSS/hr)	Specific Plate Counts (cells/mgVSSx10^{-8})
0	0.72	8.90	—
2	—	9.39	—
5	0.66	—	0.63
6	—	7.48	—
8	0.56	—	0.80
13	—	4.24	—
15	0.54	—	0.87
19	0.31	—	0.75
20	—	2.28	—
24	0.16	—	0.76
26	—	1.27	—
30	0.17	1.13	0.79

Fig. D.9 Specific Activities for Batch Reactor at 30°C.

Sludge Age (d)	Specifc ATP (µg/mg VSS)	Specific DOUR (mg O_2/g VSS/hr)	Specific Plate Counts (cells/mgVSSx10^{-8})
0	0.53	8.00	—
1	0.48	—	3.45
3	—	8.08	—
6	0.09	4.94	1.77
11	0.07	0.85	0.63
16	0.06	0.71	0.42
21	0.06	1.13	—
33	0.08	0.88	2.20
39	0.08	0.56	0.65
46	0.02	0.99	0.26
55	0.05	0.41	—

Fig. D.10 Viability and Dehydrogenase Enzyme Activity Data for Batch Reactor at 10°C.

Sludge Age (d)	Viable cells (cells/ml) $\times 10^{-8}$	Active cells (cells/ml) $\times 10^{-10}$	Total cells (cells/ml) $\times 10^{-10}$	% Activity (%)	Viable/Active cell ratio (%)	Viable/Total cell ratio (%)
2	9.6	0.6	1.9	32	16.0	5.1
5	7.7	0.8	3.3	24	9.6	2.3
13	10.5	1.6	5.7	28	6.6	1.8
17	6.5	1.5	3.7	41	4.3	1.8
24	56.0	4.9	17.3	28	11.4	3.2
29	6.7	5.4	11.0	49	1.2	0.6
32	12.3	3.3	46.2	7	3.7	0.3
36	4.0	6.1	39.6	15	0.7	0.1
43	1.7	4.7	20.2	23	0.4	0.1
51	1.0	10.1	84.7	12	0.1	0.0
61	1.2	21.0	57.1	37	0.1	0.0

Fig. D.11 Viability and Dehydrogenase Enzyme Activity Data for Batch Reactor at 20°C.

Sludge Age (d)	Viable cells (cells/ml) $\times 10^{-8}$	Active cells (cells/ml) $\times 10^{-10}$	Total cells (cells/ml) $\times 10^{-10}$	% Activity (%)	Viable/Active cell ratio (%)	Viable/Total cell ratio (%)
5	2.9	0.8	2.8	29	3.6	1.0
8	3.2	0.8	1.9	42	4.0	1.7
15	2.5	0.5	1.3	38	5.0	1.9
19	2.0	0.4	1.1	36	5.0	1.8
24	2.1	0.8	2.4	33	2.6	0.9
30	2.1	0.4	2.2	18	5.3	1.0

Fig. D.12 Viability and Dehydrogenase Enzyme Activity Data for Batch Reactor at 30°C.

Sludge Age (d)	Viable cells (cells/ml) $\times 10^{-8}$	Active cells (cells/ml) $\times 10^{-10}$	Total cells (cells/ml) $\times 10^{-10}$	% Activity (%)	Viable/Active cell ratio (%)	Viable/Total cell ratio (%)
1	19.1	7.0	13.2	53	2.7	1.5
6	7.0	5.6	15.4	36	1.3	0.5
11	2.2	1.9	14.0	14	1.2	0.2
16	1.4	—	—	—	—	—
21	—	0.7	2.8	25	—	—
33	6.0	0.2	1.2	17	30.0	5.0
39	2.1	0.2	1.2	17	10.5	1.8
46	0.7	0.3	0.7	43	2.3	1.0
55	—	0.2	1.0	20	—	—

Fig. D.13 Solids Concentrations in Semi-Continuous Reactors at 10°C, 10 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	12400	7500	4900	13090	690
4	11170	7300	3870	—	—
5	—	—	—	11750	635
9	10890	5690	5200	—	—
10	—	—	—	11475	585
12	11890	5950	5940	—	—
13	—	—	—	11560	520
20	12120	6680	5440	—	—
21	—	—	—	12060	310
24	10640	6030	4610	—	—
25	—	—	—	—	—
31	9780	5550	4230	—	—
32	—	—	—	11360	1480
36	10270	5420	4850	—	—
37	—	—	—	11200	1080
39	9810	5690	4120	—	—
40	—	—	—	11640	1770
43	10040	5540	4500	—	—
44	—	—	—	10960	900
50	9930	5050	4880	—	—
51	—	—	—	10820	905
58	9840	5000	4840	—	—
59	—	—	—	10740	905
Average :	9950	5375	4570	11120	1170
Std Dev.:	185	285	338	345	368
[2]S.S. :	9950 ± 2%	5375 ± 4%	4570 ± 6%	11120 ± 3%	1170±26%

[3]

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values at 90 % confidence level.

[3] Steady state assumed after 30 days.

Fig. D.14 Solids Concentrations in Semi-Continuous Reactors at 10°C, 20 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	12400	7500	4900	13090	620
4	10540	7210	3330	—	—
5	—	—	—	11830	1320
9	10340	5380	4960	—	—
10	—	—	—	11460	740
12	10340	5410	4930	—	—
13	—	—	—	11270	920
20	10410	5210	5200	—	—
21	—	—	—	11810	1540
24	9860	4950	4900	—	—
25	—	—	—	12000	2210
31	9340	5110	4230	—	—
32	—	—	—	11350	1950
36	9630	5380	4250	—	—
37	—	—	—	11130	1670
39	9110	5020	4080	—	—
40	—	—	—	11435	2220
43	9530	5060	4470	—	—
44	—	—	—	11130	1650
50	9180	4630	4450	—	—
51	—	—	—	10340	1190
58	8930	4550	4380	—	—
59	—	—	—	9810	800
Average :	9290	4960	4330	10870	1580
[2]Std Dev.:	264	313	173	646	514
S.S. :	9290 ± 2%	4960 ± 5%	4330 ± 3%	10870 ± 5%	1580 ± 27%

[3]

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values at 90 % confidence level.

[3] Steady state assumed after 30 days.

Fig. D.15 Solids Concentrations in Semi-Continuous Reactors at 10°C, 30 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	12400	7500	4900	13090	690
4	10050	7020	3100	--	--
5	--	--	--	10675	645
9	9950	5100	4850	--	--
10	--	--	--	10520	610
12	9840	4660	5180	--	--
13	--	--	--	10700	830
20	10090	4850	5235	--	--
21	--	--	--	11205	1210
24	9710	4380	5330	--	--
25	--	--	--	11100	1475
31	9100	4720	4380	--	--
32	--	--	--	11340	2090
36	9840	5030	4810	--	--
37	--	--	--	10860	1140
39	9470	5070	4400	--	--
40	--	--	--	11075	1625
43	9400	4840	4560	--	--
44	--	--	--	10450	1120
50	8900	4660	4240	--	--
51	--	--	--	10230	1360
58	8640	4240	4400	--	--
59	--	--	--	9970	1340
Average :	92300	4760	4470	10650	1450
Std Dev.:	432	302	197	525	365
[2]S.S. :	9230 ± 4%	4760 ± 5%	4470 ± 4%	10650±4%	1450±21%

[3]

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values at 90 % confidence level.

[3] Steady state assumed after 30 days.

Fig. D.16 Solids Concentrations in Semi-Continuous Reactors at 20°C, 10 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	15020	7500	7520	15500	480
6	11420	6130	5290	13910	2490
10	10910	4550	6410	—	—
13	10590	4570	6020	—	—
17	10910	5960	4950	12150	1240
21	11110	4770	6340	—	—
34	11070	4350	6725	—	—
37	10490	3630	6860	—	—
38	—	—	—	13280	2700
41	10840	4300	6540	—	—
43	—	—	—	12020	1240
48	10620	4680	6130	—	—
53	—	—	—	12150	1210
54	11000	4540	6460	—	—
60	11090	4710	6480	—	—
Average :	10850	4370	6530	12480	1600
Std Dev.:	249	398	251	693	850
[2]S.S. :	10850 ± 2%	4370 ± 7%	6530 ± 3%	12480 ± 9%	1600 - %

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values at 90 % confidence level.

[3] Steady state assumed after 30 days.

Fig. D.17 Solids Concentrations in Semi-Continuous Reactors at 20°C, 20 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	15020	7500	7520	15500	480
6	10990	5060	5930	12150	1160
10	11180	4870	6310	—	—
13	10230	5510	4720	—	—
17	10330	4990	5340	11850	1520
21	8050	3610	4440	—	—
34	9520	4020	5500	—	—
37	8860	3250	5610	—	—
38	—	—	—	11690	2670
41	9500	3770	5730	—	—
43	—	—	—	11370	1920
48	9330	3500	5830	—	—
53	—	—	—	11900	2225
54	9740	3660	5960	—	—
60	9790	4240	5580	—	—
Average :	9460	3740	5700	11650	2270
Std Dev.:	337	356	172	267	308
[2]S.S. :	9460 ± 3%	3740 ± 8%	5700 ± 2%	11650±4%	2270±23%

[3]

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values at 90 % confidence level.

[3] Steady state assumed after 30 days.

Fig. D.18 Solids Concentrations in Semi-Continuous Reactors at 20°C, 30 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	15020	7500	7520	15500	480
6	10540	5080	5460	11700	1160
10	11560	4540	7020	--	--
13	8960	4530	4430	--	--
17	7730	3480	4250	9280	1550
21	7370	3070	4300	--	--
[3]					
34	8020	2845	5175	--	--
37	8100	3220	4880	--	--
41	8340	3350	4990	--	--
43	--	--	--	10800	2895
48	6820	3430	3390	--	--
53	--	--	--	10500	4250
54	6140	3610	2510	--	--
60	7250	3410	3840	--	--
Average :	7450	3320	4130	10650	3570
Std Dev.:	859	243	1063	212	958
[2]S.S. :	7450 ± 9%	3320±6%	4130±21%	10650±9%	3570 -%

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values ay 90 % confidence level.

[3] Steady state assumed after 30 days.

Fig. D.19 Solids Concentrations in Semi-Continuous Reactors at 30°C, 10 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	12710	7500	5210	14160	1450
1	12420	7330	5090	--	--
6	9100	4570	4530	9205	105
11	8450	3490	4960	--	--
12	--	--	--	8590	730
16	9150	3870	5280	--	--
18	--	--	--	8960	920
21	8670	4370	4300	--	--
22	--	--	--	9800	1010
33	10150	4420	5720	11430	1280
39	9780	3920	5860	--	--
40	--	--	--	11690	1870
46	10030	4570	5460	--	--
47	--	--	--	11430	1420
55	9870	4440	5430	--	--
56	--	--	--	11520	1670
Average :	9960	4340	5620	11520	1560
Std Dev.:	165	286	208	123	262
[2]S.S. = :	9960 ± 2%	4340 ± 8%	5620 ± 4%	11520 ± 1%	1560 ± 20%

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values at 90 % confidence level.

[3] Steady state assumed after 30 days.

Fig. D.20 Solids Concentrations in Semi-Continuous Reactors at 30°C, 20 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	12220	7500	4720	13860	840
1	11520	6140	5380	—	—
2	—	—	—	10850	20
6	8960	3920	4140	—	—
7	—	—	—	8450	480
11	7620	3290	4330	—	—
12	—	—	—	8220	530
16	7980	3260	4720	—	—
18	—	—	—	8800	910
21	7520	3700	3820	—	—
22	—	—	—	8450	860
[3]					
32	—	—	—	9740	1460
33	8350	3710	4640	—	—
39	8800	3720	5080	—	—
40	—	—	—	10850	2090
46	8540	4220	4320	—	—
47	—	—	—	10880	2360
55	8340	3710	4630	—	—
56	—	—	—	10440	2100
Average :	8510	3840	4670	10480	2000
Std Dev.:	216	253	313	531	383
[2]S.S. :	8510 ± 3%	3840 ± 8%	4670 ± 8%	10480 ± 6%	2000 ± 23%

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values at 90 % confidence level.

[3] Steady state assumed after 30 days.

Fig. D.21 Solids Concentrations in Semi-Continuous Reactors at 30°C, 30 Days Detention Time.

Days from Start (d)	[1]TSS (mg/l)	VSS (mg/l)	[1]FSS (mg/l)	[1]TS (mg/l)	[1]TDS (mg/l)
0	11820	7500	4320	13320	1500
1	11660	5540	6120	--	--
6	7890	3580	4310	--	--
7	--	--	--	8170	420
11	7170	3400	3770	--	--
12	--	--	--	7960	700
16	7610	3490	4120	--	--
18	--	--	--	8240	830
21	7110	3570	3540	--	--
22	--	--	--	8355	1130
32	--	--	--	9085	710
33	8490	3920	4610	--	--
39	7690	3630	4050	--	--
40	--	--	--	9880	2220
46	7480	3340	4140	--	--
47	--	--	--	9810	2340
55	7410	3320	4090	--	--
56	--	--	--	9580	2170
Average :	7770	3550	4220	9590	1860
Std Dev.:	496	283	260	359	770
[2]S.S. :	7770 ± 8%	3550 ± 9%	4220 ± 7%	9590±4%	1860±49%

[3]

[1] Values corrected for solids variations in feed (refer to Appendix E).

[2] S.S. = Steady state values at 90 % confidence limits.

[3] Steady state assumed after 30 days.

Fig. D.22 Activity Measures in Semi-Continuous Reactors at 10°C

Det. Time (d)	D.S.[1] (d)	ATP (µg/l)	DOUR (mg/l/hr)	Viable Count (cells/ml) x10^{-8}	Specific ATP (µg/mg VSS)	Specific DOUR (mg/gVSS/hr)	Specific Viable count (cells/mg VSS) x10^{-8}
10	31	3080	41.1	--	0.55	7.41	--
10	36	2940	38.7	6.3	0.54	7.14	1.16
10	39	2920	36.0	6.6	0.51	6.33	1.16
10	43	2730	30.3	--	0.49	5.47	--
10	50	--	28.2	3.7	--	5.58	0.73
10	58	2850	24.0	3.6	0.57	4.80	0.72
Average :		2904	33.1	5.1	0.54	6.12	0.94
Std Dev.:		128	6.6	1.6	0.03	1.02	0.25
S.S.[2] :		2900	33.1	5.1	0.54	6.12	0.94
		±4%	±16%	±37%	±6%	±14%	±31%
20	31	2630	16.5	5.1	0.51	3.23	1.00
20	36	2450	24.6	5.0	0.46	4.57	0.93
20	39	2780	24.2	--	0.55	4.78	--
20	43	2760	23.1	6.4	0.55	4.57	1.26
20	50	--	20.1	2.9	--	4.34	0.63
20	58	1700	22.2	3.0	0.37	4.88	0.66
Average :		2464	21.8	4.5	0.50	4.40	0.90
Std Dev.:		447	3.0	1.5	0.08	0.60	0.26
S.S. :		2464	21.8	4.5	0.50	4.40	0.90
		±17%	±11%	±32%	±15%	±11%	±34%
30	31	2210	22.2	3.8	0.47	4.70	0.81
30	36	2730	20.7	--	0.54	4.12	--
30	39	2510	21.6	6.4	0.50	4.26	1.26
30	43	2740	16.5	--	0.57	3.41	--
30	50	--	15.0	2.0	--	3.22	0.43
30	58	2780	15.6	3.0	0.66	3.68	0.71
Average :		2594	18.6	3.8	0.54	3.90	0.80
Std Dev.:		239	3.3	1.9	0.07	0.56	0.34
S.S. :		2594	18.6	3.8	0.54	3.90	0.80
		±9%	±15%	±59%	±13%	±12%	±50%

[1] D.S. = Days from start.

[2] S.S. = Steady state values at 90 % confidence level.

Fig. D.23 Activity Measures in Semi-Continuous Reactors at 20°C

Det. Time (d)	[2]D.S. (d)	ATP (µg/l)	DOUR (mg/l/hr)	Viable Count (cells/ml) x10^{-8}	Specific ATP (µg/mg VSS)	Specific DOUR (mg/gVSS/hr)	Specific Viable count (cells/mg VSS) x10^{-8}
10	[1]33-34	3010	30.0	1.9	0.69	6.90	0.43
10	36-37	3080	41.4	3.9	0.80	11.40	1.01
10	40-41	3030	33.6	3.7	0.73	7.81	0.90
10	43-48	2680	36.6	9.8	0.61	7.82	2.22
10	50	2750	--	9.1	0.59	--	1.97
10	53-54	2950	31.8	8.9	0.65	7.00	1.95
10	58-60	2950	25.2	8.4	0.63	5.35	1.81
Average :		2920	33.1	6.5	0.67	7.71	1.47
Std Dev.:		149	5.6	3.2	0.07	2.02	0.68
[3]S.S. :		2920	33.1	6.5	0.67	7.71	1.47
		±4%	±14%	±36%	±8%	±22%	±34%
20	33-34	2910	19.5	1.4	0.73	4.85	0.35
20	36-37	1740	25.2	2.6	0.50	7.75	0.74
20	40-41	3020	19.2	2.0	0.83	5.09	0.55
20	43-48	1800	16.8	2.8	0.49	4.80	0.76
20	50	1790	--	3.5	0.59	--	0.99
20	53-54	2560	34.8	3.9	0.71	9.51	1.07
20	58-60	3180	19.2	3.3	0.79	4.53	0.81
Average :		2430	22.5	2.8	0.65	6.09	0.75
Std Dev.:		637	6.7	0.9	0.15	2.05	0.25
[3]S.S. :		2430	22.5	2.8	0.65	6.09	0.75
		±19%	±24%	±24%	±17%	±28%	±24%
30	33-34	1110	12.0	2.1	0.38	4.15	0.71
30	36-37	1590	15.6	2.7	0.51	4.84	0.87
30	40-41	1870	13.8	2.1	0.56	4.12	0.63
30	43-48	2680	15.0	3.1	0.80	4.37	0.92
30	50	2360	--	3.9	0.68	--	1.12
30	53-54	2250	12.6	7.3	0.63	3.41	2.04
30	58-60	2310	12.6	5.6	0.66	3.70	1.61
Average :		2025	13.6	3.8	0.61	4.10	1.13
Std Dev.:		536	1.5	1.9	0.14	0.50	0.52
[3]S.S. :		2025	13.6	3.8	0.61	4.10	1.13
		±19%	±9%	±37%	±17%	±10%	±34%

[1] First number refers to day of ATP and Plate Count tests, whereas second number refers to day of DOUR test.

[2] D.S. = Days from start.

[3] S.S. = Steady state values at 90 % confidence level.

Fig. D.24 Activity Measures in Semi-Continuous Reactors at 30°C

Det. Time (d)	[1]D.S. (d)	ATP (µg/l)	DOUR (mg/l/hr)	Viable Count (cells/ml) $x10^{-8}$	Specific ATP (µg/mg VSS)	Specific DOUR (mg/gVSS/hr)	Specific Viable count (cells/mg VSS) $x10^{-8}$
10	33	2370	27.9	--	0.54	6.31	--
10	39	1860	21.9	2.3	0.47	5.59	0.59
10	46	3430	28.8	1.5	0.75	6.30	0.33
10	55	2930	28.2	1.9	0.66	6.35	0.44
Average :		2650	26.7	1.9	0.61	6.14	0.46
Std Dev.:		680	3.2	0.4	0.12	0.37	0.10
S.S. :		2650	26.7	1.9	0.61	6.14	0.46
		±30%	±14%	±35%	±23%	±7%	±37%
20	33	1830	15.6	--	0.49	4.20	--
20	39	1920	12.0	1.6	0.52	3.23	0.43
20	46	3400	14.7	1.4	0.81	3.48	0.33
20	55	2760	14.4	1.4	0.74	3.88	0.38
Average :		2480	14.2	1.5	0.65	3.70	0.38
Std Dev.:		744	1.5	0.1	0.16	0.43	0.05
S.S. :		2480	14.2	1.5	0.65	3.70	0.38
		±35%	±12%	±11%	±29%	±14%	±22%
30	33	1740	13.5	--	0.44	3.44	--
30	39	1450	10.2	1.5	0.40	2.81	0.41
30	46	2380	8.4	0.8	0.71	2.51	0.24
30	55	1760	10.8	1.1	0.53	3.25	0.34
Average :		1830	10.7	1.1	0.52	3.00	0.33
Std Dev.:		390	2.1	0.4	0.14	0.42	0.09
S.S. :		1830	10.7	1.1	0.52	3.00	0.33
		±25%	±23%	±61%	±32%	±16%	±46%

[1] D.S. = Days from start.

[2] S.S. = Steady state values at 90 % confidence level.

Fig. D.25 Dehydrogenase Enzyme Activity Data for Semi-Continuous Reactors at 10°C.

Detention Time (d)	Days from Start (d)	Total Counts (cells/ml) $x10^{-10}$	Active Counts (cells/ml) $x10^{-10}$	%Activity (%)
10	31	31.0	8.5	27
10	36	16.1	6.9	43
10	39	35.3	5.6	16
10	43	28.7	8.7	30
10	50	53.8	23.9	44
10	58	74.3	14.5	20
Average :		39.9	11.4	30
Std Dev.:		20.8	6.9	12
[1]S.S. :		39.9±43%	11.4±50%	30±32%
20	31	29.2	6.3	22
20	36	21.0	11.9	57
20	39	55.3	8.1	15
20	43	24.3	11.2	46
20	50	39.7	15.2	38
20	58	55.8	12.0	22
Average :		37.6	10.8	33
Std Dev.:		15.3	3.2	16
[1]S.S. :		37.6±33%	10.8±24%	33±40%
30	31	12.7	3.9	31
30	36	18.2	5.5	30
30	39	41.0	13.4	33
30	43	28.6	6.9	24
30	50	34.6	10.1	29
30	58	79.2	16.1	20
Average :		35.7	9.3	28
Std Dev.:		23.7	4.8	5
[1]S.S. :		35.7±55%	9.3±42%	28±14%

[1] S.S. = Steady state values at 90 % confidence level.

Fig. D.26 Dehydrogenase Enzyme Activity Data for Semi-Continuous Reactors at 20°C.

Detention Time (d)	Days from Start (d)	Total Counts (cells/ml) $\times 10^{-10}$	Active Counts (cells/ml) $\times 10^{-10}$	%Activity (%)
10	40	4.1	1.0	24
10	43	2.4	1.5	63
10	50	2.0	0.6	30
10	53	1.6	0.5	31
10	58	2.2	0.8	36
Average :		2.5	0.9	37
Std Dev.:		1.0	0.4	15
[1]S.S. :		2.5±38%	0.9±42%	37±39%
20	40	2.9	0.9	31
20	43	1.6	1.0	63
20	50	1.2	0.5	42
20	53	1.0	0.3	30
20	58	3.2	0.8	25
Average :		2.0	0.7	38
Std Dev.:		1.0	0.3	15
[1]S.S. :		2.0±48%	0.7±41%	38±38%
30	40	8.6	2.2	26
30	43	1.1	0.5	45
30	50	1.3	0.6	46
30	53	1.2	0.7	58
30	58	3.0	0.7	23
Average :		3.0	0.9	40
Std Dev.:		3.2	0.7	15
[1]S.S. :		3.0±100%	0.9±74%	40±36%

[1] S.S. = Steady state values at 90 % confidence level.

Fig. D.27 Dehydrogenase Enzyme Activity Data for Semi-Continuous Reactors at 30°C.

Detention Time (d)	Days from Start (d)	Total Counts (cells/ml) $x10^{-10}$	Active Counts (cells/ml) $x10^{-10}$	%Activity (%)
10	33	1.6	0.6	38
10	39	1.3	0.5	38
10	46	1.7	0.5	29
10	55	1.8	0.5	28
Average :		1.6	0.5	33
Std Dev.:		0.2	0.1	6
[1]S.S. :		1.6±15%	0.5±24%	33±20%
20	33	1.8	0.6	33
20	39	1.7	0.5	29
20	46	1.5	0.4	27
20	55	1.9	0.5	26
Average :		1.7	0.5	29
Std Dev.:		0.2	0.1	3
[1]S.S. :		1.7±14%	0.5±24%	29±13%
30	33	1.8	0.5	28
30	39	1.7	0.5	29
30	46	0.8	0.4	50
30	55	1.5	0.4	27
Average :		1.5	0.5	34
Std Dev.:		0.5	0.1	11
[1]S.S. :		1.5±39%	0.5±24%	34±39%

[1] S.S. = Steady state values at 90 % confidence level.

Fig. D.28 COD Data for Batch Reactors.

Temp.= 10°C		20°C		30°C	
Detention Time (d)	COD (mg/l)	Detention Time (d)	COD (mg/l)	Detention Time (d)	COD (mg/l)
0	10070	0	13740	0	10510
3	10710	2	8940	2	9720
6	11440	6	6100	7	5410
14	9240	13	5610	12	4950
18	9660	20	5550	18	6830
25	10080	26	4590	22	5680
30	8400			32	4870
33	10080			40	5000
37	7480			47	4920
44	10000				
52	9000				
61	8880				

Fig. D.29 COD Data (in mg/l) for Semi-Continuous Reactors at 10°C.

Days from Start (d)	Detention Time		
	10 days	20 days	30 days
0	10070	10070	10070
10	9660	8190	7980
13	9680	9240	8360
21	10710	8820	7980
25	10080	8800	7960
32	10080	8800	8400
37	9600	8400	8000
40	8640	8280	9360
44	8500	7480	8160
51	9000	9000	8000
59	9010	8000	8500

Fig. D.30 COD Data (in mg/l) for Semi-Continuous Reactors at 20°C.

Days from Start (d)	Detention Time		
	10 days	20 days	30 days
0	13740	13740	13740
10	10530	11060	11480
13	9650	9210	8770
17	7610	8040	6740
21	7330	5600	6860
34	7440	4960	4550
37	9760	5690	6860
41	6910	4880	4880
48	7480	6075	5610
54	7340	6190	4590
60	7570	6190	5730

Fig. D.31 COD Data (in mg/l) for Semi-Continuous Reactors at 30°C.

Days from Start (d)	Detention Time		
	10 days	20 days	30 days
0	10810	10530	10750
2	10650	9720	10650
7	7210	4950	4950
12	6760	5860	5860
18	6830	5510	5950
22	6330	5460	6770
32	6780	5720	5300
40	7310	6150	5390
47	6820	6440	7200

Fig. D.32 pH Data for Batch Reactors.

Temp.= 10°C		20°C		30°C	
Detention Time (d)	pH	Detention Time (d)	pH	Detention Time (d)	pH
3	8.0	2	7.2	2	7.8
6	8.2	6	6.6	7	8.3
14	8.3	20	4.9	12	5.9
18	8.3	26	4.4	18	5.9
25	8.3			32	6.0
29	8.3			40	5.5
33	8.3			47	5.5
37	7.5				
44	5.5				
61	5.1				
62	5.1				

Fig. D.33 pH Data for Semi-Continuous Reactors at 10°C.

Days from Start (d)	Detention Time		
	10 days	20 days	30 days
10	7.2	7.3	7.3
13	7.0	7.0	7.0
21	6.3	6.1	6.1
25	6.2	6.0	6.0
32	6.2	6.0	5.7
36	6.2	5.9	6.2
40	5.9	6.2	6.2
44	5.9	5.7	5.7
51	5.8	5.8	5.7
59	5.8	6.4	5.9

Fig. D.34 pH Data for Semi-Continuous Reactors at 20°C.

Days from Start (d)	Detention Time		
	10 days	20 days	30 days
3	6.0	6.0	6.1
6	5.9	5.9	5.8
17	5.7	5.1	5.2
21	6.0	5.6	5.7
34	5.0	5.9	5.2
37	5.0	5.8	5.2
41	4.9	5.7	5.1
54	4.9	5.3	5.0
60	4.9	5.3	5.0

Fig. D.35 pH Data for Semi-Continuous Reactors at 30°C.

Days from Start (d)	Detention Time		
	10 days	20 days	30 days
2	7.7	7.7	7.0
7	8.4	8.4	8.5
12	6.3	6.2	6.1
18	5.4	5.9	5.8
32	5.1	4.8	5.1
40	5.4	5.2	5.2
47	4.9	4.6	4.5

APPENDIX E

CORRECTION OF SOLIDS CONCENTRATIONS FROM FEED VARIATIONS

The feed to the semi-continuous reactors was set to have a constant VSS content of 7500 mg/l. The ratio of VSS to TSS in the testing media not being constant resulted in variations of TSS as the volatile matter in the feed was been fixed. To account for these variations, which are independent of the treatment process, a correction procedure was used based on the assumption that the suspended solids in excess of the reactors' start-up concentrations were for the most part composed of inert solids and therefore could only accumulate in the reactors.

The corrections were estimated from a mass balance around the digester as follows :

IN - OUT = ACCUMULATION

or in terms of solids concentrations :

$$Q_{in}C_{in} - Q_{out}C_{out} = V\ (\Delta C/\Delta T) \qquad (D.1)$$

where C_{in} = difference in TSS between feed and start-up concentrations, mg/l

C_{out} = difference in TSS between output and start-up concentrations, mg/l

$\Delta C = C_{i+1} - C_i$

with C_{i+1} = difference in TSS between reactor and start-up concentrations at time t+Δt, mg/l

C_i = difference in TSS between reactor and start-up concentrations at time t, mg/l

V = reactor volume, l

Q = flowrate, l/d.

Equation D.1 can be rewritten as :

$$Q_{in}\,\Delta t\,C_{in} - Q_{out}\Delta t\,C_{out} = V(C_{i+1}-C_i)$$

or

$$V_{in}C_{in} - V_{out}C_{out} = V(C_{i+1}+C_i) \qquad (D.2)$$

Since the reactors were fed by withdrawing the reactors' mixed liquour before addition of feed, C_{out} can be equated to C_i giving :

$$V(C_{i+1}) = (V-V_{out})C_i + V_{in}C_{in} \qquad (D.3)$$

The above equation was used to evaluate the corrections for TSS. The corrections were done by substracting the correction calculated for the previous day from the measured TSS concentration. This was done because sampling was performed during the day while feeding was performed at night.

Total solids (TS) were corrected with the same values obtained above. Lack of TS data for the feed did mot permit an estimation on the variations in dissolved solids. However due to the low content of dissolved solids and the way the feed sludge was prepared (i.e., by decanting settled sludge and diluting to

required concentration with distilled water) variations in dissolved solids were assumed to have a negligeable effect on TS variations.

Below are tabulated the daily feed TSS concentrations and their respective corrections.

Fig. E.1 Solids Corrections for Semi-Continuous Reactors at 10 °C.

Day from Start (d)	TSS feed ($\overline{mg}$/l)	Corrections at detention times of : 10 d (mg/l)	20 d (mg/l)	30 d (mg/l)
0	12400	--	--	--
1	--	--	--	--
2	--	--	--	--
3	15420	302	151	101
4	12440	276	145	99
5	12120	220	124	86
6	--	170	104	74
7	15950	508	276	190
8	13700	587	327	227
9	13570	646	370	258
10	13530	694	408	287
11	12200	605	377	271
12	--	524	348	255
13	--	452	321	240
14	12860	453	328	247
15	--	454	335	255
16	--	454	341	261
17	--	455	347	268
18	11860	355	302	241
19	--	266	260	215
20	15370	536	396	307
21	15970	840	554	416
22	13830	899	598	449
23	--	952	640	482
24	14090	1026	692	522
25	--	1092	742	561
26	16000	1343	885	663
27	--	1569	1021	761
28	12630	1435	981	743
29	15630	1614	1094	826
30	11650	1378	1002	773
31	15840	1584	1123	862
32	13180	1504	1106	859
33	13730	1486	1117	875
34	11810	1279	1032	826
35	--	1092	951	779
36	--	924	874	733
37	12390	830	830	709
38	13740	881	855	730

Fig. E.1 Solids Corrections for Semi-Continuous Reactors at 10°C. (Continuation)

Day from Start	TSS feed	Corrections at detention times of : 10 d	20 d	30 d
(d)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
39	14870	1040	936	788
40	12320	928	885	759
41	12210	816	831	727
42	--	716	780	697
43	12570	661	750	679
44	--	612	721	662
45	12660	577	698	649
46	14230	702	754	688
47	--	815	808	726
48	11560	649	726	674
49	--	500	648	623
50	11510	361	571	573
51	--	236	498	524
52	11760	149	441	485
53	--	70	387	448
54	11780	1	336	412
55	--	-61	289	378
56	12160	-79	262	357
57	--	-95	237	337
58	12740	-52	242	337

Fig. E.2 Solids Corrections for Semi-Continuous Reactors at 20°C.

Day from Start	TSS feed	Corrections at detention times of :		
		10 d	20 d	30 d
(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
0	15020	0	0	0
1	15230	21	11	7
2	--	40	20	14
3	15650	99	51	34
4	--	152	80	54
5	13680	3	9	8
6	--	-131	-54	-37
7	--	-252	-123	-81
8	15030	-226	-116	-78
9	14930	-212	-115	-78
10	16240	-69	-48	-35
11	16200	56	13	6
12	--	168	72	45
13	12950	-109	-62	-43
14	12120	-326	-173	-118
15	13500	-445	-240	-165
16	12500	-653	-354	-243
17	12180	-871	-478	-330
18	14460	-840	-482	-337
19	12190	-1039	-600	-420
20	12040	-1233	-719	-506
21	--	-1408	-832	-588
22	13800	-1389	-851	-609
23	11480	-1604	-986	-707
24	13100	-1636	-1032	-747
25	15680	-1406	-948	-701
26	11060	-1662	-1098	-809
27	--	-1891	-1242	-914
28	--	-2098	-1377	-1016
29	15900	-1800	-1265	-953
30	--	-1532	-1157	-891
31	13040	-1577	-1198	-928
32	11800	-1741	-1300	-1004
33	12430	-1826	-1364	-1057
34	12340	-1912	-1430	-1111
35	11930	-2030	-1513	-1177
36	--	-2136	-1592	-1241
37	15210	-1903	-1503	-1193
38	15940	-1616	-1379	-1121

Fig. E.2 Solids Corrections for Semi-Continuous Reactors at 20 °C. (Continuation)

Day from Start	TSS feed	Corrections at detention times of : 10 d	20 d	30 d
(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
39	16000	-1356	-1261	-1051
40	12240	-1499	-1337	-1109
41	12140	-1637	-1414	-1168
42	15500	-1425	-1319	-1113
43	11980	-1587	-1405	-1177
44	11310	-1799	-1521	-1261
45	16000	-1521	-1396	-1187
46	15780	-1293	-1288	-1122
47	11350	-1531	-1407	-1207
48	11580	-1722	-1509	-1281
49	12960	-1755	-1536	-1307
50	12210	-1861	-1600	-1357
51	11620	-2015	-1690	-1425
52	11670	-2148	-1773	-1489
53	11660	-2269	-1852	-1552
54	15530	-1992	-1734	-1483
55	12370	-2057	-1780	-1522
56	14530	-1901	-1715	-1488
57	15110	-1702	-1625	-1435
58	11730	-1860	-1708	-1497
59	12800	-1896	-1734	-1521
60	—	-1929	-1758	-1544

Fig. E.3 Solids Corrections for Semi-Continuous Reactors at 30°C.

Day from Start (d)	TSS feed (mg/l)	Corrections at detention times of : 10 d (mg/l)	20 d (mg/l)	30 d (mg/l)
0	*	0	0	0
1	13400	69	59	53
2	12320	23	61	68
3	12140	-36	54	76
4	--	-90	47	84
5	13240	-28	96	129
6	13400	49	150	177
7	12670	36	165	199
8	12600	21	176	219
9	--	8	186	238
10	12620	-2	197	257
11	12300	-43	191	269
12	12490	-60	195	278
13	12650	-60	207	296
14	--	-60	218	314
15	12630	-62	227	331
16	--	-64	237	347
17	12880	-41	258	370
18	12820	-26	275	391
19	12790	-15	290	411
20	12870	3	308	432
21	--	18	325	453
22	14120	157	403	514
23	--	282	478	574
24	--	395	549	631
25	14380	523	630	695
26	--	637	706	758
27	--	741	779	818
28	14020	798	830	864
29	--	849	879	908
30	14040	897	926	952
31	11700	706	853	916
32	11600	525	780	878
33	--	361	710	842
34	12880	392	707	849
35	--	325	705	856
36	--	309	703	863
37	12850	292	699	868
38	--	277	696	974

Fig. E.3 Solids Corrections for Semi-Continuous Reactors at 30°C. (Continuation)

Day from Start (d)	TSS feed (mg/l)	Corrections at detention times of : 10 d (mg/l)	20 d (mg/l)	30 d (mg/l)
39	--	263	692	879
40	13210	287	707	896
41	--	308	721	913
42	--	328	735	928
43	13210	395	748	944
44	--	360	760	959
45	--	374	771	973
46	12800	346	762	973
47	--	320	753	974
48	12910	308	749	977
49	--	297	746	981
50	--	288	744	985
51	13100	298	750	995
52	--	307	757	1004
53	13200	325	768	1017
54	--	342	779	1020
55	--	357	789	1041

* For this run the three reactors had different initial TSS concentrations but were fed with same feed sludge. The initial TSS concentrations were : 12710, 12220, 11820 mg/l at 10, 20, and 30 days detention time respectively.

APPENDIX F

CORRECTION OF BVSS FOR SOLUBILIZATION FROM CELL LYSIS

Variations in TDS and FSS with detention time were presented in Figure 6.4. The variations indicated that a large portion of cellular solids had been solubilized and not metabolized. To account for such reductions in suspended solids a correction procedure was used based on the assumption that for a specific detention time, the amount of BVSS solubilized could be approximated by the difference between TDS increase and FSS decrease from their initial respective values.

The corrections were estimated from the equations :

$$BVSS_{ci} = BVSS_i + \Delta BVSS_i \qquad (F.1)$$

with

$$BVSS_i = \Delta TDS_i - \Delta FSS_i \qquad (F.2)$$

and

$$\Delta TDS_i = TDS_o - TDS_i \qquad (F.3)$$

$$\Delta FSS_i = FSS_o - FSS_i \qquad (F.4)$$

where $BVSS_{ci}$ = corrected BVSS concentrations at detention time $t = t_i$, mg/l

$BVSS_i$ = uncorrected BVSS concentration at detention time $t = t_i$, mg/l

$\Delta BVSS_i$ = correction for BVSS at time $t = t_i$, mg/l

ΔTDS_i = change in TDS from t = 0 to t = t_i, mg/l

ΔFSS_i = change in FSS from t = 0 to t = t_i, mg/l

TDS_o = initial TDS concentration, mg/l

TDS_i = TDS concentration at time t = t_i, mg/l

FSS_o = initial FSS concentration, mg/l

FSS_i = FSS concentration at time t = t_i, mg/l

The plots presented in Figure 6.4 were obtained from least squares approximations with polynomials of second degree. Values for TDS and FSS in above equations were obtained from these plots. Figures F.1 and F.2 summarize the corrections performed.

Fig. F.1 BVSS Corrections for Batch Reactor at 20°C.

Detention Time (d)	BVSS (mg/l)	TDS (mg/l)	FSS (mg/l)	Δ BVSS (mg/l)	BVSS ci (mg/l)
0	4900	0	4515	--	4900
2	3260	365	4320	170	3430
6	1730	1190	3980	655	2385
13	370	2015	3535	1035	1405
20	30	2050	3285	820	850
26	230	2050	3220	755	985
30	50	2030	3260	795	845

Fig. F.2 BVSS Corrections for Batch Reactor at 30°C.

Detention Time (d)	BVSS (mg/l)	TDS (mg/l)	FSS (mg/l)	Δ BVSS (mg/l)	BVSS ci (mg/l)
0	4770	790	4960	—	4770
1	2860	--	--	—	2860
6	1220	775	4860	--	1220
11	790	780	4780	—	790
16	640	800	4720	—	640
21	720	840	4670	--	720
33	0	1005	4595	15	15
39	510	1125	4585	100	610
46	10	1300	4590	230	240
55	1	1565	4640	440	440