VERIFICATION OF THE BISUBSTRATE CONCEPT IN MODELLING OF THE ACTIVATED SLUDGE PROCESS

by

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DECLARATION BY CANDIDATE

I hereby declare that this thesis is my own work and has not been submitted for a degree at any other University.

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SYNOPSIS

This investigation was concerned with two problems: (1) verification of the bisubstrate concept proposed by Dold, Ekama and Marais (1980), in which the biodegradable component of wastewater consists of readily and slowly biodegradable fractions, and (2) physical separation techniques for estimating the readily biodegradable fraction.

Intensive research, since 1970, into the behaviour of activated sludge systems at the University of Cape Town has culminated in the formulation of a general kinetic model, by Dold and Marais (1986). The model is highly complex, incorporating a number of different processes; however extensive simulation studies have shown that the model simulates the behaviour of activated sludge systems very closely.

A crucial concept incorporated in the general model is that the biodegradable fraction of a wastewater can be subdivided into two fractions with sharply different characteristics (bisubstrate concept), a readily biodegradable fraction which is directly utilized by the organism mass at a high rate and a slowly biodegradable fraction which requires to be solubilized extracellularly to readily biodegradable material for subsequent use by the organism. Solubilization is hypothesized to be a relatively slow process. These characteristics were inferred from the oxygen utilization rate response of a system fed in a cyclic square wave fashion. No work has been done to verify if substrates of specific chemical structures can be allocated to one or the other of the fractions. One of the objectives of this investigation was to check if pure and mixtures of specific selected substrates reflected these fractions when fed to activated sludge systems.

Glucose and maize starch were selected as representative of readily biodegradable and slowly biodegradable substrate respectively. Systems were run under steady state and square wave cyclic state with glucose only, starch only and glucose/starch mixtures. From the steady state response the specific yield values could be determined and the reliability of the data checked by doing mass balances on the COD. From the cyclic

response the specific rate constants for growth and solubilization respectively could be determined by trial simulation using the general model and specifying the concentrations of readily and slowly biodegradable fractions equal to the stoichiometric concentrations of glucose and starch in the feed. The constants thus determined were compared with the "standard" constants for municipal wastewaters.

Using the derived constants close correspondence between the observed and simulated responses was observed. With a purely readily biodegradable substrate (glucose) it was necessary to increase only the maximum specific growth rate constant for heterotrophs, $\hat{\mu}_H$, from 2,50 to 3,0/day and the half saturation coefficient, K_S, from 5,0 to 10,0 mgCOD/ℓ; the specific yield constant, Y_H, remained as before, Y_H = 0,666 mg cell COD yield/mg COD utilized. For a purely particulate slowly biodegradable substrate (maize starch) and a mixture of glucose and maize starch, the growth rate constant $\hat{\mu}_H$ remained at 2,5/d, the solubilization rate for particulate substrate, K_h, had to be reduced from 2,20 to 1,80 mgCOD/mg cell COD/day and the specific yield, Y_H, from 0,666 to 0,592 mg cell COD yield/mg COD utilized. With these constants reasonably good simulated fits to the observed data were obtained. Kinetically the effects of the changes are relatively minor and not outside the ranges obtained on different municipal waste flows. Some deviation from the mean standard values is not unlikely, due to the specificness of the substrates - it is to be expected that both the readily and slowly biodegradable COD fractions would be influence in some degree by the chemical structure or the organic material in each fraction.

The conclusion formed was that the observed response data of the glucose, starch and glucose/starch substrates and the response predicted by the bisubstrate model appeared to be consistent thus lending support to the validity of the bisubstrate hypothesis.

With regard to the second objective, the determination of the readily biodegradable fraction in a wastewater, three biological assay methods already exist and have been tested and evaluated under the conditions to which these respectively apply. However these methods demand considerable skill and are time-consuming. In consequence, alternative methods of

evaluation have been sought. One suggested method is physical separation by ultrafiltration techniques. This method is based on the hypothesis that in general readily biodegradable organic molecules should be smaller than the more complex slowly biodegradable ones.

Considerable effort had to be expended to develop the technique; problems with blinding of the ultra-filters had to be resolved and procedures for cleaning the membrane for re-use had to be developed. To limit extraneous variables the investigation was undertaken on one wastewater only, that from Mitchell's Plain, Cape Town.

It was found that ultra-filters with molecular weight cut-off values of 500, 5000 and 10000 significantly underestimated the readily biodegradable COD concentration, but the 100000 molecular weight cut-off filter gave values that were reasonably well correlated with the values determined by the biological method. All the filters gave results that were consistent in that the values were proportional to those of the biological method. It was found further that 0,45 µm filters also gave rise to consistent results but overestimated the biologically derived value by approximately 20 percent.

It was concluded that both the molecular weight cut-off filters and the 0,45 µm filter can be used to estimate the readily biodegradable COD fraction. However before this method can be accepted for general use it needs to be checked for a number of waste flows, of different origin and composition.

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TABLE OF CONTENTS

LIST OF SYMBOLS

In the list of symbols below two symbol systems are given. In the first column, the symbol system recommended by the IAWPRC (Grau et al., 1982) is given for those parameters and variables that have been allocated symbols. In the second column the symbol system used in the previous papers on activated sludge process modelling is given. In this report, the former symbol system is used, except in Chapter Five, where the more common expression (at present) for readily biodegradable COD, S_{bs}, has been retained.

-	AVSS	=	active volatile suspended solids
S_{ALK}	Alk	=	Alkalinity in mg/ℓ as $CaCO_3$ i.e. mg/ℓ as $CaCO_3$ strong acid added to titrate down to the equivalent carbonic acid solution.
-	b_h*	=	heterotrophic organism endogenous respiration/mass loss rate in the steady state model of Marais and Ekama (1976)
		=	0,24/d.
b_H	b'_h*	=	heterotrophic organism death rate in the general kinetic model of Dold et al., (1980)
		=	0,62/d.
-	f	=	endogenous residue fraction in the steady state model of Marais and Ekama (1976)
		=	0,20.
f_E	f'	=	unbiodegradable fraction of the heterotrophic cell mass in the general kinetic model of Dold et al., (1980)
		=	0,08.
-	f_{cv}	=	COD to VSS ratio of the sludge mass
		=	1,48 mgCOD/mgVSS.

- f_{bs} = readily biodegradable COD fraction with respect to the biodegradable COD ($f_{bs} = S_{bsi}/S_{bi}$) (mgCOD/mgCOD).
- f_{ma} = maximum mass of COD (in terms of VSS i.e. mgVSS = mgCOD/f_{cv}) that can be adsorbed onto the active organism mass (mgVSS/mgAVSS)
  = 1,0 mgVSS/mgAVSS.
- f_{up} = unbiodegradable particulate COD fraction of the influent (mgCOD/mgCOD).
- f_{us} = unbiodegradable soluble COD fraction of the influent (mgCOD/mgCOD).
- f_x = fraction of the total hydraulic retention time that the digester is under anoxic conditions.
- K^* = denitrification rate constant ($mgNO_3$-N/mgAVSS/d).
  Subscripts 1, 2 and 3 refer respectively to the first and second rates in the primary anoxic reactor and the rate in the secondary anoxic reactor of the single sludge system.
  Subscript 4 refers to the rate in the anoxic-aerobic digestion system for waste activated sludge.
- K_a = particulate COD adsorption rate (ℓ/mgAVSS/d)
  = 0,25 ℓ/mgAVSS/d.

$\hat{\mu}_H$ K_{ms} = maximum specific readily biodegradable COD utilization rate
= 5,6 mgCOD/mgAVSS/d. $\hat{\mu}_H = K_{ms}Y_h = 5,6.0,45 = 2,5/d$.

K_s K_{ss} = half saturation coefficient for readily biodegradable COD utilization
= 5 mgCOD/ℓ.

K_H	K_{mp}	=	maximum specific adsorbed COD utilization rate under aerobic conditions
		=	2,2 mgCOD/mgAVSS/d.
-	K'_{mp}	=	maximum specific adsorbed COD utilization rate under anoxic conditions
		=	ηK_{mp} mgCOD/mgAVSS/d where η = 0,30 to 0,38.
K_x	K_{sp}	=	half saturation coefficient for adsorbed COD utilization under aerobic and anoxic conditions
		=	0,15 mgCOD/mgVSS.
S_{NHi}	N_{ai}	=	influent free and saline ammonia concentration (mgN/ℓ)
-	N_{ti}	=	influent TKN concentration (mgN/ℓ).
-	N_{ui}	=	influent unbiodegradable organic nitrogen concentration (mgN/ℓ).
S_{NO}	N_n	=	nitrate nitrogen concentration ($mgNO_3$-N/ℓ).
-	OCR	=	oxygen consumption rate (mgO/ℓ/d).
S_O	O	=	general symbol for oxygen concentration (mgO/ℓ).
-	dO_c/dt	=	carbonaceous (COD) oxygen consumption rate for synthesis in the general kinetic model of Dold et al., (1980) (mgO/ℓ/d).
-	dO_e/dt	=	endogenous respiration oxygen consumption rate in the steady state theory of Marais and Ekama (1976) (mgO/ℓ/d).
-	R_h	=	hydraulic retention time (d).
θ_x	R_s	=	sludge age (d).
-	SSGR	=	specific sludge growth rate (mgVSS/mgVSS/d).
-	SLR	=	sludge loading rate (mgCOD/mgVSS/d).
-	SOCR	=	specific oxygen consumption rate (mgO/mgVSS/d).
-	S_b	=	biodegradable COD concentration (mgCOD/ℓ).
X_s	S_{bp}	=	particulate biodegradable COD concentration (mgCOD/ℓ).

S_S	S_{bs}	=	readily biodegradable COD concentration (mgCOD/ℓ). additional subscript i refers to the concentrations in the influent (i.e. S_{bi}, S_{bsi} and S_{bpi}).
-	S_{ti}	=	total influent COD concentration (mgCOD/ℓ).
-	t	=	time.
-	T	=	temperature in °C.
X	X	=	general symbol for volatile suspended solids (VSS) concentration (mgVSS/ℓ).
$X_{B,H}$	X_a	=	active VSS concentration (mgAVSS/ℓ).
X_E	X_e	=	endogenous residue VSS concentration (mgVSS/ℓ).
X_I	X_i	=	inert VSS concentration (mgVSS/ℓ).
-	X_v	=	total VSS concentration (mgVSS/ℓ)
		=	$X_a + X_e + X_i$.
X_S	X_s	=	concentration of COD (in terms of VSS) adsorbed on the active VSS with respect to the bulk liquid (mgVSS/ℓ).
Y_H	Y_h	=	yield coefficient for active mass (mgAVSS/mgCOD)
		=	0,45 mgAVSS/mgCOD. Note that Y_H is specified as mgCOD cells formed/mgCOD consumed i.e. $Y_H = f_{cv}Y_h$ = 0,66 mgCOD/mgCOD.
-	Δ	=	symbol denoting change.
η_G/η_S	η	=	reduction factor for anoxic conditions for the maximum specific stored COD utilization rate i.e. K'_{mp}(anoxic) $= \eta K_{mp}$(aerobic)
		=	0,30 to 0,38.
$\hat{\mu}_A$	μ^*_{nm}	=	maximum specific growth rate of the nitrifiers (/d).

* symbols with additional subscript T or 20 denotes rate at T°C or 20°C.

CHAPTER ONE

INTRODUCTION

Since 1970 the Marais Group at the University of Cape Town has been engaged in intensive research into the behaviour of activated sludge systems. The overall objective has been to develop a general kinetic model which accurately describes the dynamic behaviour of activated sludge systems, incorporating carbonaceous material oxidation, nitrification, denitrification and biological excess phosphorus removal. The outcome of this research is a general kinetic model by Dold and Marais (1986).

The general model appears to simulate the behaviour of activated sludge systems very closely. The model is a complex one incorporating a large number of processes and based on certain key hypotheses as to (i) the mechanisms operating in some of the processes,* and (ii) the nature of the substrate. In regard to the practical application of models the assumptions usually are judged to be satisfactory if an adequately close correspondence of the simulated and observed responses is obtained. However this does not imply, by any means, that the assumptions have substance - the causes for the behaviour possibly could be explained as satisfactorily using a different hypothesized structure. An examination of the historical development of the general model will show that this is indeed so - four versions have been proposed each in some measure with a different hypothesized structure, yet each model gave reasonably close predictions to the observed behaviour. It is most desirable therefore that the hypothesis incorporated in the model be evaluated as independently of the model as possible to give substance to the hypothesis or casting doubt on it.

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*An historical description of the evolution of the model is briefly given in Chapter Two.

Amongst the assumptions incorporated in the model of Dold et al. there are two that merit critical investigation. These are the nature of the substrate, and, the kinetic mechanisms of utilization of the substrate. The model hypothesizes that the substrate can be split into two sharply defined fractions, (the so-called bisubstrate hypothesis), into readily biodegradable (soluble) and slowly biodegradable (particulate) COD fractions. It is hypothesized further that the organism mass can only utilize the readily biodegradable soluble form directly, it being presumed that this substrate fraction consists of organic molecules that can be absorbed i.e. are translocatable across the cytoplasmic membrane. The slowly biodegradable (particulate) COD is presumed to consist of molecules that cannot pass through the membrane - the molecules require to be hydrolyzed to smaller, simpler units before absorption can take place. Hydrolysis is presumed to take place extracellularly by enzymes released by the organism mass. The hydrolysed material in effect becomes of the same nature as the readily biodegradable COD originally present; hydrolysis merely adds to the pool of readily biodegradable material.

The differentiation of the two fractions in a wastewater at present is based completely on the observed kinetic response of the oxygen utilization rate. For example, the rate of oxygen utilization is measured in a system under a square-wave feed pattern; at feed termination the rate shows a precipitous decrease, hypothesized to be due to cessation of addition of the readily biodegradable COD fraction. The precipitous decrease allows an estimate to be made of the readily biodegradable fraction. Such an estimation clearly is completely defined in terms of the conceptual model devised to explain the observed response. This does not imply that the assumptions have a foundation in reality.

The concept of readily and slowly biodegradable substrate fractions has implications beyond its value in kinetic description of systems response with respect to carbonaceous oxygen utilization rates. There are strong indications that this concept is valuable also in understanding the biological excess phosphorus removal phenomenon, and more recently, in adding to the understanding of bulking phenomena. It would be most valuable therefore if experimental evidence could be produced that verifies that the bisubstrate concept in fact has a basis in reality.

Enquiring into the substantiation or rejection of the bisubstrate hypothesis has formed the main subject of this investigation. From the discussion above the objective of the investigation can be set out as follows:

(1) The magnitudes of the readily and slowly biodegradable fractions of a wastewater at present are determined by the biological assay procedure. Is there a correlation between the magnitudes as indicated by the chemical compositions of the fractions and the bioassay estimate. For example, glucose and starch subjectively are representative of readily and slowly biodegradable substrates respectively. Does the biological assay procedure reflect the magnitude of the glucose in the influent?

(2) Assuming that (1) above is found to be true, is the response of an activated sludge system receiving the artificial bisubstrate under dynamic conditions of flow and load adequately simulated by the general activated sludge model?

(3) The biological assay method for determining the readily biodegradable COD fraction is tedious and requires adequate laboratory facilities; for field application the method, in consequence, is not convenient. Is it possible to identify and estimate the readily biodegradable component by some physical-chemical separation technique such as centrifugation, filtration or ultrafiltration?

In Chapter Two, a brief history of the antecedents to the activated sludge model is set out, and the latest version is explained in detail. This historical development is fruitful in that it highlights the relevance of the present investigation. The description of the general model also is important for two reasons: (i) the model as constituted at present has not been written up in its latest form and (ii) a quantitative statement of the model is necessary in order to evaluate critically its capacity in simulation of observed responses of experimental activated sludge systems.

In Chapter Three, the experimental design and procedure for the kinetic investigation is set out. The results and analysis of results are presented in Chapter Four. In Chapter Five the studies of the physical-chemical separation technique are reported and a discussion of the findings of the investigation is presented in Chapter Six.

CHAPTER TWO

DYNAMIC MODEL DEVELOPMENT AND DESCRIPTION

INTRODUCTION

In this chapter we will first review very briefly the historical developments that led to the dynamic model as it exists at present. After this, the latest version of the model will be considered in detail.

In the historical development we will restrict the discussion to the processes involved in the conversion of the carbonaceous material; nitrogen conversion will not be dealt with. Although the processes with respect to nitrogen and carbon conversion are interlinked, the linkage is not essential to understanding the kinetics of either.

1. MODEL EVOLUTION

Steady state model of Marais and Ekama (1976): The Marais and Ekama steady state model (i.e. for constant input flow rate and concentration) constituted a development of the model proposed by Lawrence and McCarty (1970). The latters' model accepted the Monod equation which relates the specific organism growth rate with the concentration of substrate surrounding the organism. They also accepted the Herbert (1958) approach that concomitant with growth there is a continuous loss of volatile mass (endogenous respiration) at a rate directly proportional to the organism mass. Ekama and Marais extended the model further by incorporating some of the concepts proposed by McKinney (1962) viz. the generation and accumulation of endogenous residue due to endogenous respiration, and. the accumulation of inert volatile solids due to the presence of this material in the influent. Finally, they rejected the Biochemical Oxygen Demand (BOD) as a valid parameter for defining the carbonaceous material strength, and proposed instead the Chemical Oxygen Demand (COD). In this respect they accepted the equivalence between substrate (COD) and volatile solids (VSS). They proposed that the influent COD be divided into 3 fractions: (1) biodegradable, (2) unbiodegradable particulate and (3)

unbiodegradable soluble. Based on these assumptions they developed equations describing the steady state response for a single completely mixed reactor as a function of sludge age i.e. volatile active, endogenous and inert solids concentration and oxygen consumption rate for synthesis and endogenous respiration.

The Marais and Ekama (1976) steady state model differed substantially from any other model available at the time. Perhaps the most important aspects of the biological model related to (1) distinction between the active, endogenous and inert sludge fractions, (2) formulation of the biological reactions in terms of the active mass concentration, (3) linking oxygen utilization to the synthesis and endogenous reaction rates, and (4) the subdivision of the influent COD into the three fractions.

A steady state kinetic model of the activated sludge process facilitates the determination of parameters which will adequately describe the operation of an activated sludge plant under steady state conditions; the various equations for effluent quality, sludge concentration and oxygen requirements provide a basis for design of activated sludge plants. However, the usual input pattern to a plant is daily cyclic, hence a need exists for a dynamic model of the activated sludge process. Such a model would enable consideration of the effects of cyclic input patterns (flow and concentration, and thus load) to be predicted. By considering the system response under the input patterns expected in practice important information for design could be derived. For example, the peak oxygen utilization rate would define the peak aeration requirement. Furthermore a dynamic model would allow the development of effective process control procedures for prevention of process failure and for improving process performance.

Dynamic model of Ekama and Marais (1979): Ekama and Marais (1979) incorporated the biological processes identified in the steady state model of Marais and Ekama (1976) in a dynamic model. This model was utilized to predict, inter alia, the response observed in cyclically loaded Completely Mixed Activated Sludge (CMAS) systems receiving municipal wastewater as influent. In comparing observed and predicted responses the experiment

which was found to be most valuable was one in which a single reactor short sludge age (2,5 days) system was subjected to a square wave cyclic input pattern (12 hours feed/12 hours no feed, on a daily cyclic basis). In this system the oxygen utilization rate (OUR) response was found to be a particularly useful parameter for highlighting the system response. On termination of the feed an immediate, sharp drop in oxygen utilization rate was observed; thereafter, for several hours, the rate continued at a value of about 90 percent of that measured before feed termination. Subsequently over a period of about 5 to 6 hours the oxygen utilization rate decreased gradually, eventually reaching a constant value which could be ascribed to the oxygen requirement for endogenous respiration.

Simulation of the OUR response, by the dynamic model derived from the steady state theory, is compared to that observed in Fig 2.1. The model essentially predicts a square wave OUR response to the square wave influent pattern, and differs substantially from that observed. The difference between observed and predicted behaviour was thought to result from the nature of the influent substrate. Accordingly Ekama and Marais proposed a new hypothesis with regard to the constitution of the biodegradable influent COD. Marais and Ekama (1976) had accepted that the influent biodegradable COD consists of a homogeneous mix of organic matter and can be regarded as being "soluble" in the sense that it is absorbed and metabolized at a rate defined by Monod's rate equation. Instead Ekama and Marais (1979) noted that approximately two-thirds of the biodegradable COD content of municipal wastewater is in a fine particulate form; they observed that this material would require extracellular enzymatic breakdown before transfer through the cell wall. Furthermore, they proposed that a major portion of the soluble biodegradable COD consists of large complex molecules also requiring enzymatic breakdown i.e. very little of the biodegradable COD, whether soluble or not, could be utilized directly by the organisms. Therefore it was hypothesized that all the biodegradable material could be regarded as being of a particulate nature and initially is adsorbed and stored on the organism before being degraded extracellularly by enzyme action - and that the breakdown products then are absorbed through the cell wall. The utilization of particulate COD was

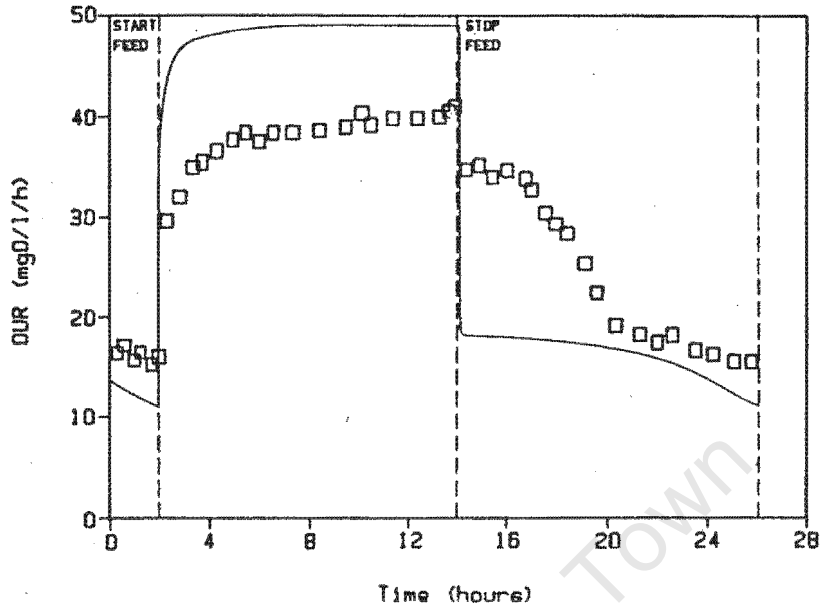

Fig 2.1: Comparison of the theoretical oxygen utilization rate response predicted by the Marais and Ekama (1976) model with that observed experimentally for daily cyclic square wave loading conditions at 2,5 days sludge age, pH = 7,0 and temperature 20°C. (after Dold, Ekama and Marais, 1980).

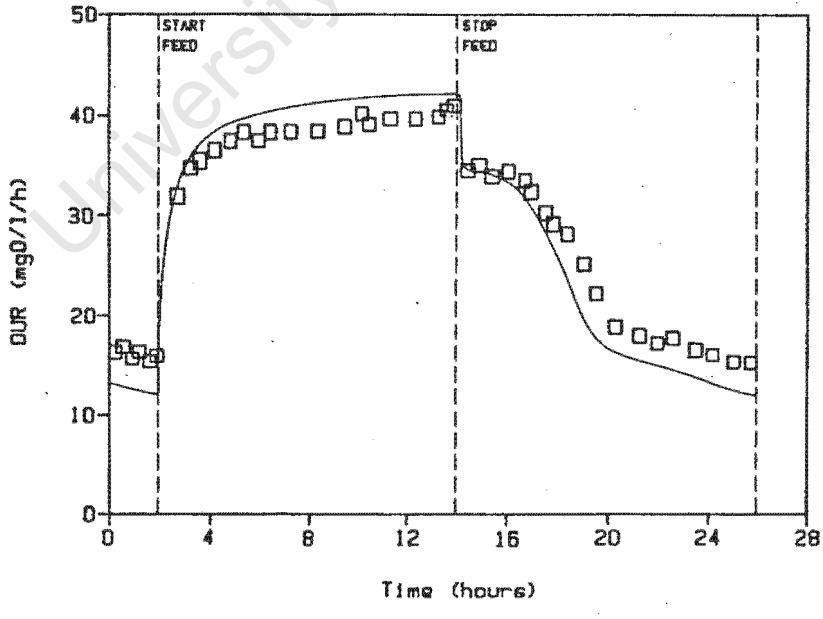

Fig 2.2: Comparison of the oxygen utilization rate response predicted by the theoretical model (Dold, Ekama and Marais, 1980) with that observed experimentally under daily cyclic loading conditions at 2,5 days sludge age, pH = 7,0 and temperature 20°C. (after Dold, Ekama and Marais, 1980).

modelled as a two-stage process: (2) adsorption for storage of substrate on the organism, according to adsorption kinetics and (2) substrate solubilization cum absorption cum utilization via a Monod-type function with respect to the stored substrate concentration.

In an attempt to explain the sharp step change in oxygen utilization rate on feed termination Ekama and Marais hypothesized that this may be due to an energy requirement for substrate adsorption. The step change in oxygen consumption rate often was used to determine the energy required for adsorption and storage of COD. The model incorporating this hypothesis gave reasonable agreement between predicted and observed OUR responses in the square wave tests (Fig 2.2).

Dold, Ekama and Marais (1981) model: The model of Ekama and Marais (1979) provided satisfactory simulation of observed and experimental data for a range of process conditions; however, there were several aspects in the model which were open to criticism, [Dold, Ekama and Marais (1981)]. The first of these related to the hypothesis on the adsorption of COD, and more particularly, to the energy requirement for this adsorption. In terms of thermodynamics, the adsorbed state is at a lower energy level than the unadsorbed one. If this also were true for the adsorption of particulate material onto organisms, then the hypothesis that the adsorption of COD is an energy-demanding process was incorrect. Furthermore the step-change in OUR in the square wave test was observed also in square wave cyclic loading experiments identical to those for Fig 2.1, but using a soluble readily biodegradable substrate (glucose) instead of municipal wastewater. On termination of the glucose feed the oxygen utilization rate decreased almost immediately to the steady value measured prior to commencement of the feed period, (i.e. to the "endogenous" rate) [see Fig 2.3]. The fact that the OUR response for glucose was so different to that for municipal wastewater, and that the glucose OUR response could be predicted reasonably well by the normal Monod growth rate expression, provided the basis for a new hypothesis. It was postulated that the biodegradable portion of municipal wastewater should be regarded as consisting of two fractions: (a) a readily assimilable (soluble) fraction which is utilized at a very rapid rate and (b) a slowly biodegradable (particulate) fraction

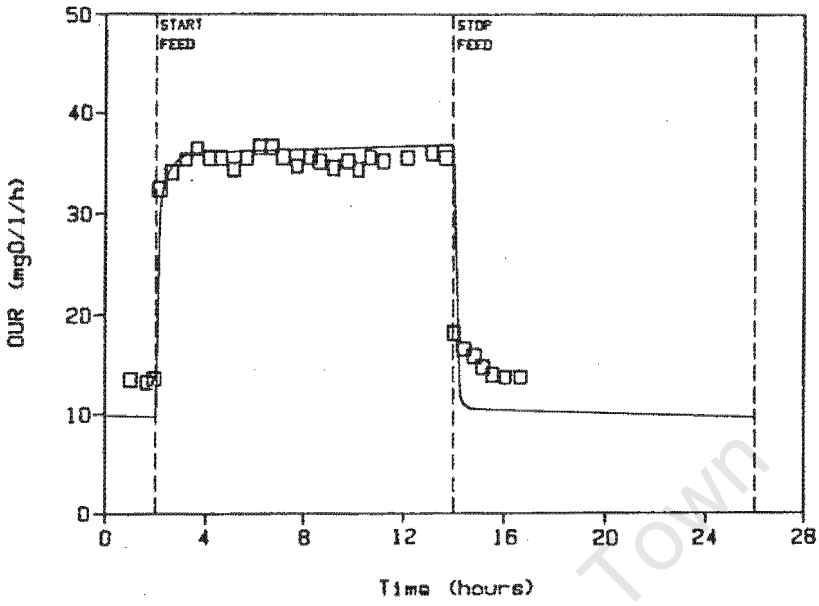

Fig 2.3: Comparison of the theoretical oxygen utilization rate response predicted by the Marais and Ekama (1976) model with that observed experimentally for daily cyclic square wave loading conditions at 2,5 days sludge age and temperature 20°C. with glucose as substrate. (after Dold, Ekama and Marais, 1980).

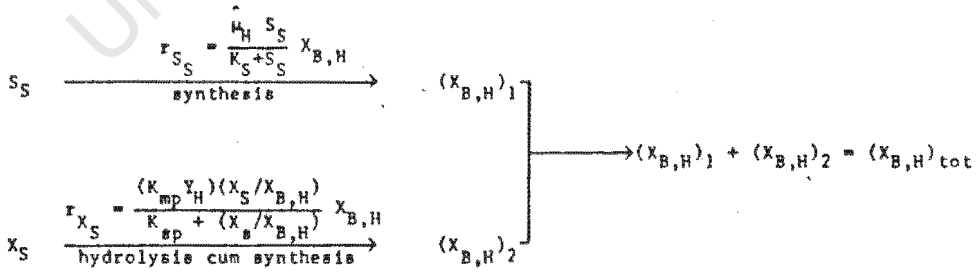

Fig 2.4: Diagram illustrating the fate of the soluble rapidly assimilable and particulate slowly biodegradable carbonaceous material fractions via the kinetic expressions of the Dold, Ekama and Marais (1980) model.

which requires storage and enzymatic breakdown prior to transfer through the cell wall and utilization for growth; this was the bisubstrate concept. In terms of this bi-substrate hypothesis the sharp step change observed in the oxygen utilization rate at feed termination using domestic wastewater could be attributed to the cessation of the oxygen requirement for the metabolism of the rapidly biodegradable substrate (COD) fraction in the influent. After feed termination the observed behaviour is due to the storage and utilization of the slowly biodegradable particulate fraction of the influent - the mechanism of storage and utilization remains unchanged and there is no energy requirement for adsorption. In order to account for the complete insensitivity of the effluent COD to severe cyclic load variations, an additional hypothesis was proposed in that all particulate material not adsorbed was assumed to be enmeshed in the sludge mass, densified in the settling tank and recycled to the reactor thus excluding this material from the effluent.

Acceptance of the bi-substrate hypothesis introduced the problem of the reaction of the organism mass to the two substrates. Although the exact mechanism was unknown, extensive simulations seemed to indicate that the two substrates are acted on independently by the same active mass and that the sludge synthesized is the sum of these two metabolic reactions as depicted in Fig 2.4:

Readily biodegradable substrate: The synthesis of active mass from the readily biodegradable substrate fraction was formulated according to the normal Monod relationship which links the specific growth rate of the active mass to the readily biodegradable (soluble) substrate concentration in the liquid phase.

Slowly biodegradable substrate: Utilization of particulate substrate was hypothesized to involve three distinct phases: (a) adsorption and storage, (b) extracellular enzymatic breakdown of the complex organic molecules to simpler components which can easily pass through the cell wall, and (c) synthesis of new cell mass by the organism. The overall reaction rate is slow - the limiting rate appears to be the rate of extracellular breakdown of the stored particulate material, rather

than the rate of metabolism of the material which passes through the cell wall. Hence the utilization of particulate substrate was modelled as a two stage process, i.e. two separate rate expressions were used to model (1) the adsorption and storage of the particulate material by the organism, and (2) the enzymatic breakdown of the stored substrate, and subsequent metabolism of the hydrolysis products by the active organisms for synthesis.

The mathematical description of the rate of hydrolysis of stored particulate substrate of Ekama and Marais (1979) was modified by Dold et al. (1981) to account for the observation that when the food to micro-organism ratio is high, the specific growth rate should be very high, in fact close to or at its maximum, because there is a large amount of stored substrate relative to active mass, i.e. the rate should depend upon the surface concentration of substrate rather than the bulk concentration as assumed by Ekama and Marais (1979). This was achieved by assuming that the surface concentration may be approximated by the ratio of the bulk concentrations of stored COD and active organism mass; the rate of utilization was described using an empirical expression for the rate of surface-mediated reactions developed by Levenspiel (1972). When this expression was incorporated in the mathematical model, application of the model to the suspension mixed aerated lagoon system predicted an acceptably high sludge growth rate even at retention times as low as 12 hours. Similarly, a significant improvement in predictions of the BOD-time curve observed in a BOD test was obtained. Consequently the Levenspiel expression was accepted as the rate equation for utilization of particulate biodegradable substrate.

A second modification to the model of Ekama and Marais (1979) relating to the endogenous respiration process, was proposed by Dold et al. (1981). Endogenous respiration is the mechanism proposed to explain the phenomenon of organism decay i.e. a reduction in active volatile mass per unit active mass per unit time. This process has been attributed to an energy requirement for organism maintenance, where a fraction of the organism mass disappears to provide energy for the maintenance of the mass remaining. As in

all biological systems an electron acceptor is a prerequisite for making the energy available; in the activated sludge process this requirement is served by either oxygen or nitrate. However in nitrification-denitrification systems, in situations where the concentrations of oxygen and nitrate are zero, maintenance energy apparently cannot be made available. Thus there was a need to develop an alternative model which could accurately reflect the situation with regard to endogenous respiration in aerobic and anoxic conditions, and also where aerobically generated organisms were placed in an anaerobic state for a short period. This led to the formulation of the death-regeneration model.

In the death-regeneration model an attempt is made to separate out the reactions which may take place during the organism's "death phase". The percentage of live mass that disappears is hypothesized to be due to death (natural or predation); through lysis of the dead cell, biodegradable substrate is transferred back into the liquid and a fraction remains as unbiodegradable endogenous residue. The lysed biodegradable substrate becomes part of the slowly biodegradable COD in the liquid, returning to the same cycle of adsorption, storage, hydrolysis and, finally, synthesis of new cell mass (i.e. regeneration). The main implication of this approach is that "maintenance energy" per se (the oxygen requirement for maintenance) was considered to be small so that it can be lumped with, and completely swamped, by the oxygen demand for the synthesis of new cell mass from the lysed substrate.

Simulation studies utilizing respectively the endogenous respiration and the death-regeneration approaches showed that for dynamic modelling of the aerobic activated sludge process there is little difference between the two approaches. However when modelling series reactor activated sludge systems incorporating denitrification, the death-regeneration approach appeared more suitable. When the nitrate concentration becomes zero in an unaerated reactor, synthesis ceases but organism death continues with the associated release of biodegradable substrate back into the liquid; this results in a build up of biodegradable material in the reactor. When the liquid passes to a downstream aerated reactor, the utilization of the accumulated biodegradable material results in a high oxygen utilization

rate, much higher than that obtained when using the endogenous respiration approach.

Model extension by van Haandel, Ekama and Marais (1981): Extra strength was lent to the bisubstrate/death-regeneration modelling approach by the work of van Haandel and Marais (1981) on denitrification systems. They showed that it was possible to incorporate the effects of anoxic zones in single sludge systems by making only one non-qualitative change to the aerobic model; namely, when nitrate serves as electron acceptor the rate of utilization of the particulate COD apparently was reduced to 40 percent of the rate under aerobic conditions. The rate of utilization of the readily biodegradable COD was presumed also to be reduced to 40 percent of the aerobic rate. The parallel between aerobic and anoxic behaviour is highlighted by Fig. 2.5. In a laboratory scale single sludge nitrification-denitrification system consisting of a series of three reactors with a plug flow anoxic reactor (pre-denitrification reactor) preceding a completely mixed aerobic reactor and another plug flow reactor subsequent to the aerobic reactor (post-denitrification reactor) three linear nitrate reduction phases were observed. In the pre-denitrification reactor the first, rapid denitrification rate in Fig. 2.5(b) is due to the rapid utilization of the readily biodegradable substrate in the influent and the second, less rapid, rate to the slower utilization of particulate substrate derived both from the influent and from death and lysis of the active organisms mass. The rate of denitrification is even lower in the post-denitrification reactor, being due almost entirely to the utilization of slowly biodegradable particulate COD from cell death and lysis. A further important observation relates to the amount of denitrification observed during the initial phase in the predenitrification reactor, due to the utilization of the readily biodegradable COD. In terms of the bisubstrate theory it should be possible to determine the amount of influent readily biodegradable COD from the nitrate reduction during the first phase. This in fact was the case in that this readily biodegradable COD concentration calculated from the nitrate reduction corresponded to that determined from the square wave aerobic system where the readily biodegradable COD is determined from the magnitude of the step change in OUR.

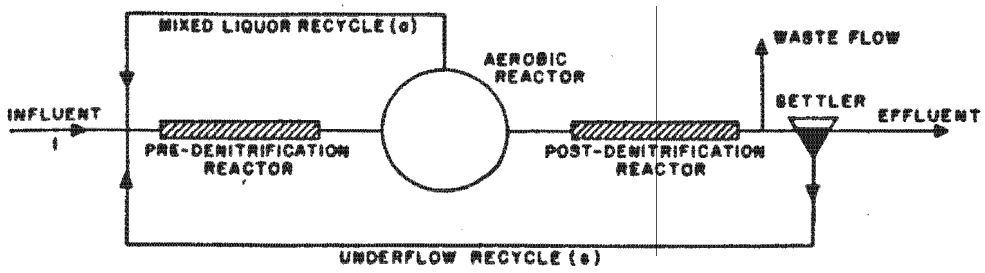

Fig 2.5(a): Configuration including pre- and post-denitrification plug flow reactors.

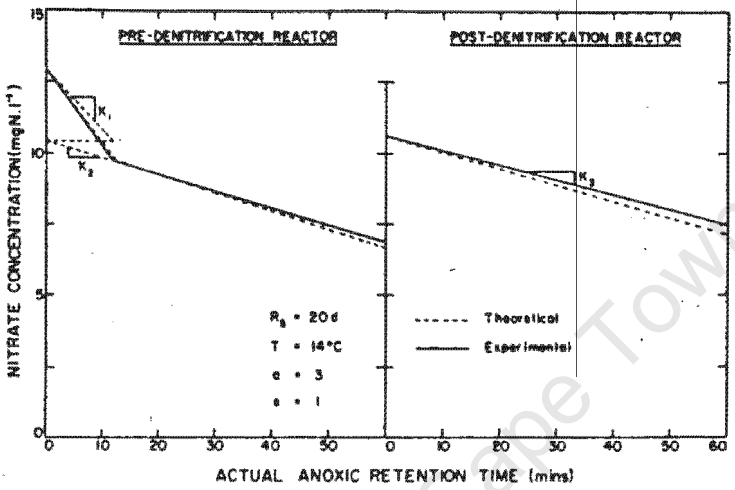

Fig 2.5(b): Comparison of experimentally observed and theoretically predicted nitrate concentration-retention time profiles in the pre- and post-denitrification reactors.

The aerobic activated sludge system model of Dold et al., as modified by Van Haandel and Marais to incorporate unaerated reactors, was very successful in predicting the behaviour observed in a wide range of systems operated over a wide range of conditions. The model was utilized without change until 1985. At this point modifications to the model were proposed by a Task Group on mathematical modelling of wastewater treatment of the International Association on Water Pollution Research and Control (IAWPRC).

IAWPRC Task Group model (1985): The IAWPRC Task Group (which included Marais together with Grady of the USA, Gujer of Switzerland, Henze of Denmark and Matsuo of Japan) was appointed in 1982 to review modelling of activated sludge systems; their deliberations culminated in 1985 with the proposal of a revised model. Two changes in the Dold et al./Van Haandel et al. model were put forward, in the enmeshment-adsorption (storage) and in the solubilization (hydrolysis) concepts. The Group agreed that all

particulate material is enmeshed almost completely by the sludge mass and thus would not be present in the secondary settled effluent even if unmetabolised. However the Group rejected the hypothesis that the biodegradable particulate COD is adsorbed and stored on the organism mass and then utilized directly for growth; instead they proposed that the biodegradable substrate is stored virtually instantaneously and hydrolysed to readily biodegradable substrate by extracellular enzymes; the hydrolysis products released into the bulk liquid which together with the readily biodegradable influent COD, constitutes a single pool of substrate to be utilized for synthesis by the active organisms, via Monod's relationship. They restricted the growth rate equation of the particulate COD to an hydrolysis function only. The differences between the two models are evident from Fig 2.6.

With the modifications described above the expression for the rate of particulate substrate storage in the model of Dold et al. falls away. Even in the Dold et al. model the necessity to include a rate of storage expression is doubtful; analysis of the effect of the expression indicates that the storage rate is extremely rapid in the Dold et al. model, so much so that replacing it by immediate storage leads to inconsequential changes in the model predictions.

Bearing in mind the proposals of the IAWPRC Task Group, Dold and Marais (1985) conducted a comprehensive evaluation of the Dold et al./Van Haandel et al. model, and the Task Group model, in an effort to reconcile some of the apparent differences between the Dold model and the Task Group model. This study led to the adoption of certain of the Task Group proposals, but in turn suggested certain modifications to the IAWPRC model. Dold and Marais accepted the proposals of the Task Group that (1) immediate enmeshment-storage of particulate COD occurs, and the rate of storage expression is not necessary, and (2) solubilization of particulate COD occurs rather than direct utilization.

Comparison of Figs 2.4 and 2.6 indicates that for aerobic systems very little difference exists between the original Dold et al. model and the Task Group model. However, with respect to denitrification, major

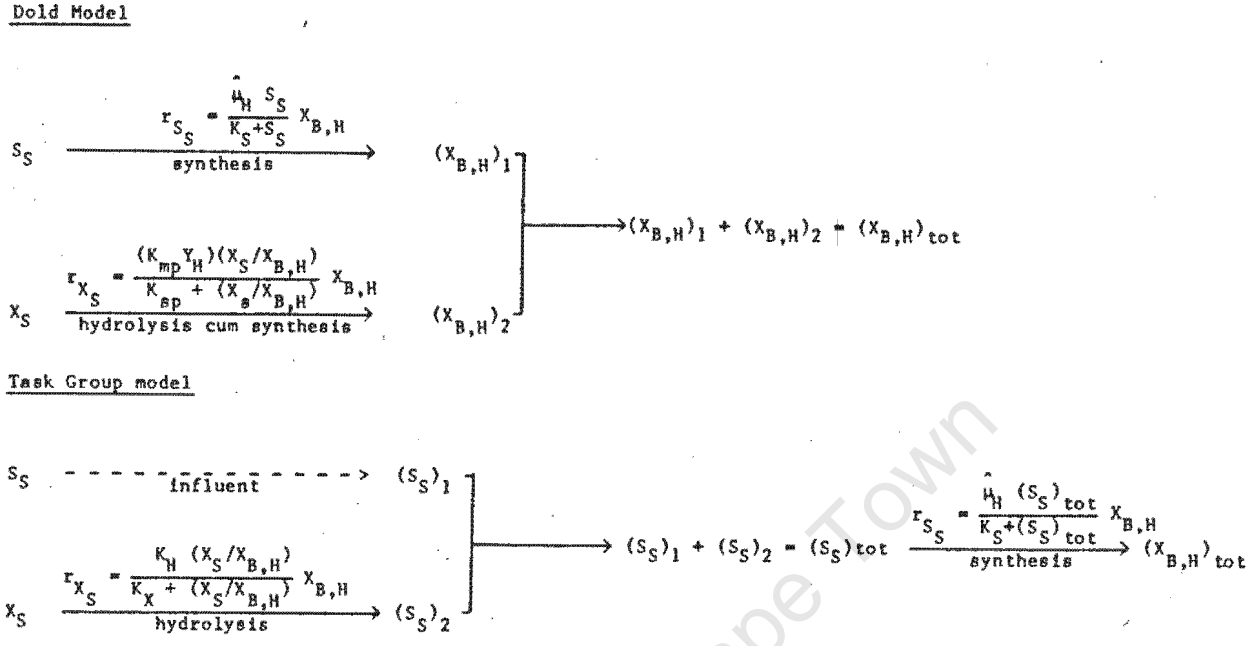

Fig 2.6: Diagram illustrating similarities/differences between the model of Dold *et al.* and the Task Group model with respect to hydrolysis/synthesis of carbonaceous material under aerobic conditions.

differences in interpretation between the two models arise. In the Dold model, hydrolysis and synthesis of stored substrate were considered in one overall reaction, with a reduced growth rate under anoxic conditions i.e. ± 40 percent. In the Group model, hydrolysis is separated from growth; there is no indication as to whether the reduced growth rate under anoxic conditions is due to a lower rate of hydrolysis or to a lower rate of synthesis of new organism mass.

From an analysis, by simulation, of the denitrification observations of Stern and Marais (1974) and Van Haandel *et al.* (1981), Dold and Marais came to the conclusion that the factor of 40 percent needs to be applied to the hydrolysis rate. This implies that solubilization is the rate limiting step in anoxic growth from particulate biodegradable COD.

Possibly the most significant feature of the recent models (since 1981) in respect to carbonaceous energy removal is the bisubstrate approach to the nature of the influent COD and the fate of the respective influent fractions. Adopting (1) the bisubstrate approach with different kinetic expressions for utilization of the different COD fractions, and (2) accepting the division of the overall influent COD into biodegradable and unbiodegradable fractions (with soluble and particulate subdivisions) has provided the basis for a comprehensive general model. That is, a single model with a single set of kinetic constants capable of reasonably accurate prediction of dynamic response in systems incorporating COD oxidation, nitrification and denitrification, and prediction of sludge production, etc., over a wide range of sludge ages.

The division of the biodegradable influent COD into readily and slowly degradable portions has been an inferential one; that is, the division has been calculated, for example, from the response of the oxygen utilization rate in the square wave test described earlier. The Marais Group has always stressed that this division is a kinetic-biological one; however, there has been the underlying suggestion that "readily biodegradable" is synonymous with "soluble" and "slowly biodegradable" with "particulate cum colloidal". This parallel was endorsed by the square wave tests in which glucose replaced municipal wastewater. However, no attempt has been made to specifically correlate the readily biodegradable COD measurement from the OUR/square wave method with measurement of the soluble COD fraction obtained by, say, filtration. Also, the models propose kinetic expressions for the concurrent utilization of the readily and slowly biodegradable COD, but evaluation of these kinetic expressions independently on single readily or slowly biodegradable substrates has been very limited.

The objective of this investigation has been to address the deficiencies referred to above. Specifically, the objectives have been:

(1) To evaluate the kinetic expressions for the utilization of readily and slowly biodegradable substrates by conducting experiments on activated sludge systems using single soluble and particulate substrates, and combinations of these.

(2) To compare the magnitude of the readily biodegradable COD in municipal wastewater derived from the "biological" assay with the magnitude of the soluble COD fraction obtained by various physical separation techniques (e.g. ultrafiltration). (This is reported on in Report No. W57).

2. DESCRIPTION OF CURRENT MODEL

2.1 Model Representation

A complex activated sludge system model incorporating carbonaceous material oxidation, nitrification and denitrification necessarily must attempt to account for a large number of conversion (reaction) processes occurring within the system. The model should quantify, for each process, both the kinetics (rate-concentration dependence) and the stoichiometry (effect on the concentrations of species involved).

Because of the number of different components in the model, and because of the number of biological conversion processes to be modelled, it is convenient to present the model in a matrix format. This ensures clarity as to what compounds, processes and reaction terms are to be incorporated and facilitates transforming the model into a computer program. The matrix method for model presentation in the context of biological systems was proposed by Dr Willi Gujer of the Swiss Federal Institute for Water Resources and Water Pollution Control, and has been utilised by the IAWPRC Task Group on mathematical modelling in wastewater treatment design. The method is based on the approach to chemical kinetic modelling of Peterson (1965).

Setting up the Matrix: The matrix method for representation of process kinetics and stoichiometry is best illustrated by using an example. Consider the information presented in Table 2.1 for a simple Monod-Herbert model for aerobic microbial growth on a soluble substrate, accompanied by organism death:

The first step in setting up the matrix is to identify the compounds of relevance in the model. The Monod-Herbert model quantifies growth of biomass (X_B) at the expense of soluble substrate (S_S). By keeping track of

Table 2.1: The activated sludge system general model presented in matrix form.

Component → 1 Process ↓	1 $X_{B,H}$	2 X_E	3 $X_{B,A}$	4 X_S	5 X_I	6 X_{ND}	7 S_S	8 S_{NH}	9 S_{ND}	10 S_{NO}	11 S_{ALK}	12 S_I	13 S_O	Process Rate, ρ_j $M\ L^{-3}\ T^{-1}$
Aerobic growth of heterotroph with ammonia-N	1						$-\frac{1}{Y_H}$	$-i_{XB}$			$-i_{XB}/14$		$-\frac{1-Y_H}{Y_H}$	$\hat{\mu}_H\left(\frac{S_S}{K_S+S_S}\right)\left(\frac{S_O}{K_{O,H}+S_O}\right)X_{B,H}$
Anoxic growth of heterotrophs	1						$-\frac{1}{Y_H}$	$-i_{XB}$		$-\frac{1-Y_H}{2.86Y_H}$	$\frac{1-Y_H}{14\times2.86\times Y_H}$ $-i_{XB}/14$			$\hat{\mu}_H\left(\frac{S_S}{K_S+S_S}\right)\left(\frac{K_{O,H}}{K_{O,H}+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)X_{B,H}\,\eta_G$
Death of heterotrophs	-1	f_E		$1-f_E$		$i_{XB}-f_E i_{XE}$								$b_H X_{B,H}$
Hydrolysis of enmeshed COD				-1			1							$K_H\frac{(X_S/X_{B,H})}{K_X+(X_S/X_{B,H})}\left[\left(\frac{S_O}{K_{O,H}+S_O}\right)+\eta_S\left(\frac{K_{O,H}}{K_{O,H}+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\right]X_{B,H}$
Hydrolysis of particulate organic N						-1			1					$\rho_6(X_{ND}/X_S)$
Ammonification of soluble organic N								1	-1		1/14			$K_R\ S_{ND}\ X_{B,H}$
Aerobic growth of autotrophs			1					$-i_{XB}-\frac{1}{Y_A}$		$\frac{1}{Y_A}$	$-i_{XB}/14$ $-1/7\ Y_A$		$-\frac{4.57-Y_A}{Y_A}$	$\hat{\mu}_A\left(\frac{S_{NH}}{K_{NH}+S_{NH}}\right)\left(\frac{S_O}{K_{O,A}+S_O}\right)X_{B,A}$
Death of autotrophs		f_E	-1	$1-f_E$		$i_{XB}-f_E i_{XE}$								$b_A X_{B,A}$
Observed Conversion Rates, $ML^{-3}T^{-1}$	$r_i=\sum_j \nu_{ij}\rho_j$													
Stoichiometric parameters:	Active heterotrophic biomass - $M(COD)L^{-3}$	Endogenous mass - $M(COD)L^{-3}$	Active autotrophic biomass - $M(COD)L^{-3}$	Enmeshed slowly degradable substrate - $M(COD)L^{-3}$	Unbiodegradable particulate substrate - $M(COD)L^{-3}$	Particulate organic degradable nitrogen - $M(N)L^{-3}$	Readily biodegradable substrate - $M(COD)L^{-3}$	NH_3-NH_4^+ nitrogen $M(N)L^{-3}$	Soluble organic degradable nitrogen - $M(N)L^{-3}$	NO_2 + NO_3 nitrogen $M(N)L^{-3}$	Alkalinity Molar units	Unbiodegradable soluble substrate - $M(COD)L^{-3}$	Oxygen - negative COD $M(-COD)L^{-3}$	

Heterotroph yield: Y_H

Autotrophic yield: Y_A

Inert fraction of biomass: f_E

Mass N/Mass COD in biomass: i_{XB}

Mass N/Mass COD in inerts: i_{XE}

Kinetic Parameters:

Heterotrophic growth and decay:

$\hat{\mu}_H$, K_S, $K_{O,H}$, K_{NO}, b_H

Correction factor for anoxic heterotrophic growth: η_G

Autotrophic growth and decay:

$\hat{\mu}_A$, K_{NH}, $K_{O,A}$, b_A

Hydrolysis: K_H, K_X

Correction factor for anoxic hydrolysis: η_S

Ammonification: K_R

X_B and S_S, it is possible to calculate the oxygen requirement, so oxygen (S_O) can be included as a third component. The compounds are presented as symbols across the top of the table, and are defined (with units) at the bottom of the corresponding matrix columns. The index "i" is assigned to the range of compounds. In this case, "i" ranges from 1 to 3 for the three compounds considered in this simple model. It should be noted that the recommended symbol notation of the IAWPRC has been followed; namely, X for particulate matter and S for soluble materials. (IAWPRC, 1982).

The second step in developing the matrix is to identify the biological processes occurring in the system. These are any conversions or transformations which affect any of the compounds considered in the model. Only two processes are considered in this simple model - aerobic growth of organisms at the expense of soluble substrate and organism death. These are itemised one above the other at the left of the matrix. The index "j" is assigned to the range of processes; in this case "j" can only take on a value of 1 or 2.

The kinetic expressions (rate equations) for each process are recorded down the right hand side of the table in the appropriate row. These are given the symbol ρ_j with j denoting the biological process. The kinetic parameters incorporated in the rate expressions are defined at the lower right corner of the matrix. The elements in the matrix comprise the stoichiometric coefficients, υ_{ij}, which relate the rate of conversion, r_{ij}, of a compound to the process rate, ρ_j. For example, aerobic growth of heterotrophs occurs at the expense of readily biodegradable substrate; oxygen is utilised in the metabolic process. The stoichiometric parameters are defined at the lower left of the table.

The coefficients υ_{ij}, are simplified by working in consistent units; in this case all concentrations are expressed as COD equivalents. The sign convention used in the matrix is "negative for consumption" and "positive for production".

Whilst entering the stoichiometric coefficients, cognisance must be taken of the units used in the rate equation. For example, the rate equation for

aerobic growth of biomass, ρ_j, is written as a growth rate (and not as a substrate utilisation rate) with units of (mg cell COD growth)(mg substrate COD utilised)$^{-1}$(day)$^{-1}$. The stoichiometric values are thus normalised with respect to the biomass concentration i.e. for growth, the stoichiometric coefficients for X_B and S_B are 1 and -1/Y respectively, and not Y and -1.

Use in Mass Balances: Within a system, the concentration of a single compound may be affected by a number of different processes. An important benefit of the matrix representation is that it allows rapid and easy recognition of the fate of each component, which aids in the preparation of mass balance equations. The basic equation for a mass balance within any defined system boundary is:

$$\begin{Bmatrix} \text{Rate of} \\ \text{Accumulation} \end{Bmatrix} = \begin{Bmatrix} \text{Rate of} \\ \text{Input} \end{Bmatrix} \begin{Bmatrix} \text{Rate of} \\ \text{Output} \end{Bmatrix} + \begin{Bmatrix} \text{Rate of} \\ \text{Reaction} \end{Bmatrix} \quad (1)$$

The input and output terms are transport terms and depend upon the physical characteristics of the system being modelled. The reaction term is obtained by summing the products of the stoichiometric coefficients υ_{ij} times the process rate expression, ρ_j, for the component i being considered in the mass balance i.e. moving down the column for the specific component i. For example, the process conversion rate for biomass would be:

$$\hat{\mu} S_S/(K_S + S_S)\ X_B - b\ X_B \quad (2)$$

The observed conversion rate would be combined with the appropriate advective (flow) terms to create the mass balance equation for a particular system.

Switching Functions: At this point, it is worth introducing an aspect of the kinetic equations utilized extensively in the model - namely the use of "switching functions". Consider the aerobic growth of biomass. In this case, the Monod growth rate equation has been utilised, viz:

$$\mu = \hat{\mu}\left(S_S/(K_S + S_S)\right) X_B \qquad (3)$$

In an environment where the dissolved oxygen concentration (S_O) is zero (or perhaps close to zero) the rate of this aerobic process also should decrease to zero. Mathematically, this can be achieved by multiplying the Monod rate expression by a "switching" factor which is zero when S_O is zero, and unity when the organisms' environment is aerobic. In this case, the switching function is of the form:

$$S_O/(K_O+S_O) \qquad (4)$$

where K_O = switching constant of small magnitude (say 0,1 mgO/ℓ).

With this "switching function" operating on the growth rate equation, when S_O is zero, the value of the function is zero and the process rate, ρ_1, will be zero. However, if S_O is say 3 mgO/ℓ, then the value is close to unity and the process rate will then be that given by the Monod equation. The selection of a small value for K_O means that the value of the switching function decreases from near-unity to zero only at very low S_O values, i.e. when the D.O. value decreases below, say, 0,2 mgO/ℓ. However, the function is mathematically continuous, which helps to eliminate problems of numerical instability in simulating system behaviour; such problems can arise if the rate is switched "on" and "off" discontinuously.

2.2 Model Description

A quantitative description of the current version of the general model and the interrelationships between the parameters is facilitated by consideration of Table 2.1, which is the matrix representation of the model. The matrix representation incorporates the processes relating to nitrification (growth and death of autotrophs) even though this aspect has been excluded from the current investigation. Inclusion of these is merely for completeness. The stoichiometric, kinetic and switching function constants for use in the model at 20°C are listed in Table 2.2.

1. Aerobic growth of heterotrophs using ammonia as synthesis nitrogen source: This process is responsible for a large portion of the removal of organic matter (and its oxygen requirement) and production of the

Table 2.2: Kinetic, stoichiometric and switching function parameters for general model.

Symbol	Value	Units
Kinetic parameters:		
$\hat{\mu}_H$	2,4-5,0	day^{-1}
K_S	5,0	gCOD m^{-3}
b_H	0,62	day^{-1}
K_H	2,2	gCOD (g cell COD.day)$^{-1}$
K_X	0,15	gCOD (g cell COD)$^{-1}$
K_R	0,016	m^3 (g cell COD.day)$^{-1}$
$\hat{\mu}_A$	0,2-0,65*	day^{-1}
K_{NH}	1,0	g NH_3-N m^{-3}
b_A	0,04	day^{-1}
η_S	0,38	-
η_G	0,7-1,0	-
Stoichiometric parameters:		
Y_H	0,666	g cell COD yield (g COD utilized)$^{-1}$
Y_A	0,15	g cell COD yield (g N utilized)$^{-1}$
f_E	0,08	-
i_{XB}	0,068	gN (gCOD)$^{-1}$ in active organisms
i_{XE}	0,068	gN (gCOD)$^{-1}$ in endogenous residue
Switching function parameters:		
$K_{O,H}$	0,002	g O_2 m^{-3}
$K_{O,A}$	0,002	g O_2 m^{-3}
K_{NO}	0,15	g NO_3-N m^{-3}

* With regard to the rate of nitrification from ammonia, there is conclusive evidence that the maximum specific growth rate constant for the autotrophs ($\hat{\mu}_A$) is a characteristic of the waste flow. The value of μ_H at 20°C can range from 0,20 to 0,65 day^{-1}. In consequence its value needs to be determined by calibration for each waste flow. One procedure for the determination has been set out (Water Research Commission, 1984).

bulk of the MLVSS. Concomitant with growth, the small nitrogen requirement for synthesis purposes is supplied from the pool of ammonia nitrogen, and there is an associated alkalinity change. The rate of growth reduces to zero in the absence of dissolved oxygen or when the ammonia concentration decreases to low values i.e. two switching functions. At low S_O concentrations, there is a switch to anoxic growth (Process 2), and when ammonia is limiting there is a switch to aerobic growth with the nitrogen requirement for synthesis being supplied from the nitrate pool (Process 7).

2. Anoxic growth of heterotrophs using ammonia as synthesis nitrogen source: In the absence of dissolved oxygen, a fraction, η_G, of the heterotrophic organism population is capable of using nitrate, if available, as terminal electron acceptor. This process occurs at the expense of organic matter and results in the production of MLVSS; the product of nitrate reduction is N_2 gas. As with aerobic growth, ammonia nitrogen is incorporated in the new cells and there is an alkalinity change. At low ammonia concentrations, the rate decreases to zero and there is a switch to anoxic growth with the nitrogen requirement for synthesis being supplied from the nitrate pool (Process 8).

3. Decay of heterotrophs: The process is modelled according to the "death-regeneration" hypothesis. That is, the heterotrophic organism mass decays at a certain rate; a portion of the material from the decay is non-degradable and adds to the endogenous cell residue while the remainder adds to the pool of particulate biodegradable COD. Particulate nitrogen associated with the particulate biodegradable COD becomes available as soluble organic nitrogen. The process of organism death is assumed to continue under aerobic, anoxic and anaerobic conditions.

4. "Hydrolysis" of particulate COD: Particulate COD enmeshed in the sludge mass is broken down extracellularly, with the products of breakdown adding to the pool of readily biodegradable substrate available to the organisms for synthesis purposes. This "hydrolysis"

process is modelled on the basis of Levenspiel's surface reaction kinetics, and occurs only under aerobic or anoxic conditions; the rate of hydrolysis under anoxic conditions is reduced to a level of η_s times the rate in an aerobic environment. Hydrolysis does not occur under anaerobic conditions.

5. "Hydrolysis" of particulate organic nitrogen: Biodegradable particulate organic nitrogen is broken down to soluble organic nitrogen at a rate defined by the hydrolysis reaction for carbonaceous particulate matter (4 above). The product of breakdown adds to the pool of soluble organic nitrogen.

6. Ammonification of soluble organic nitrogen: Soluble organic nitrogen is converted to free and saline ammonia, a process mediated by the active heterotrophs. Acidity produced in the conversion process results in an alkalinity change.

7. Aerobic growth of heterotrophs using nitrate as synthesis nitrogen source: Under certain loading patterns in nitrifying systems, ammonia is not available in sufficient quantity at all times to supply the nitrogen requirement for synthesis. In this situation, the heterotrophs can use nitrate as a nitrogen source for synthesis. The stoichiometry of this process parallels that for growth of heterotrophs using ammonia nitrogen for synthesis (Process 1).

8. Anoxic growth of heterotrophs using nitrate as synthesis nitrogen source: In the event of anoxic growth (Process 2) being limited by the availability of ammonia, there is a switch from ammonia to nitrate as the nitrogen source for synthesis purposes. (This switch parallels the switch from Process 1 to 7).

CHAPTER THREE

EXPERIMENTAL PROGRAM

1. INTRODUCTION

In Chapter One it was stated that two of the objectives of this investigation were to (i) check if the general activated sludge model of Dold, Ekama and Marais had validity and if so to (ii) check if the general activated sludge model of Dold, Ekama and Marais (1980), as modified by Dold and Marais (1985), is a reliable instrument to simulate the response of aerobic systems under dynamic loading conditions receiving an artificial substrate.

The bi-substrate hypothesis subdivides the biodegradable fraction of the influent into two fractions, readily biodegradable and slowly biodegradable. The readily biodegradable fraction is hypothesized to be directly absorbed and metabolized by the organism at a relatively high rate. The slowly biodegradable fraction is hypothesized to consist of large molecules that cannot pass through the cytoplasmic membrane but need to be hydrolyzed extracellularly to simpler organic units that can pass through the membrane, that is, hydrolysis must produce readily biodegradable COD extracellularly from the slowly biodegradable COD. Hydrolysis, it is hypothesized, takes place at a relatively slow rate compared to the rate of utilization of the readily biodegradable COD. The readily biodegradable substrate produced by hydrolysis, together with the readily biodegradable substrate from the influent, forms the "pool" of readily assimilable material available for synthesis. If slowly biodegradable COD only is available the rate of production of readily biodegradable material by hydrolysis will in effect govern the rate of synthesis. If readily biodegradable COD is available in the influent then the "pool" of readily biodegradable COD around the organisms will not be limited by that produced from hydrolysis and the rate of synthesis will reflect the presence of the additional readily biodegradable material.

By selecting two substrates, one readily biodegradable and one slowly biodegradable, from the responses of a system receiving each substrate

separately, and a combination of the two substrates, one would be able to check via the general model (based on the bisubstrate hypothesis) whether these substrates indeed reflect the hypothesized behaviour.

2. BISUBSTRATE CHOICE

Two substrates that immediately come to mind as readily and slowly biodegradable possibilities, are glucose and starch. Glucose has a history as a substrate that is readily utilized by a wide range of micro-organisms; subjectively one has little difficulty in accepting glucose as readily biodegradable. With respect to starch, as a slowly biodegradable entity, it consists of chains of glucose molecules, it does not dissolve in water at 20°C. On mixing with water its behaviour appears to be influenced by the raw material from which it is manufactured and, possibly, the method of manufacture. This became evident when evaluating two starch samples: The first one was of Analar quality ("soluble starch", UnivAR registered) made by SaARchem. This starch would not mix in suspension but tended to form globules and drift to the surface. On boiling it dissolved, but subsequent experiments indicated that with boiling the starch chains apparently were broken down partially into smaller units possibly even single glucose units; on utilizing the boiled starch the response was analogous to that expected from a mixture of readily and slowly biodegradable substrates (see Section 4.4). The second starch was of General Reagent quality (maize starch laboratory reagent) made by BDH. It readily formed a suspension when mixed with cold water and in this respect was a more suitable choice of a particulate substrate. Consequently maize starch was selected as representative of the particulate substrate fraction.

The nutrients recipe, to be added to the single pure substrates or two-substrate mixtures initially was that proposed by Gaudy (1969). His recipe does not provide for a possible deficiency of "growth factors". With glucose as influent in the initial experiments, slime formation (see later) was so severe that it was ascribed to a deficiency of growth factors; to supply these yeast extract powder was added to the substrate solution, which resolved the slime problem. The nutrient concentrations, for a substrate concentration of 1500 mgCOD/ℓ, are given in Table 3.1.

In preparing the substrate feed a nutrient solution, without the yeast extract, of high concentration was made up using tap water, and stored at 20°C in a closed container. Each day the feed was made up with the required masses of substrate, yeast extract and nutrients; the mixture was then diluted to the required concentration using tap water. When "Soluble Starch" served as substrate the required daily mass of the starch was weighed and mixed with a small quantity of tap water, boiled for 5 minutes and cooled, and the nutrients added.

Table 3.1: Nutrient medium composition for substrate concentration of 1500 mgCOD/ℓ

Component	Concentration (mg/ℓ)
Substrate	1500
Ammonium Sulphate $(NH_4)_2SO_4$	750
Magnesium Sulphate $MgSO_4.7H_2O$	100
Iron (II) Sulphate $FeSO_4.7H_2O$	1
Manganese Sulphate $MnSO_4.4H_2O$	20
Calcium Chloride $CaCl_2.2H_2O$	15
Potassium dihydrogen phosphate KH_2PO_4	790
Dipotassium hydrogen phosphate K_2HPO_4	1600
Yeast extract	100

With maize starch as substrate, the starch mixed readily into a fine suspension, but the suspension slowly settled out on standing - the starch particles were kept in suspension by gentle stirring in the feed container.

3. REACTOR CONFIGURATION

3.1 Feed pattern

The Oxygen Utilization Rate (OUR) has been shown to be a useful parameter for delineating the system response to given input and load

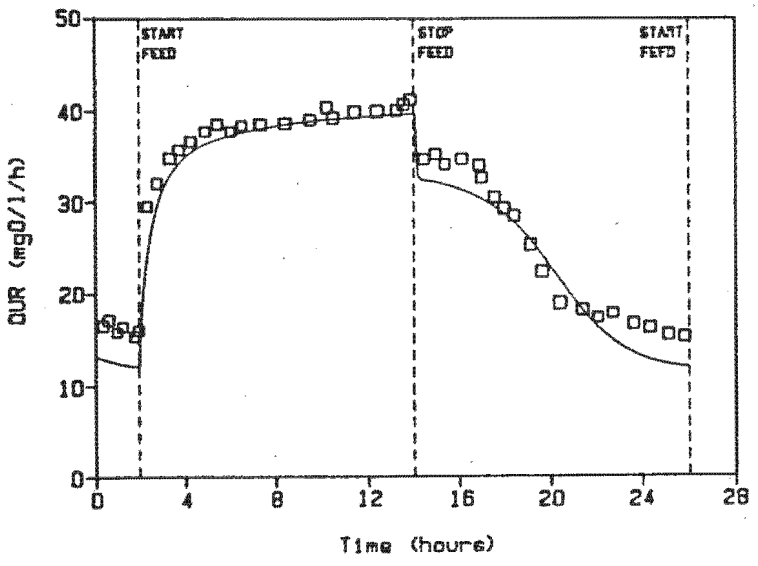

Fig 3.1: Experimental (and predicted) oxygen utilization rate in a single completely mixed aerobic activated sludge system at 2,5 days sludge age and 20°C under daily cyclic square wave loading conditions (12 hours feed/12 hours no feed). (after Dold, Ekama and Marais, 1980).

conditions - the OUR response of a short sludge age system under a square wave input pattern of flow and load (12h on/12h off), at feed termination, in fact formed the basis for proposing the bisubstrate hypothesis, see Fig 3.1. To separate out the effects of the particulate and readily biodegradable COD fractions, experience had shown that this was best done under the square wave cyclic feed pattern at relatively short sludge ages, 2-4 days. At longer sludge ages the responses of the two fractions tend to merge so that their respective effects are blurred. From these considerations it was decided to use a square wave-type feed pattern, influent at constant flow and load for a defined interval during a 24 hour period, and no feed for the remainder. By trial the length of the feed period and the substrate loading was to be determined taking account of the nature of the artificial wastewater (whether it was composed solely of readily biodegradable or slowly biodegradable substrate, or a mixture), for optimal discrimination in the response to the two substrates. For example, for a purely readily biodegradable substrate feed, the OUR response should drop precipitously on feed termination to a measured value approximately equal to that before commencement of the feed; for a feed composed solely of slowly biodegradable substrate, after feed termination the observed OUR should remain at the same constant level for some time before gradually

decreasing to the pre-feed or endogenous respiration level, and so on. The observed OUR response then could be checked against the predicted response of the bisubstrate general model.

3.2 System design

When dealing with pure substrate experimentation it is desirable to use the simplest apparatus possible, consistent with obtaining the required data to an acceptable degree of accuracy - one needs to reduce to a minimum the number of external factors which could have an influence on the system's response. The usual aerobic activated sludge system is a single completely mixed aerobic reactor with a secondary settling tank and sludge recycle. However at short sludge ages experience has shown that such a system is prone to sludge bulking; this causes a problem with the control of the mixed liquor concentration in the reactor and, if bulking is severe, sludge is likely to be lost in the effluent outflow. For this reason, it was decided to dispense with the settling tank and use a single completely mixed flow-through reactor system. In this system the flow from the reactor equals the flow to the reactor; there is no sludge recycling and the sludge retention time or sludge age is equal to the hydraulic retention time.

3.3 Experimental Apparatus

3.3.1 Reactor construction. A single 10ℓ reactor was constructed from 190 mm diameter perspex tube. Two vertical baffle plates were mounted inside the reactor to improve the mixing action by preventing the formation of a vortex. The mixed liquor in the reactor was kept thoroughly mixed by means of perspex paddles mounted along the submerged length of a stainless steel shaft driven by an electric motor which was mounted on the lid of the reactor (see Fig 3.2). The motor was geared to provide a stirring speed of 30 rpm. The size of the paddles and their position on the shaft were found by trial, to give complete mixing independent of the bubble aeration mixing effect without causing turbulence at the surface of the mixed liquor, yet provide sufficient turbulence so that the oxygen probe when immersed in the liquid mass would give stable response. The lid of the reactor, which was removable to allow brushing of the inside walls,

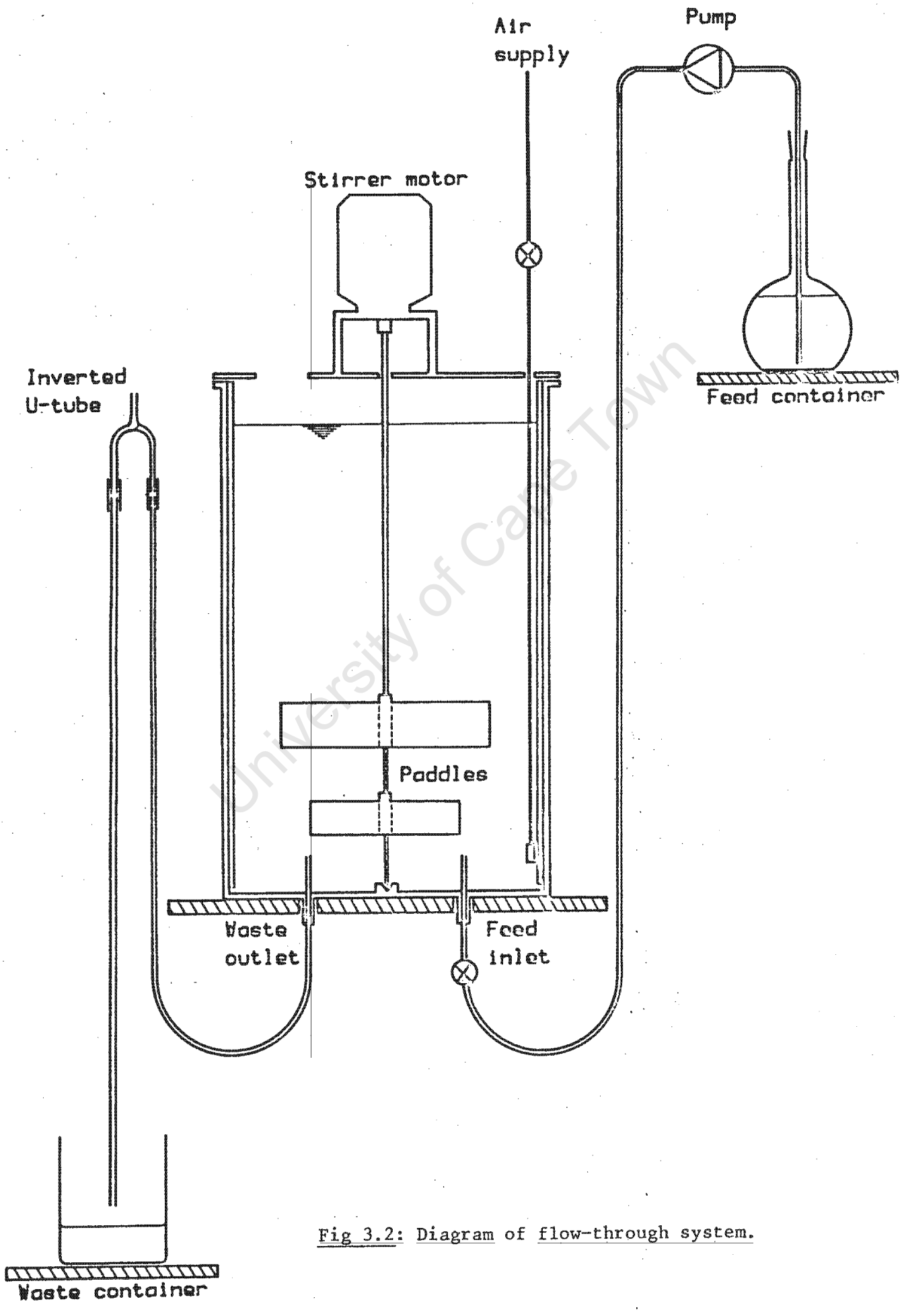

Fig 3.2: Diagram of flow-through system.

was provided with a 40 mm diameter access port through which the D.O. probe could be immersed in the mixed liquor. The inlet and outlet ports were located at the bottom of the reactor. The reactor volume was controlled, on the outlet line, by having an adjustable inverted U-tube overflow which could be set to give, and maintain, the desired mixed liquor volume.

Aeration of the mixed liquor was by means of a porous stone attached to a perspex tube which could be adjusted to a suitable depth in the reactor. A fish tank pump provided sufficient air to maintain a dissolved oxygen concentration of greater than 2,0 mgO/ℓ in the reactor at all times. During the feed period the D.O. level could rise to as high as 6,0 mgO/ℓ.

The diameter of the inlet port (approximately 3 mm) was chosen to ensure that the influent stream was injected into the mixed liquor at a reasonable velocity, so that it would be quickly dispersed throughout the reactor by the mixing action of the paddles. The reasons for this were twofold: (1) the feed flow rates varied between 11 mℓ/min for glucose and 1,5 mℓ/min for maize starch. The top of the inlet port was positioned in such a manner that the influent stream was directed straight up into the arc of the lowest, and largest, of the paddles. This ensured that however small the volume of feed entering the reactor per unit time, it was rapidly and efficiently dispersed throughout the mixed liquor, (2) in the case of the maize starch feed, short intermittent high speed delivery was used (e.g. 5 seconds on 20 seconds off) to scour starch particles in the inlet tube that settled during the 20 second intervals when the pump was switched off.

The waste flow exit port was also of small diameter (3 mm) to ensure a fairly high outflow of waste mixed liquor during the feed period. This was (1) to prevent possible blockage of the overflow by settlement of large sludge flocs, at low flow rates and (2) to allow abstraction of a constantly uniform concentration of mixed liquor.

The unit was operated in a laboratory maintained at a temperature of 20°C.

3.3.2 Pump and tubing: The feed pump was of the peristaltic type manufactured by Watson-Marlowe. With this pump it is possible to set the pump to deliver an exact volume of feed per unit time, by adjusting the peristaltic roller mechanism, the pump speed or with on/off operation of the pump. Silicon latex tubing with an internal diameter of 0,6 mm was used. The small bore of the tubing was selected to provide a high flow velocity during pumping and to minimise deposition of particulate matter and promoted scouring of such deposited material. The tubing was periodically checked and replaced when necessary as the tube had a tendency to wear into an oval shape under the roller action and deliver feed at lower rates than required. When pumping the glucose feed, the pump was operated continuously over the feed period. However with soluble starch, maize starch and mixture of glucose and maize starch in the feed, the pump was operated on/off on a fixed cycle time to ensure high flow rates during pumping; two electric timer devices controlled the lengths of the pump-on and pump-off periods.

3.3.3 Daily feed containers: The glucose feed was contained in a 5ℓ pyrex bottle. Pressure equalization with regard to the atmosphere was via a long thin glass tube which appeared to be effective in preventing air-borne bacteria from contaminating the feed. Maize starch feeds and glucose/maize starch mixtures were contained in a 2ℓ pyrex volumetric flask, also suitably stoppered. During the feed period the starch particles were kept in suspension by means of a small magnetic stirrer. This was important in order to maintain a uniform suspension concentration. Each day at the end of the feed period the feed container was cleaned and sterilized with boiling water.

3.4 System operation

3.4.1 Control of sludge retention time: For the special case of a flow-through system, under steady state, the sludge age of the system is exactly equal to the hydraulic retention time: as the influent feed is pumped in, an equivalent volume of mixed liquor is removed from the reactor via the waste overflow. Thus for a 10ℓ reactor receiving 4ℓ of influent per day; the sludge age (or sludge retention time) is fixed at 10/4 = 2,5 days, since every day 4 litres of mixed liquor is displaced.

In a flow-through system under cyclic flow and load conditions, the volume of mixed liquor removed still remains equal to the volume of feed discharged but now the sludge age is no longer exactly given by the hydraulic retention time. The reason for this is that during the feed period the concentration of active mass will increase so that the mass of sludge removed per unit of flow changes over the feed period. Indeed sludge age, determined by volume of reactor/volume of discharge per day, serves now only as an approximate parameter, for defining the loading condition approximately. However when comparing the response observed with that simulated, this difficulty does not affect the comparison because the exact same conditions of flow can be imposed in the simulation as in the experiment. Consequently when we speak of a sludge age of, say, 10 days in cyclic loading experiments, it is merely for fixing approximately the loading state imposed.

3.4.2 Experimental procedure: The feed was made up every day by using tap water. The feed was not sterilized for the following reasons: With maize starch this would have caused breakdown of some of the starch to glucose. But even with glucose as the only substrate it was found that sterilizing the substrate-nutrient mixture caused some precipitation of the nutrients. Using tap water did not appear to give rise to growth in the feed containers; this was checked by doing COD tests on the feed at the beginning and at the end of the feed period. However it was found most important to wash the feed container and the stirrers with boiling water every day to minimise innoculation from growth on the walls of the container.

The inside of the reactor was brushed twice daily to remove any sludge which may have adhered to the walls, and to return any sludge spattered on the walls above the 10ℓ level mixed liquor volume surface. The feed lines were regularly flushed out with hot water to remove any deposited substrate and to sterilise the tubes against wall growths. During the day regular gentle squeezing of the lines between the fingers was sufficient to dislodge stuck particles. Measurement of the waste sludge concentration in the waste bucket was performed in parallel with the measurement of the reactor sludge concentration to check if the two were the same.

Every two to three weeks, the mixed liquor was filtered through a fine brass gauze sieve to break up any large sludge flocs. At the same time the reactor and paddles were brushed down and cleaned with boiling water. The reactor was cooled by flushing with tap water before the sludge was returned to the reactor.

3.4.3 Experimental tests

The following tests were done:

(1) MLVSS
(2) COD of unfiltered influent, effluent and reactor contents
(3) COD of filtered effluent and reactor contents
(4) TKN of influent, and TKN and NO_3 of reactor contents from a filtered sample.
(5) OUR
(6) pH

Each day a 50 mℓ sample was pipetted from the reactor and the waste bucket respectively, a few drops of mercuric chloride solution added to terminate bacterial activity, and the sample centrifuged for a minimum of 20 minutes at 2000 rpm. The supernatant was drawn off and retained; the solids in the centrifuge tube were washed with distilled water into weighed evaporating crucibles in preparation for the MLVSS test. The supernatant from the centrifuged samples was vacuum filtered, firstly through two sheets of Whatmans No. 1 filter paper and then through a 0,45 µm Millipore filter membrane, and a COD test performed on the filtrate.

The COD of the unfiltered influent, reactor contents and waste bucket contents were done as follows: 10 mℓ samples were abstracted and suitably diluted to concentrations within the range 100 - 800 mgCOD/ℓ and COD tests done. The diluted concentration range was selected because in this range the standard COD test is the most accurate. [Standard Methods, 1971].

Throughout the experimental program nitrification in the reactor was suppressed by the addition of fresh thiourea solution (20 mg/ℓ reactor volume) at 2 day intervals. This effectively suppressed nitrification.

Continuous addition of thiourea was not practised as observation by Van Haandel et al. (1979) indicated that continuous addition affected heterotrophic growth whereas intermittent addition did not. Suppression of nitrification avoided the complication of the oxygen requirement for nitrification when monitoring oxygen utilization rates of the mixed liquor. To check if nitrification was effectively suppressed, samples were periodically removed from the mixed liquor to test for TKN and NO_3^- concentrations.

The pH of the mixed liquor was measured periodically and adjusted when necessary by the addition of sodium bicarbonate powder to the influent feed.

The oxygen utilization rate (OUR) measurement was of crucial importance to the experimental investigation. Over the experimental period every few days the OUR was measured on the system over 24 hours at half-hour intervals. The OUR was measured with a standard oxygen probe (Yellow Springs Instrument Co.); the D.O meter was connected to a Phillips chart recorder. The air flow to the reactor was increased to raise the D.O concentration to approximately 6,0 mgO/ℓ; thereupon aeration was stopped and the chart recorder plotted the change in D.O concentration with time. During measurement of the OUR, stirring and feeding were maintained. Aeration was restarted when the D.O concentration reached a lower level of 2,0 mgO/ℓ. The slope of the line plotted by the chart recorder provided the rate of oxygen consumption (in mgO/ℓ/hr).

The tests to determine the MLVSS, COD and TKN concentrations were performed in accordance with the procedures laid down in "Standard Methods for the examination of water and wastewater", 13th Edition (1971). Nitrate concentrations were determined by auto-analyser in accordance with the Industrial Methods 33,68 and 33,69 W test techinques as set out in the Technicon Auto-Analyser methodology. Mixed liquor pH was measured using a Radiometer type 80 pH meter.

CHAPTER FOUR

EXPERIMENTAL RESULTS AND ANALYSIS

It was stated in Chapter One that two of the objectives of this experimental program were to (i) test the validity of the bisubstrate hypothesis of Dold, Ekama and Marais and (ii) check whether the observed kinetic response of aerobic activated sludge systems receiving artificial wastewater under dynamic conditions of flow and load input is adequately simulated by the latest version of the general activated sludge model. These objectives are addressed in this Chapter.

Using the apparatus and following the experimental procedure described in Chapter Three, four sets of experiments were undertaken in which glucose, starch, glucose-starch and boiled "soluble starch", respectively, served as substrate.

The following presentation is employed in dealing with the four experimental sets: For each set, experimental procedure, results and evaluation are dealt with independently. The Chapter closes with a summary of the general findings and conclusions.

4.1 EXPERIMENT No.1: CYCLIC FEED WITH GLUCOSE AS SUBSTRATE

The objective of this experiment was to determine the response of an activated system receiving glucose as substrate under cyclic flow and load conditions.

The activated sludge system selected was a flow-through system at a sludge age of approximately 2,5 days. The reactor volume was set up at 10ℓ and the proposed flow was 4ℓ/day over an 8 hour feed period.

Waste sludge from an anaerobic-anoxic-aerobic system, operated at 20 days sludge age on unsettled municipal wastewater, served as an inoculum to start the process. A volume of 1,8ℓ of mixed liquor, with a concentration of approximately 3000 mgVSS/ℓ was added to the reactor. The initial glucose feed was made up according to the recipe of Gaudy (1969), which

did not include yeast extract; the daily volume of feed was 4ℓ at a COD of 1500 mg/ℓ.

The reactor was fed daily for 4 days, without wasting any sludge, as follows: Before the start of a feed period the mixed liquor was allowed to settle for about one hour, or until 4ℓ of clarified supernatant was available. Four litres of supernatant was abstracted and 4ℓ of the feed added to the reactor over the 8 hour feed period. This feed procedure was repeated for 4 days, a period subjectively assessed as adequate for adaption of the organisms to the glucose substrate.

On completion of the adaption period the operational mode was changed to a flow-through system: In this mode the reactor volume was maintained at 10ℓ and 4ℓ of substrate is fed per day, so that 4ℓ of mixed liquor discharges from the system during the feed period. (This gives an approximate sludge age of 10/4 = 2,5 days). The influent COD strength was maintained at 1500 mg/ℓ. The volume of substrate was fed over an 8 hour feed period i.e. over the remaining 16 hours no feed was added.

After about one week's operation on the flow-through mode the following behavioural patterns and measurement difficulties became apparent:

(1) Slime formation. On centrifugation of the mixed liquor, "slime" was present as a transparent jelly-like layer on the solid mass. The appearance of the mixed liquor also was gelatinous. From a search of the literature it seemed that the following factors could be implicated as causes of slime formation.

(i) Too high a food/micro organism (F/M) ratio
(ii) Nutrient deficiency
(iii) Inadequate oxygen supply.

Of these factors, nutrient deficiency was accepted to be the most likely cause. High F/M ratios might appear to be a cause, but slime formation in fact may be still due to nutrient deficiency - if the nutrient cannot diffuse into the organism at the same rate as the

substrate, it creates an artificial nutrient deficiency inside the organism. This situation had been positively identified as a cause for slime formation in aerobic treatment of apple juicing wastes in an activated sludge system with a selector. In the selector slime formation developed when the ammonia concentration was about 1,25 the concentration required for synthesis; when the ammonia concentration was increased to 3 times that needed for synthesis no slime formation was observed (Ekama, 1983).

Lack of oxygen was an unlikely cause because the dissolved oxygen concentration was maintained at ± 6 mgO/ℓ.

To reduce the possibility that a nutrient deficiency or the absence of some essential growth factor, was responsible for slime formation, the substrate was augmented by yeast extract (100 mg yeast extract powder/1500 mgCOD substrate). The system was cleaned and the process restarted as before. With yeast augmented substrate, slime production disappeared and was noted only sporadically in the mixed liquor. In subsequent experiments with starch and glucose-starch substrate no evidence of any slime formation was detected. The improvement in the condition of the mixed liquor, with yeast augmentation of the feed, was so dramatic that it was accepted that no further nutrient deficiency was present and that the sporadic (and rather minor) slime formation could be neglected as unimportant.

(2) Nitrification. One of the reasons for choosing to operate the system at a short sludge age of approximately 2,5 days was to avoid the added complications of nitrification and the associated nitrification oxygen demand. Assuming a maximum specific growth rate for nitrifiers at 20°C of 0,36/day the minimum sludge age for nitrification should have been about 3,1 days. However periodic checks of filtered effluent samples for nitrate and of the nitrogen balance between influent, sludge requirement for synthesis and effluent concentration showed that nitrification was taking place. Consequently it was decided to suppress nitrification by the addition of thiourea. It was found that if 20 mg/ℓ of thiourea per litre of reactor contents was added at 2

day intervals nitrification was suppressed. Continuous addition of thiourea was not considered advisable as previous experience in the laboratory had indicated that the continuous presence of thiourea affected the heterotrophic growth rate (Van Haandel and Marais, 1979). The spaced addition of thiourea was maintained with all the tests in this investigation.

(3) Measurement of VSS concentration. The inclusion of yeast extract in the nutrient medium prevented the formation of slime, yet the mixed liquor did not settle well. Furthermore, the concentration of mixed liquor was low and the organisms did not agglomerate into floc particles - most of the bacterial cells appear to be present as free organisms, individually suspended in the liquid. This made the measurement of VSS concentration very difficult, since even with prolonged centrifuging, it was not possible to remove all of the organisms by deposition. The volatile solid measurement therefore tended to be imprecise. To counter this the COD of the total mixed liquor was measured as an added feature and the COD of the volatile mass determined by subtracting the filtered COD from the total COD. This had the advantage in that it allowed an estimate of the COD/VSS ratio to be made.

(4) Measurement of filtered effluent COD concentration. Filtered samples of the effluent were initially obtained by filtering the centrifuge supernatant through a 0,45 μm membrane. However the free bacterial cells present in the supernatant tended to cause blockage of the membrane by deposition of a "blinding" layer on the surface. This problem was overcome to a degree by passing the centrifuge supernatant through several Whatman's No. 1 filter papers before passing through the 0,45 μm filter membrane. The series filtration was the more effective procedure in removing the free bacterial cells so that less blinding was experienced on the 0,45 μm filter. On some occasions however it was likely that some free bacterial cells still passed through the whole filter system, since several high COD values were recorded for the filtered (<0,45 μm) effluent COD, e.g. 200 mgCOD/ℓ, compared with the usual average value of about 80 mgCOD/ℓ.

The system, with the yeast augmented substrate, was operated in the flow-through mode under the cyclic feed pattern for 6 weeks with the following tests performed each day:

(1) on the influent, total COD and on the reactor contents, at the end of each load/no load cycle, total COD, filtered COD, and VSS

(2) on the effluent in the waste collection bucket, at the end of each load/no load cycle, total COD, and VSS

(3) once or twice a week, tests for TKN and NO_3^- on the filtered samples from (1) and (2) above and on the unfiltered influent

(4) every day, during the feed period and for 3 to 6 hours during the no feed period, the OUR response of the system was measured at 1 hour intervals.

The daily results of this set of tests (and indeed for the whole investigation) on the total COD of the influent, total COD and filtered COD of the effluent were plotted daily (see Fig 4.1). When the VSS and OUR data indicated that the system appeared to have attained a dynamic steady state (about 2 weeks after commencement of the flow-through dynamic mode of operation), 24 hours intensive testing was undertaken. Three sets of 24 hour test data were obtained, the following parameters being measured at the time intervals indicated:

Mixed liquor COD	End of no-feed period
Filtered COD	End of no-feed period
VSS	End of no-feed period, after 2 hours feed.
Waste overflow COD	End of no-feed period
OUR	at $\frac{1}{2}$ hour intervals during feed and no-feed periods

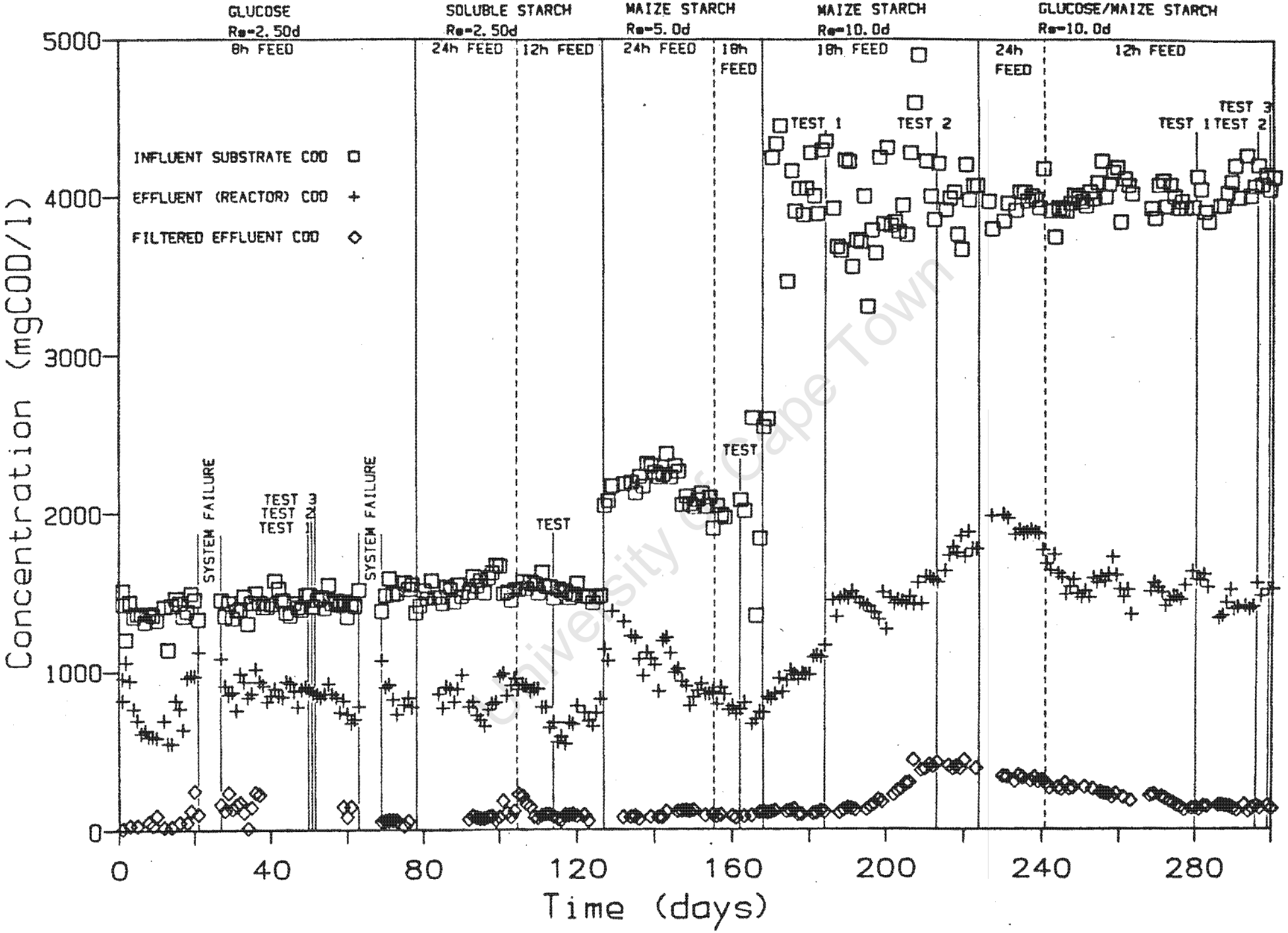

Fig 4.1: Observed daily system response during the period of investigation.

The results of the three 24 hour tests are listed in Appendix A and shown plotted in Figs 4.2 (a), (b) and (c).

Data Acceptability. Before a set of data can be taken as reliable it is essential to check if a mass balance on the COD is within acceptable limits. Consequently a COD mass balance on each of 24h experimental results was performed as follows:

Take experiment No. 1.2

COD in:

Measured influent COD	= 1450 mg/ℓ
Flow = 4ℓ/d	
Hence MCOD/d into system	= 5800 mg/d

COD out:

(1) COD of discharge (in bucket)	= 855 mg/ℓ
Flow	= 4 ℓ/d
Hence MCOD/d in discharge	= 3420 mg/d
(2) Oxygen demand/day	
This was determined from the area below the OUR curve over 24h.	
MOUR	= 2935 mg/d
Hence MCOD/d out of system	= 6355 mg/d
i.e. COD out/COD in	= 1,10

Mass balances of experiments 1.1 to 1.3 are listed in Table 4.1.

All the mass balances (Table 4.1) were greater than 100 percent. Mass balances of greater than 100 percent, i.e. more COD from the system than to the system, are not usual, recoveries normally range from 90 to 105 percent with the major number below 100 percent. Clearly some experimental error either in the COD or the OUR measurement (or both) introduced bias into the results. The data therefore could not serve as reliable measures to determine yield values or COD/VSS ratios but still is quite suitable to check the form of the response against that simulated.

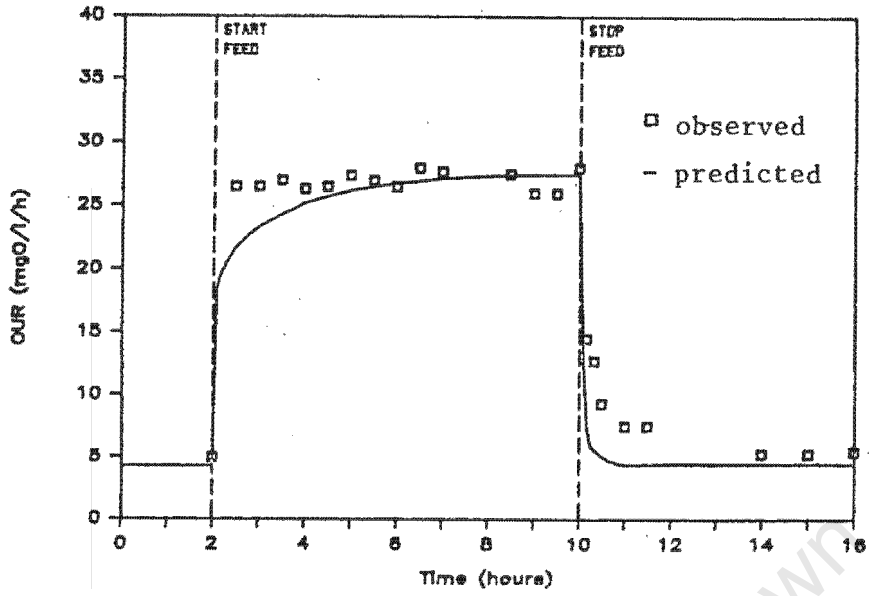

Fig 4.2(a): Observed (and predicted) oxygen utilization rate in a flow-through completely mixed aerobic activated sludge system at 2,5 days sludge age and 20°C under daily cyclic square wave loading conditions (8 hours feed/16 hours no feed) with glucose as substrate.
Test 1: influent COD = 1488 mg/ℓ; pH = 6,53.

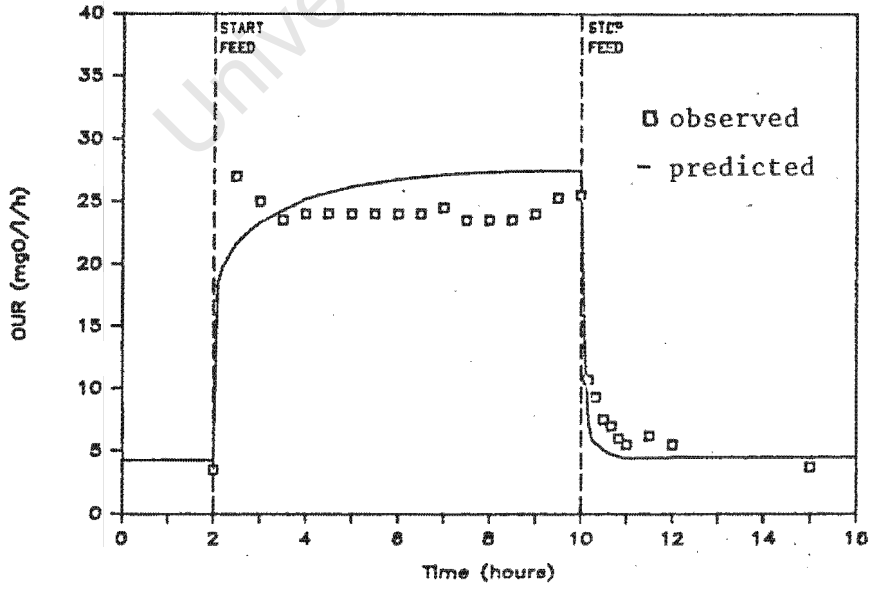

Fig 4.2(b): Observed (and predicted) oxygen utilization rate at 2,5 days sludge age and 20°C under daily cyclic square wave loading conditions (8 hours feed/16 hours no feed) with glucose as substrate.
Test 2: influent COD = 1410 mg/ℓ; pH = 6,57.

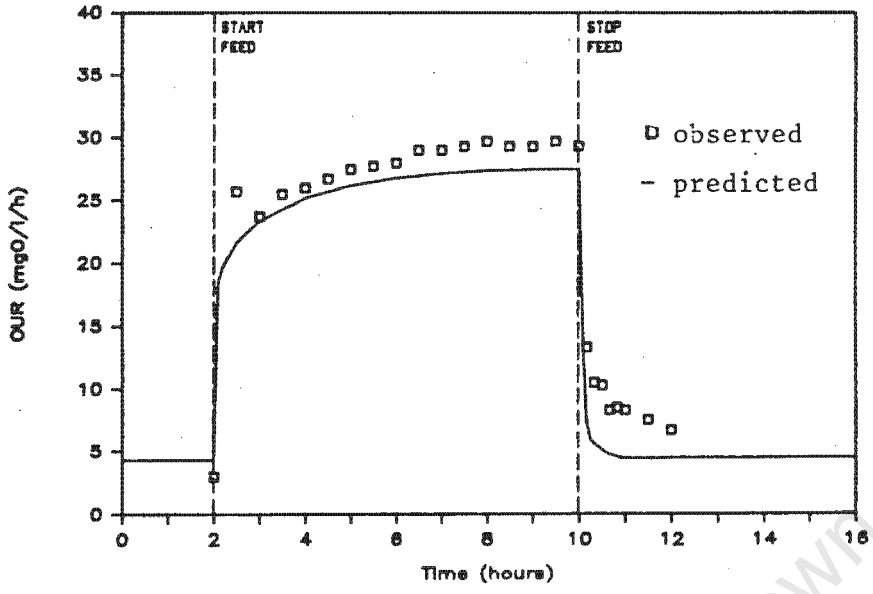

Fig 4.2(c): Observed (and predicted) oxygen utilization rate in a flow-through completely mixed aerobic activated sludge system at 2,5 days sludge age and 20°C under daily cyclic square wave loading conditions (7,75 hours feed/16,25 hours no feed) with glucose as substrate.
Test 3: influent COD = 1452 mg/ℓ; pH = 6,61.

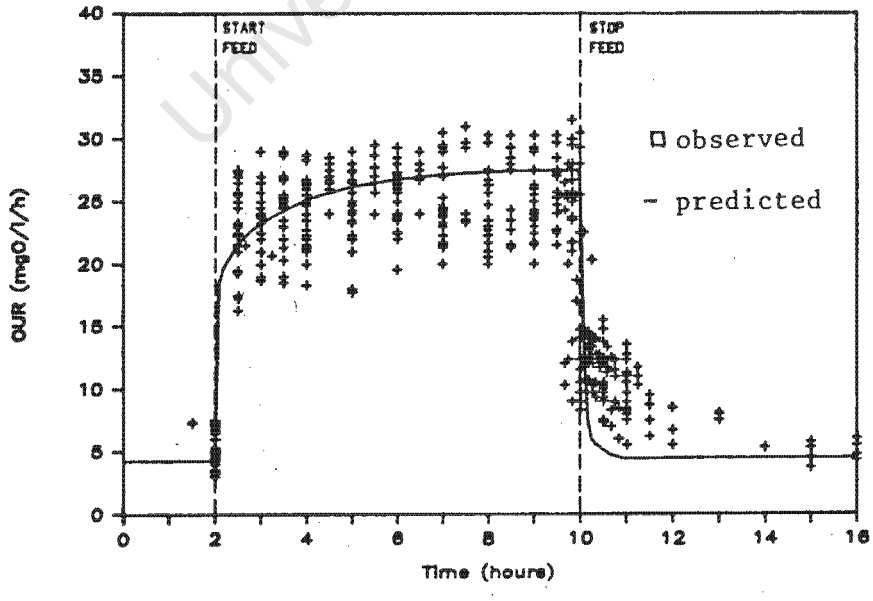

Fig 4.2(d): Daily observed oxygen utilization rate data measured at 2,5 days sludge age and 20°C under cyclic square wave loading conditions (8 hours feed/16 hours no feed) with glucose as substrate.
Mean (simulation) influent COD = 1447 mg/ℓ;
pH range = 6,20-6,90.

Table 4.1: Mass balances on COD for Experiments 1.1 to 1.3

Experiment	COD recovery
1.1	1.16
1.2	1.10
1.3	1.06

To simulate the responses of Experiments 1.1 to 1.3 using the modified IAWPRC model the kinetic and stoichiometric constants that gave the best fits are listed in Table 4.2. The constants accepted by Dold and Marais (1985) are also listed. The constants that needed to be adjusted, in so far as these affected the kinetic behaviour, were $\hat{\mu}_H$ and K_S. Both of these constants relate to the growth rate of the heterotrophic organisms. Ekama and Marais (1985) have shown that $\hat{\mu}_H$, and to a lesser degree K_S, is dependent on the feed pattern, the $\hat{\mu}_H$ values tending to increase as the feed pattern approaches a batch mode. As the feed pattern imposed in this set tended to batch mode operation, a $\hat{\mu}_H$ of 3,0 (as compared to the general mean value of 2,5) is in conformity with expected behaviour. The specific yield of 0,66 is the same as that accepted as "standard" in the general model.

Evaluating the response using the OUR, the system response follows the same pattern as other investigations using glucose and is in conformity with the behaviour expected from a readily biodegradable COD e.g. Dold, Ekama and Marais (1981). The prediction of the general model using the modified values for $\hat{\mu}_H$ and K_S also is very close to the observed.

The significant conclusion from this set of experiments, therefore, is that in terms of the response behaviour as hypothesized in the bisubstrate model, glucose sorts into the readily biodegradable category.

Table 4.2: Kinetic and stoichiometric constants used in simulation of observed response in experiments 1.1 to 1.3.

Constant	Dold and Marais (1985)	Experimental Simulation	Units
Kinetic			
$\hat{\mu}_H$	2,5	3,00	/day
K_s	5,00	10,00	$gCOD/m^3$
b_H	0,62	0,62	day
K_H	2,20	2,20	gCOD/(g cell COD/d)
K_X	0,15	0,15	gCOD/(g cell COD)
Stoichiometric			
Y_H	0,666	0,666	g cell COD yield/ (gCOD utilized)
f_E	0,08	0,08	-

4.2 EXPERIMENT No. 2: CYCLIC FEED WITH MAIZE STARCH AS SUBSTRATE

As a guide to establish an experimental protocol, when using only particulate COD as influent, simulations via the general model using the "standard" constants showed that for a steady state sludge age of 2,5 days, washout of organisms would take place. However, simulation of the system under steady state, with 5 days sludge age, indicated that stable operation should be achievable, that this would also be so if the proposed conditions for the cyclic experiment were imposed - a feed period of 18 hours and a sludge age of about 5 days i.e. 10/5 = 2ℓ of feed/day. Furthermore, from a practical point of view to obtain reasonably high oxygen utilization rates (for more accurate OUR measurements), the influent COD needed to be increased, from 1500 to 2000 mg/ℓ.

Experiment with 5 day sludge age:

For start up the previous glucose substrate system was fed on "soluble-starch" that had been dissolved previously by boiling. [As shall be shown later (Section 4.4) boiling appeared to cause a partial breakdown of the starch chains to glucose]. Steady state conditions were imposed i.e. feed

period extended over 24 hours. When the system appeared to have adapted, after two weeks, the substrate was changed to maize starch; the maize starch powder was mixed with nutrient solution without boiling and fed as a suspension. This substrate was fed under steady state conditions for 4 weeks i.e. about 6 sludge ages.

The imposition of steady state conditions prior to cyclic flow and load conditions was decided on because it was felt that adaption of the organisms to the substrate would be facilitated and that stable dynamic behaviour under cyclic conditions would be achieved more readily. Steady state also was likely to provide more accurate information on the specific yield constant, a parameter which experience with the glucose test had shown to be difficult to evaluate accurately under dynamic conditions, due to problems with filtration.

The system achieved steady state only after 3 weeks operation (see Fig 4.1). It was run for a further 10 days. The experimental values over the 10 day period, listed in Table 2.1 in Appendix A2, were analysed by a graphical statistical technique [see Fig 4.3(d)]. The mean values and their standard deviations are listed in Table 4.3.

The acceptability of the data was tested by doing a mass balance on the observed mass of influent COD, mass of effluent COD and the mass of oxygen. This gave a COD recovery to COD input of 1,01 to 1,00. Considering the large standard deviations, from the statistical distributions of the observations, (see Table 4.3 or Fig 4.1), a one percent lack of balance is insignificant.

After the steady state work was completed the system was run under cyclic dynamic conditions (18 hours feed/6 hours no feed) for a further two weeks to acclimate the organisms to cyclic flow conditions; thereupon a 24 hour intensive test was done. In the 24h dynamic state investigation the frequency of sampling for mixed liquor COD, VSS and filtered effluent COD was increased compared with the frequency of sampling in the glucose investigation [see Table 2.2 in Appendix A2 and Fig 4.5(b)]. This allowed a greater surety in delineating the behaviour of the parameters than was

Table 4.3: Experimental and theoretical mean steady state results for maize starch substrate investigation.

Parameter	Mean (std deviation)	Theoretical steady state value
Influent (mgCOD/ℓ)	2074 (66)	2074
Reactor contents (mgCOD/ℓ)	874 (52)	856
Waste overflow (mgCOD/ℓ)	835 (84)	856
Filtered effluent (mgCOD/ℓ)	105 (28)	104
Reactor mixed liquor (mgVSS/ℓ)	434 (54)	448*
Waste mixed liquor (mgVSS/ℓ)	459 (102)	448
Reactor COD/VSS (mgCOD/mgVSS)	1,68 (0,40)	1,68
Waste COD/VSS (mgCOD/mgVSS)	1,53 (0,52)	1,53
OUR (mgO/ℓ/hr)	11,1 (3,0)	10,1

* VSS (theoretical) = (856 - 104)/1,68

possible in the glucose investigation. Again a mass balance on the COD was performed which gave a COD out to COD in of 1,06 to 1,00; thus this data also appeared to be acceptable.

Simulating the data for the steady and dynamic steady states, using the average influent COD value as input, the best fits were obtained by reducing the hydrolysis rate constant K_h from 2,20 to 1,80 and accepting a fraction of 0,05 percent of the maize starch COD component as unbiodegradable particulate and reducing the yield for the heterotrophs (Y_H) from 0,666 to 0,592 mgCOD/mgCOD. The simulated response for the steady state together with the mean observed response are listed in Table 4.3: The simulated response for the dynamic system and the observed data, are shown plotted in Fig 4.3(a).

The model determines all the parameters in terms of the COD so that in fact it does not predict an estimate of VSS. This must be determined from an estimate of the COD/VSS ratio. Every time COD and VSS tests were performed a COD/VSS ratio was determined. A graphical statistical analysis of the COD/VSS ratio is shown in Fig 4.3(d) from which the mean COD/VSS

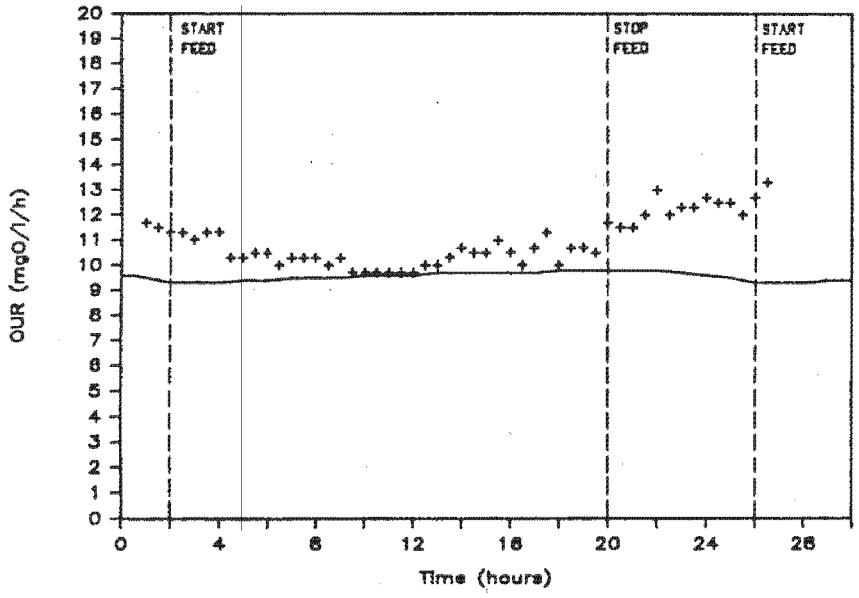

Fig 4.3(a): Observed (and predicted) oxygen utilization rate in a flow-through completely mixed aerobic activated sludge system at 5,0 days sludge age and 20°C under cyclic square wave loading conditions (18 hours feed/6 hours no feed) with maize starch as substrate. Test influent COD = 2008 mg/ℓ; pH = 7,05.

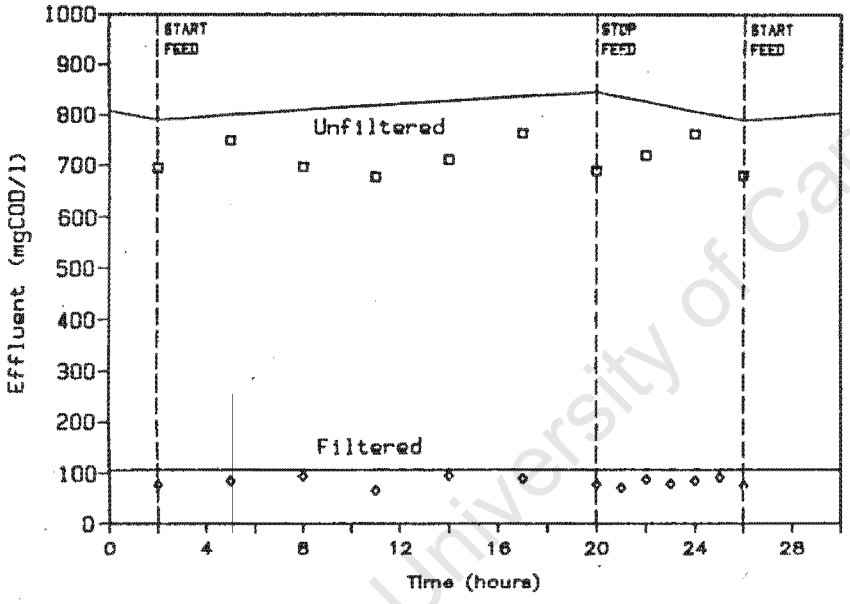

Fig 4.3(b): Experimental (and predicted) unfiltered and filtered (<0,45µm) effluent concentrations measured at 5,0 days sludge age and 20°C under cyclic square wave loading conditions with maize starch as substrate.

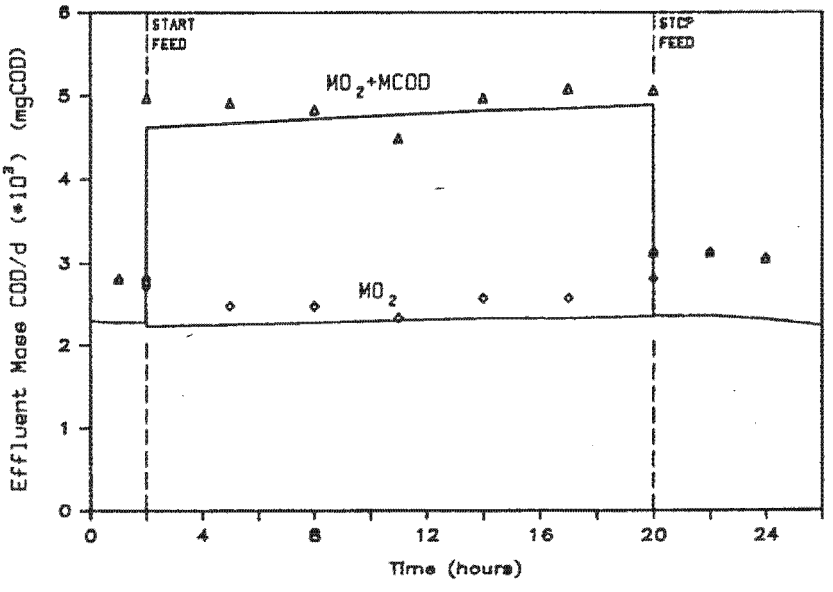

Fig 4.3(c): Experimental (and predicted) effluent mass COD response in the flow-through system at 5,0 days sludge age and 20°C under cyclic square wave loading conditions with maize starch as substrate.

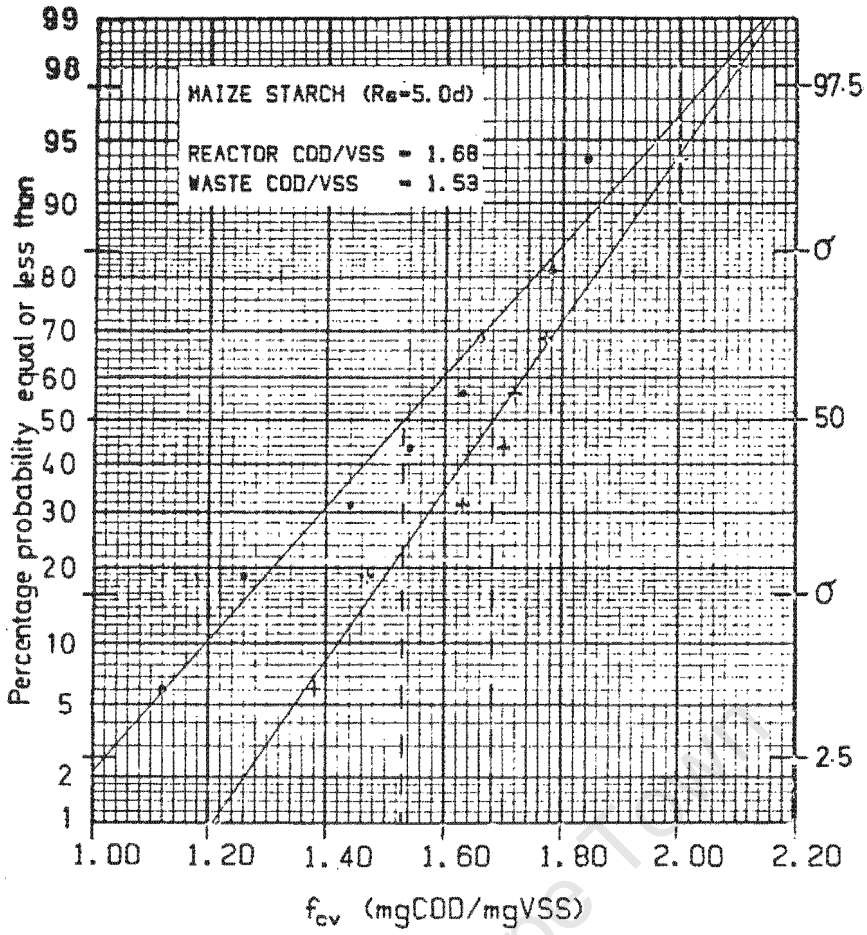

Fig 4.3(d): Statistical graphical estimation of mean COD/VSS ratios for observed reactor effluent and waste concentrations measured under steady state conditions at 5,0 days sludge age and 20°C with maize starch as substrate.

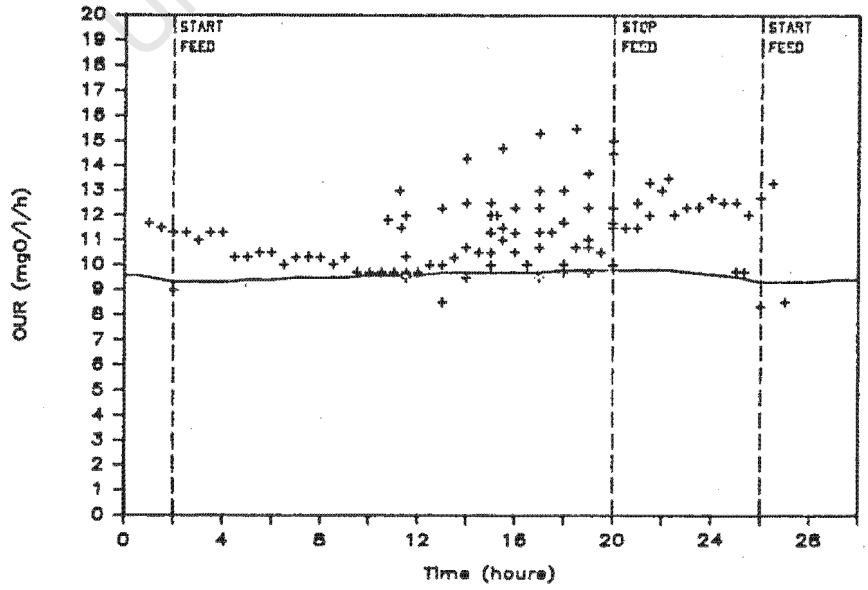

Fig 4.3(e): Daily observed oxygen utilization rate data measured at 5,0 days sludge age and 20°C under cyclic square wave loading conditions (18 hours feed/6 hours no feed) with maize starch as substrate. Mean (simulation) influent COD = 2074 mg/ℓ; pH range = 6,80-7,00.

ratio of 1,68 was determined, as listed in Table 4.3. Accepting this ratio, the theoretically estimated VSS was calculated from the theoretical COD of the VSS, also listed in Table 4.3.

Figures 4.3(a) and 4.3(b) allow a direct comparison between the simulated and predicted data for the OUR, MLVSS and the filtered (soluble) effluent COD. The OUR shows some deviation during the no-load period. It is likely that this was partly an hydraulic effect. However the relative deviation is relatively minor if one takes into account the masses of COD and O_2 passing in and out of the system; this is illustrated in Fig 4.3(c) where the simulated and observed masses of COD + O_2 passing out of the system are compared. The experimental data for the mass OUR/day plotted in Fig 4.3(c) were those corresponding to the time values of the experimental COD determinations.

In the 5 day starch experiment the focus was, in effect, on the rate of solubilization as this rate becomes the limiting one for the rate of growth. Provided the growth rate is faster than the solubilization rate, the simulated OUR response would remain the same, governed by the solubilization rate. In the experiments above in order to obtain good correspondence the maximum specific solubilization rate constant (K_h) had to be reduced from the "standard" 2,20 to 1,80; one should remember that the solubilization rates for different particulate COD organic material are likely to differ also so that the standard rate is probably a weighted mean value, whereas the rate for the starch is a specific one.

Examining the OUR observed and predicted in the cyclic 24h tests the observed OUR showed a decreasing tendency from the initial rate over the first half of the loading period. This effect is apparent also in the OUR measurement taken hourly during the feed period on 7 sets of OUR observed prior to the intensive 24h tests [see Fig 4.3(e)]. This behaviour coupled with the reduced solubilization specific rate constant necessary to simulate the data, led to the conclusion that perhaps the system could not achieve stability with a sludge age of 5 days. Hence it was decided to repeat the cyclic test but at 10 days sludge age, where one should achieve stable operation.

Experiment with 10 day sludge age: In order to establish a 10 day sludge age in the flow through system, without modifying either the volume, the mass of COD fed per day, or the feed period (as used in the 5 day sludge age system), the feed volume was halved i.e. from 2 to 1 ℓ/day and the COD concentration of the starch increased from 2000 to 4000 mg/ℓ. The system continued to be operated under the cyclic flow regime i.e. 18 hours feed/6 hours no feed. With a sludge age of 10 days the system attained a dynamic steady state only after about 2 sludge ages (see Fig 4.1). At this point a 24 hour intensive test was undertaken, the results listed in Appendix A.3. The system was then run for a further period in order to do a second 24 hour test, 30 days later. However about 15 days after the first 24 hour test the data for the filtered effluent COD (< 0,45 μm filter pore size) showed a gradual increase from an average of about 110 to 400 mgCOD/ℓ. Thereafter this high value decreased gradually until after about 50 days it again settled to a steady state value of about 150 mgCOD/ℓ (see Fig 4.1). The causes for this increase in the filtered effluent COD concentration, and its subsequent decrease, could not be established - the procedures for testing and running the unit did not change. From Fig 4.1, in fact the higher value persisted into subsequent investigations when a mixture of glucose and starch formed the influent.

The second 24 hour set of data was obtained during the period when the filtered effluent COD was high. A listing of the results is given in Appendix A.3.

To analyse the results of the two tests, first a mass balance was performed as set out in the description of the glucose experiment. Mass balances were calculated using the average input COD over the prior ten days to a test. The average data for the 10 day period prior to each 24 hour test are listed in Table 4.4. The two tests gave respective recovery ratios of 0,96 to 1,00 and 1,09 to 1,00.

To simulate the data the same constants as for the five day test were used, that is, the standard constants for the general model (see Table 4.2), but with a maximum specific solubilization rate, K_H, of 1,80, a heterotrophic organism yield, Y_H, of 0,592 and unbiodegradable COD

fraction for the starch of 0,05 percent. Again, as for the 5 day test, the system was simulated utilizing the average influent COD for the previous 10 days. The simulated and observed responses for the two 24 hour tests are shown plotted together in Figs 4.4(a) and 4.4(c). The combined masses of COD and O_2 out of the system are shown in Fig 4.4(d). The occasional OUR data taken prior to the 24h test are shown plotted in Fig 4.4(b).

Table 4.4: Dynamic steady state data with maize starch as substrate for 10 day sludge age.

Parameter	Test 1 Observed response	Test 1 Simulated response	Test 2 Observed response	Test 2 Simulated response
Influent (mgCOD/ℓ)	4075 (410)	4075	4108 (386)	4108
Reactor contents (mgCOD/ℓ)	1030 (107)	1178	1574 (18)	1400
Waste overflow (mgCOD/ℓ)	1060 (120)	1178	1645 (10)	1400
Filt. effluent (mgCOD/ℓ)	108 (27)	122	403 (32)	405
Reac.mixed liq.(mgVSS/ℓ)	741 (136)	857	821 (91)	711
Waste mixed liq.(mgVSS/ℓ)	716 (24)	857	830 (90)	896
Reac.COD/VSS (mgCOD/mgVSS)	1,23 (0,17)	1,23	1,40 (0,16)	1,40
Waste COD/VSS (mgCOD/mgVSS)	1,23 (0,19)	1,23	1,43 (0,30)	1,43
OUR (mgO/ℓ/hr)		See Fig 4.4(a)		

4.2.1 Discussion on the starch tests

The significant points that arose from the starch tests are the following:

(1) The rate of utilization of the starch is virtually an order of magnitude lower than that for glucose; In terms of the general model the limiting rate is that due to solubilization. The observed response can be simulated reasonably well via the general model with only one substantive change in the kinetic constants, that of the maximum specific rate of solubilization, K_h, which needs to be reduced from 2,20 to 1,80 mgCOD/mg cell COD/day, and in one stoichiometric constant, the specific heterotrophic organism yield, Y_H, which had to

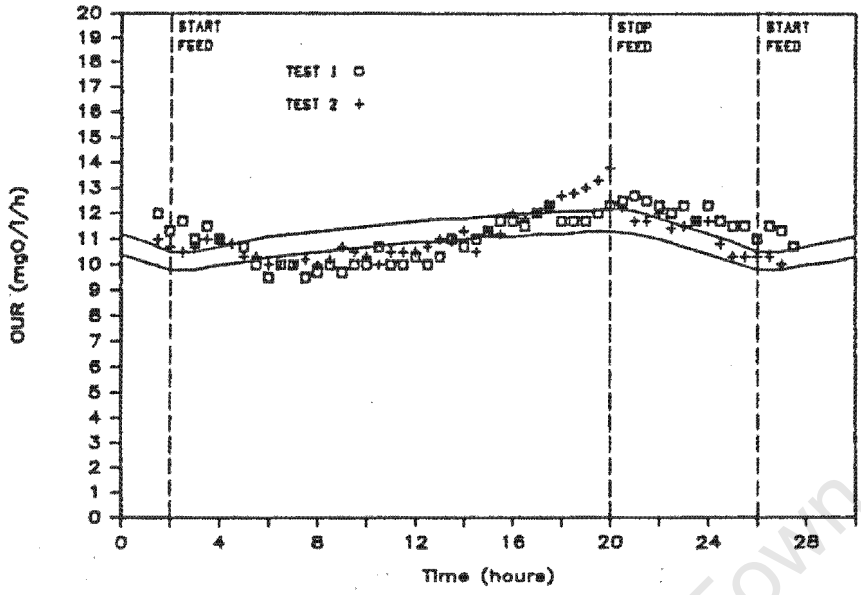

Fig 4.4(a): Observed (and predicted) oxygen utilization rate in a flow-through completely mixed aerobic activated sludge system at 10 days sludge age and 20°C under cyclic square wave loading conditions (18 hours feed/6 hours no feed) with maize starch as substrate.
Test 1: influent COD = 4075 mg/ℓ; pH = 7,00;
Test 2: influent COD = 4108 mg/ℓ; pH = 7,01.

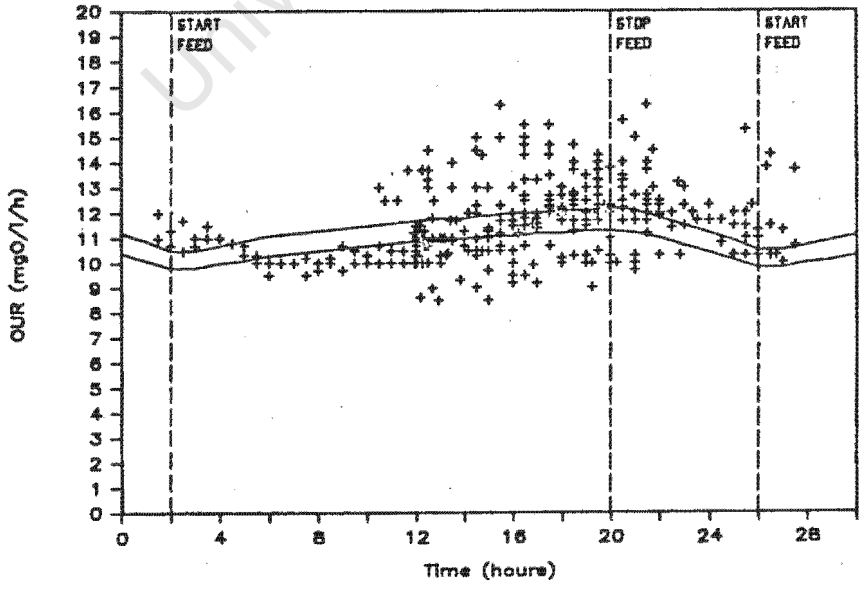

Fig 4.4(b): Daily observed oxygen utilization rate data measured at 10 days sludge age and 20°C under cyclic square wave loading conditions with maize starch as substrate (pH range = 6,90-7,10).

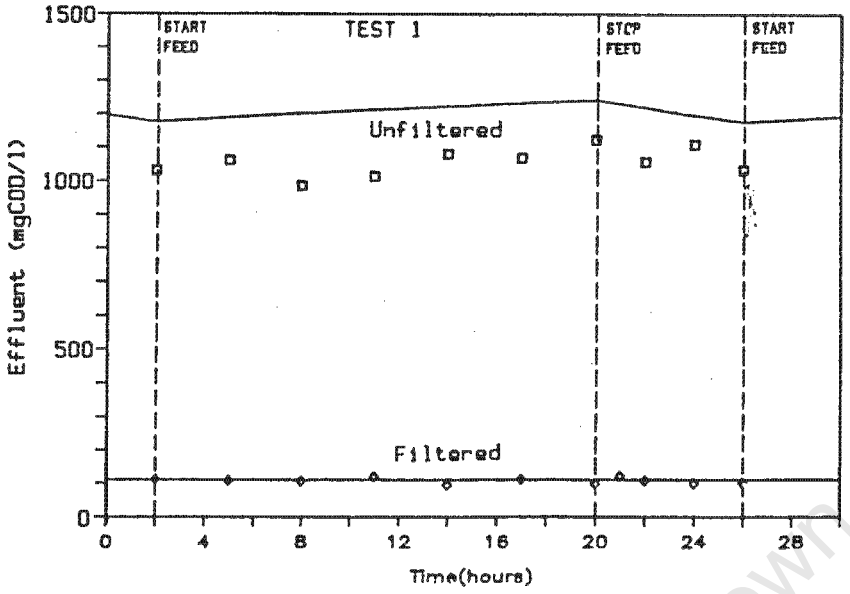

Fig 4.4(ci) : Experimental (and predicted) unfiltered and filtered (<0,45 μm) effluent concentrations measured at 10 days sludge age and 20°C with maize starch as substrate, (Test 1).

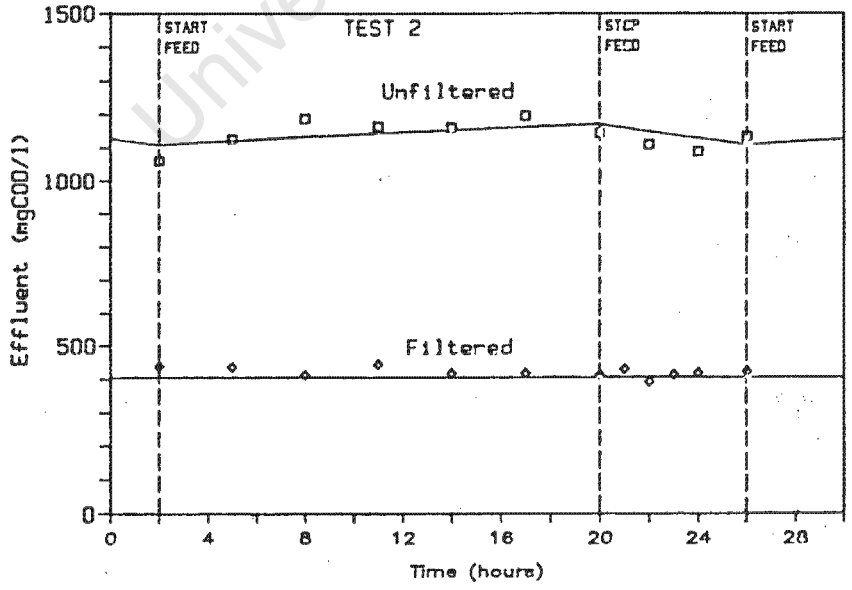

Fig 4.4(cii) : Experimental (and predicted) unfiltered and filtered (<0,45 μm) effluent concentrations measured at 10 days sludge age and 20°C with maize starch as substrate, (Test 2).

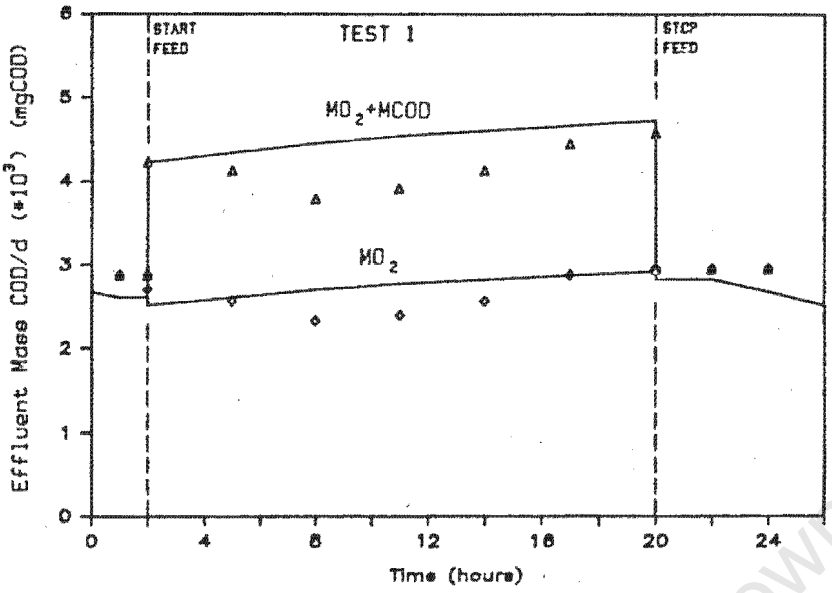

Fig 4.4(di) : Experimental (and predicted) effluent mass COD response at 10 days sludge age and 20°C with maize starch as substrate, (Test 1).

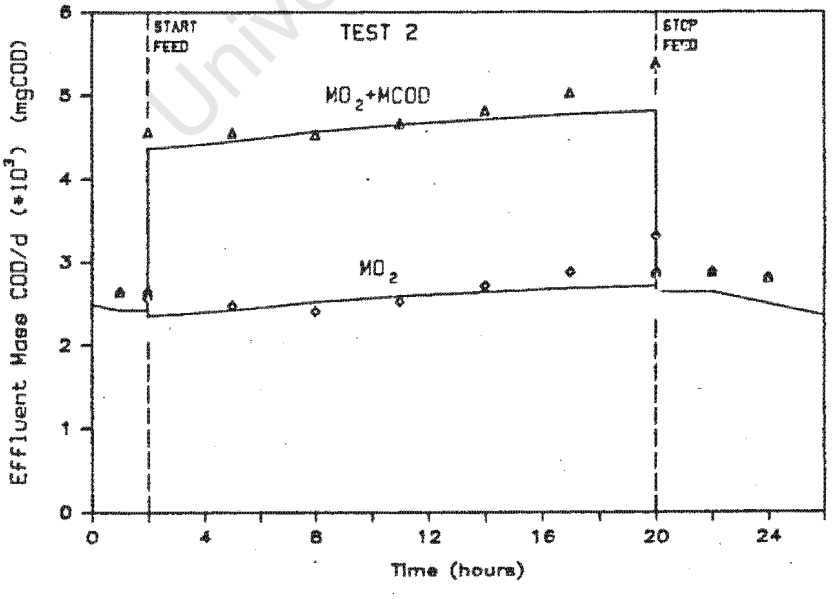

Fig 4.4(dii): Experimental (and predicted) effluent mass COD response at 10 days sludge age and 20°C with maize starch as substrate, (Test 2).

be reduced from 0,666 to 0,592 mg cell COD yield/mg COD utilized. Both these changes do not count against the hypothesized structure of the general model because the substrate used in these tests was a very specific one - starch - whereas the standard constants in the general model were developed by curve fitting against tests on municipal wastewater containing particulate organic materials of diverse chemical structures. In particular it should be noted that the yield observed in these tests i.e. 0,592 mg cell COD/mgCOD is very near that observed by other investigators for some specific substrates (Payne, 1970).

(2) The 10 day sludge age cyclicly loaded system again exhibited the tendency to a slight decline in the OUR for a period after feeding commenced, as noted previously in the 5 day experiments. In the 5 day experiments this was thought to be as a result of a near washout state in the reactor but such a conclusion could not apply for a 10 day sludge age. An explanation that could account for the deviatory behavour may lie in the procedures used to prepare the substrate: the substrate was weighed out immediately prior to mixing into the influent volume; it is possible that the starch granules absorbed water (wetted) relatively slowly. With absorption, the granules would expand making the starch chains more available to enzymatic action. This would explain the rise in oxygen utilization rate towards the end of the feed period - with hindsight a more appropriate method for preparing the starch substrate would have been to do the preparation 12-24 hours before feeding, to allow the substrate to "stabilise".

4.3 EXPERIMENT NO.3: CYCLIC FEED WITH GLUCOSE AND MAIZE STARCH AS SUBSTRATE

In the experiments using glucose and starch as substrate it was demonstrated that these two substrates react significantly differently and that the respective response in an activated sludge system can be simulated reasonably accurately using the bisubstrate general model. In this experiment it was proposed to check if a mixture of the two substrates also could be simulated accurately by the general model.

The experimental set up, loading and operational procedures remained the same as for the 10 day sludge age starch experiment, i.e. volume of reactor 10ℓ; feed rate 1ℓ/day; influent substrate 4000 mgCOD/ℓ but divided into 2000 mgCOD/ℓ glucose and 2000 mgCOD/ℓ maize starch; feeding period 18 hours. The approximate sludge age remained at V/(Q/d) = 10/1 = 10 days. The mixed liquor of the starch experiment served as the inoculum for the glucose-starch experiment. To acclimate the system to this feed, it was run under steady state (24h feed) for a period of 20 days on the glucose-starch substrate. Thereafter, a cyclic feed pattern was imposed, 12h feed, 12h no feed. The system was run for a further 40 days whereupon a 24 hour intensive test was done, repeated 10 and 15 days later, using the same test procedures as before. (See Fig 4.1 for dates of tests). The data observed are listed in Appendix A4.

The 24 hour response data obtained on the three tests are shown plotted in Fig 4.5(a) (Test No.1) and Fig 4.5(b) (Tests Nos 2 and 3).

To check the acceptability of the data, mass balances on the 24 hour data were performed using the statistical average influent concentration i.e. 4047 mgCOD/ℓ. The mass balances gave recoveries of 97 percent, 104 percent and 107 percent respectively.

The response observed in Fig 4.5(a) (Test No. 1) differs significantly from those in Fig 4.5(b) (Tests Nos. 2 and 3). No explanation for the deviatory behaviour in Fig 4.5(a) could be established - however it does illustrate that often unpredictable responses can arise and emphasizes the need for more than one 24 hour test.

The data was simulated using the same constants as for the starch i.e. Y_H = 0,592, K_h = 1,80. The f_{us} was selected as 0,034 to give the observed filtered effluent COD; the unbiodegradable fraction of the starch COD was maintained at 0,05 percent giving a f_{up} value of 0,025 for the 50:50 glucose-starch mixture. To determine the volatile solids the experimental COD/VSS ratios were analysed statistically and gave a mean value of 1,41 mgCOD/mgVSS. (This ratio differed significantly from that observed with starch only as substrate, where the COD/VSS ratio = 1,68 mgCOD/VSS).

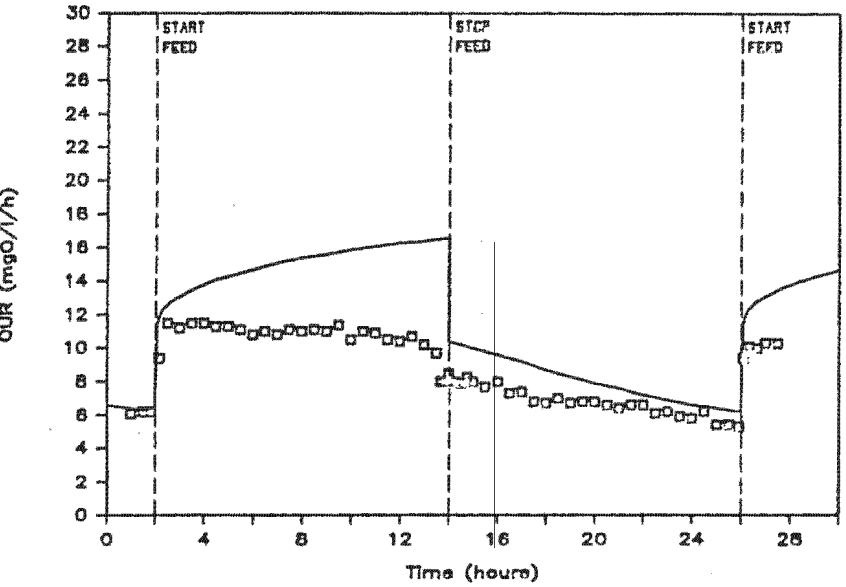

Fig 4.5(a): Observed (and predicted) oxygen utilization rate in a flow-through completely mixed activated sludge system at 10 days sludge age and 20°C under cyclic square wave loading conditions (12 hours feed/ 12 hours no feed) with a mixture of glucose and maize starch as substrate.
Test 1: influent COD = 4125 mg/ℓ;
pH = 7,00.

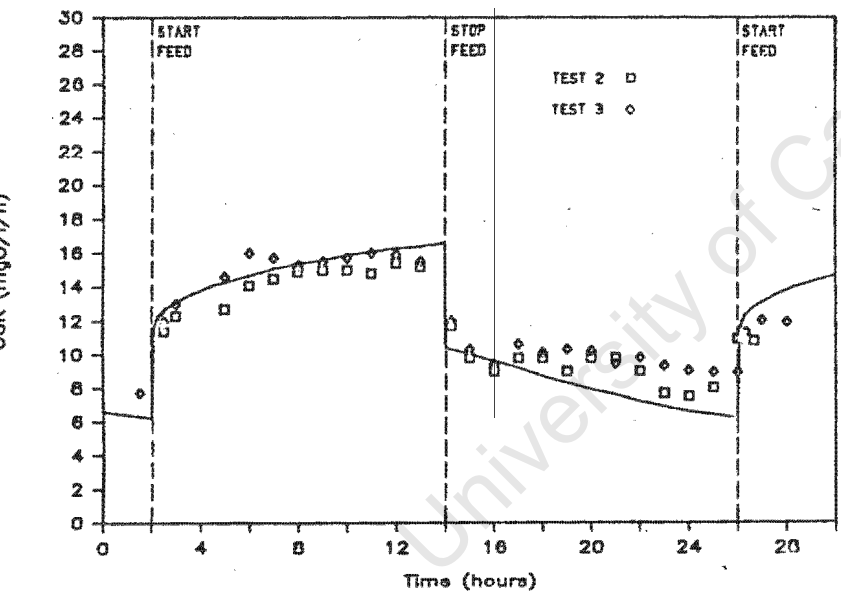

Fig 4.5(b): Observed (and predicted) oxygen utilization rate at 10 days sludge age and 20°C with glucose and maize starch as substrate.
Test 2: influent COD = 4165 mg/ℓ;
pH = 7,06
Test 3: influent COD = 4040 mg/ℓ;
pH = 6,95.

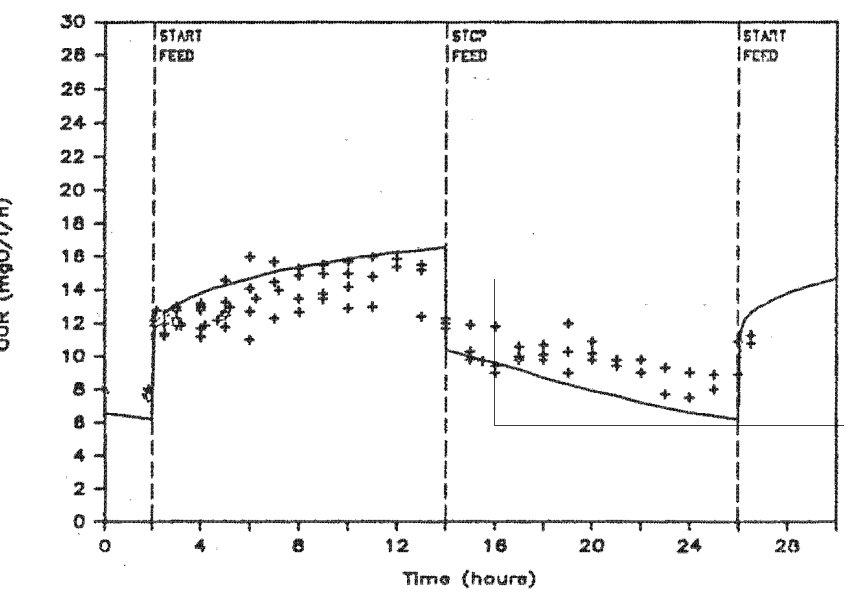

Fig 4.5(c): Daily observed oxygen utilization rate data measured at 10 days sludge age and 20°C with glucose and maize starch as substrate. Mean (simulation) influent COD = 4047 mg/ℓ;
pH range = 6,90-7,10.

The simulated and observed data for the 3 tests are shown in Figs 4.5(a) (Test No.1) and Fig 4.5(b) (Tests No. 2 and 3). The observed behaviour for Test No. 1 differs so significantly from the experimental and predicted behaviour of Tests No. 2 and 3 that one must accept that it did not reflect the mean behaviour. This is further supported by the correspondence between the predicted OUR behaviour and that measured intermittently on a number of occasions during the glucose-starch investigation, see Fig 4.5(c).

Although the correspondence between observed and predicted behaviour is not exceptionally good the experimental response follows the predicted response in the main features predicted by the bisubstrate hypothesis.

4.4 EXPERIMENT No.4: CYCLIC FEED WITH BOILED STARCH AS SUBSTRATE

When the experimental protocol for starch as substrate was being considered it was found that the "soluble starch" would not form a uniform suspension in cold tap water but tended to stick together in lumps that rose to the surface. To obtain a uniform suspension, it was thought that mixing would be promoted by boiling; this did give an opaque milky suspension/solution. A cyclic test program was undertaken with the boiled starch solution as substrate. In preparing the substrate two approaches were tested: (1) adding the micronutrient with the starch powder and then heating slowly and boiling, and (2) addition of the starch powder only, then heating and boiling; thereafter cooling the solution and adding the nutrients. The second approach was accepted when it became apparent that some form of precipitation occurred when the micronutrients were boiled. In order to maintain the composition of the substrate as constant as possible, the mass of starch always was made up in the same mass of water and boiled for exactly five minutes and thereafter left to cool without disturbance before adding the nutrients.

The following experimental conditions were imposed: Volume of reactor 10ℓ; volume of influent 4ℓ; system operated on a flow-through system so that the sludge age was approximately 10/4 = 2,5 days; feeding period 12 hours, non-feed period 12 hours; influent COD 1500 mg/ℓ.

To acclimate the system it was run for 25 days under steady state conditions i.e. the volume of feed was fed over 24 hours. Thereafter cyclic feeding was imposed and the system run for a further 10 days before a 24 hour test was undertaken.

The mean response under steady state operation is listed in Table 4.5; the cyclic response is shown plotted in Fig 4.6(b). The observed response under cyclic flow conditions was very similar to that for a pure glucose solution, whereupon it was inferred that the boiling must have broken down the starch chains, in some unknown measure, to simple glucose molecules. In order to evaluate the fraction of starch converted to glucose, a series of simulations were undertaken with increasing fractions of the starch being accepted as converted to glucose. In these simulations the kinetic and stoichiometric model constants were accepted to be the same as for glucose, that is, Y_H = 0,666, $\hat{\mu}_H$ = 3,00, K_s = 10,0, K_H = 2,20, K_X = 0,15. An unbiodegradable soluble fraction, f_{us} = 0,0554 was selected to give the observed filtered effluent COD over the 12 hours unloaded period. As the soluble starch was of analar quality the unbiodegradable particulate fraction, f_{up}, was accepted as zero.

The best fit between observed and simulated data was obtained by accepting that 70 percent of the starch was converted to glucose on boiling. Simulated data under steady state is listed in Table 4.5 and the simulated response under dynamic conditions is plotted in Fig 4.6(a). To check the experimental volatile solids concentrations, an analysis of the experimental COD/VSS ratio data gave a value of 1,47, and this value was used to determine theoretically the volatile solids concentration from the theoretical COD representing the volatile solids fractions.

The correspondence between observed and simulated data is reasonably close. It would be possible to obtain even closer correspondence by adjusting the various constants but such improvements would not add, in a significant manner, to the two main objectives of the investigation i.e. to check the validity of the bisubstrate hypothesis and to check if the observed behaviour could be modelled adequately by the general bisubstrate model.

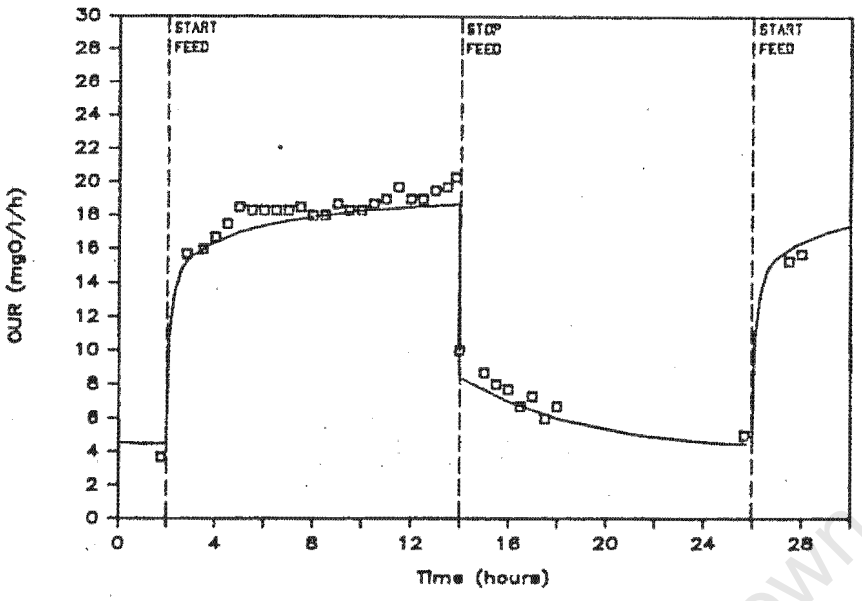

Fig 4.6(a): Observed (and predicted) oxygen utilization rate in a flow-through completely mixed activated sludge system at 2,50 days sludge age and 20°C under cyclic square wave loading conditions. (12 hours feed/12 hours no feed) with boiled soluble starch as substrate. Influent COD = 1535 mg/ℓ; pH = 6,78.

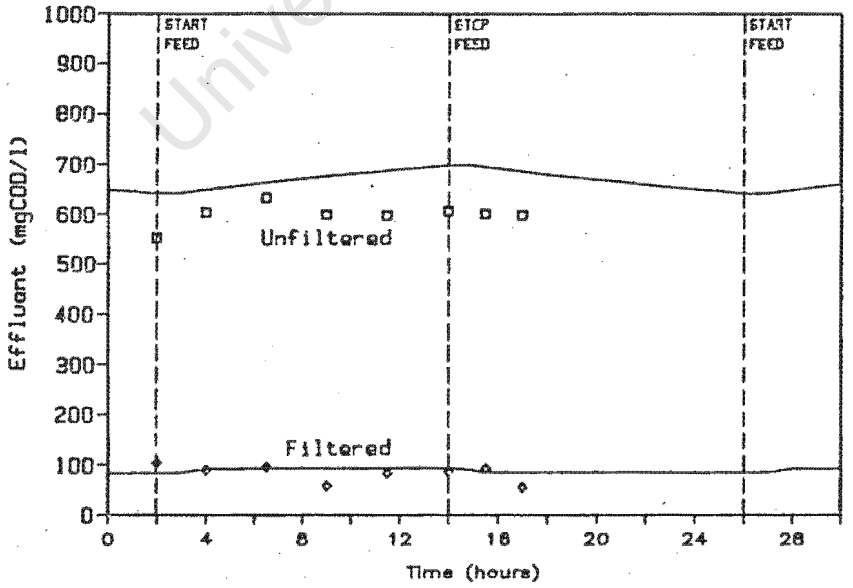

Fig 4.6(b): Experimental (and predicted) unfiltered and filtered (<0,45 µm) effluent concentrations measured at 2,5 days sludge age and 20°C with boiled soluble starch as substrate.

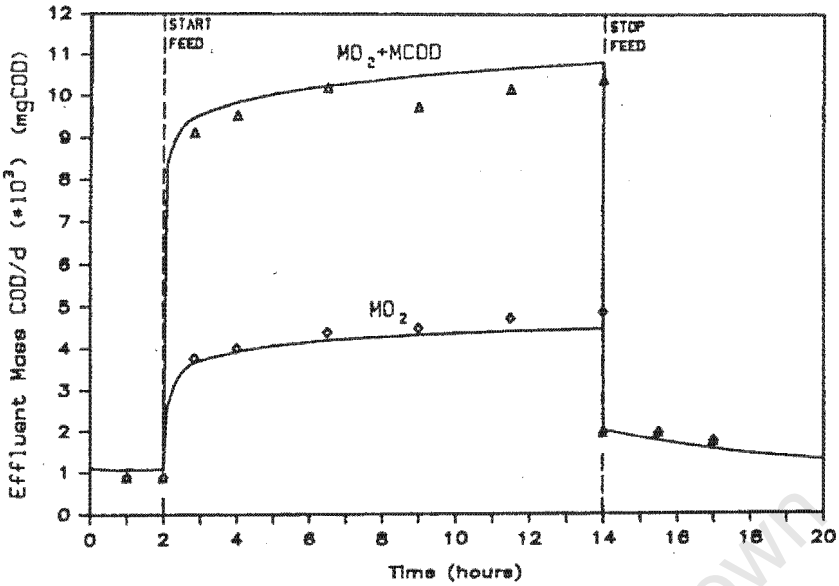

Fig 4.6(c): Experimental (and predicted) effluent mass COD response at 2,5 days sludge age and 20°C under cyclic square wave loading conditions (12 hours feed/12 hours no feed) with boiled soluble starch as substrate.

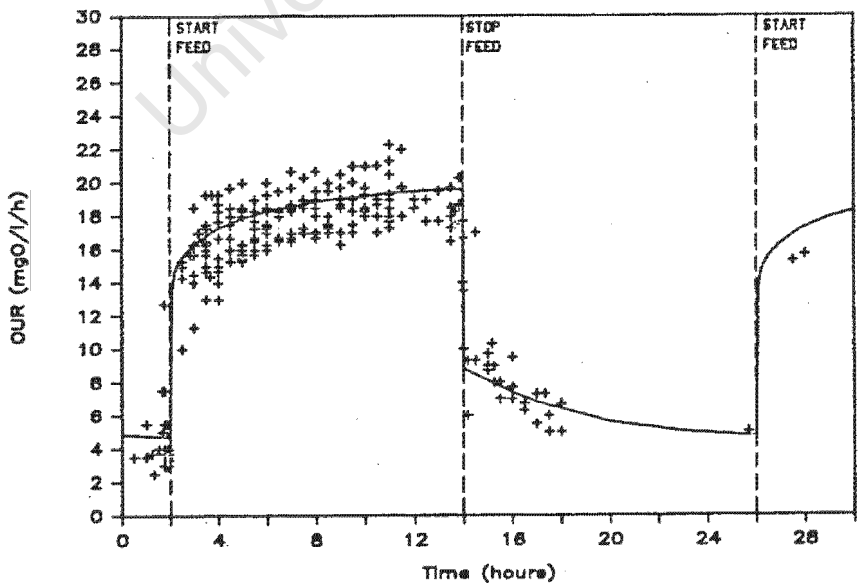

Fig 4.6(d): Daily observed oxygen utilization rate data measured at 2,5 days sludge age and 20°C with boiled soluble starch as substrate.
Mean (simulation) influent COD = 1554 mg/ℓ;
pH range = 6,60-6,85.

Table 4.5: Mean experimental and theoretical steady state results for "soluble starch" substrate investigation.

Parameter	Experimental Mean (Std. deviation)	Theoretical steady state value
Influent (mgCOD/ℓ)	1554 (136)	1554
Reactor contents (mgCOD/ℓ)	820 (190)	802
Filtered effluent (mgCOD/ℓ)	82 (30)	82
Reactor mixed liquor (mgVSS/ℓ)	510 (154)	490 *
Reactor COD/VSS (mgCOD/mgVSS)	1,47 (0,56)	1,47
OUR (mgO/ℓ/hr)	11,7 (4,0)	12,5

* VSS (theoretical) = (802-82)/1,47

There is no doubt that the model simulates the observed response adequately but the experimental results sort into the same category as for normal wastewaters in that it does not provide explicit proof that there are two substrates present, only that if the two substrate concept is accepted the model can predict the correct response. In retrospect explicit information could have been obtained if, for example, the glucose fraction had been determined in the substrate after boiling.

The main conclusion from this test is that in terms of the observed and simulated response it would appear that on boiling starch a fraction of the starch is broken down to glucose-like i.e. readily biodegradable material.

4.5 DISCUSSION AND CONCLUSIONS

1. Substrate composition:

(a) Growth factors

The initial experiments utilized the substrate nutrient recipe from Gaudy et al (1969). At short sludge ages under cyclic flow conditions, the glucose nutrient recipe gave rise to slime formation, a condition often observed with unbalanced substrates. This condition was cured effectively by providing "growth factors" via the addition of yeast extract. The improvement in the system response was so significant that future research

utilising pure or specifically defined substrates the nutrients should include this undefined growth factor additive, yeast extract. Consideration should also be given to the addition of suitable trace elements to the nutrient medium. In this investigation the tap water used to make up the feed solution was assumed to contain satisfactory concentrations of elements such as Copper (II), Cobalt (II), Molybdenum (VI), Boron (III) and Zinc. The absence of these elements may have been a contributory factor to the slime formation.

(b) Effect of boiling

Starch as a substrate appears to undergo breakdown to glucose on boiling; consequently the preparation procedures in making up the substrate, such as boiling, can be an important factor in the subsequent response characteristics of the system; furthermore the nutrients may also interact at a higher temperature and precipitate out, thereby creating a nutrient imbalance. The same situation may be encountered with other organic substrates.

(c) Wetting of starch

Although no specific experimental data on wetting of starch substrate was obtained, it would appear that the starch powder requires time to "wet", that is, to untangle the starch chains and thereby become more accessible to the solubilization action of the extracellular enzymes. As an empirical recommendation, the starch substrate should be prepared 24 hours before feeding.

(d) Starch origin

Starch consists of α-amylose and amylopectin units. "Soluble starch" probably consists principally of the former, i.e. long unbranched chains of glucose molecules which vary in molecular weight from 3000 to 500000. In contrast, maize starch is a complex molecule of amylopectin, highly branched, with molecular weights in excess of 10^6.

It is very likely that depending on the starch, different starches may have different responses to wetting and to extracellular solubilization by enzymes. The starches may also possess different fractions of

unbiodegradable (cellulose fractions?) material.

2. System response

(a) Oxygen utilization rate

The oxygen utilization rate response under cyclic flow conditions was the most important response parameter in elucidating the process characteristics in the system. It allowed clear distinction to be made between glucose and starch as substrates. In contrast, the filtered effluent COD and the volatile solids concentration were most insensitive parameters. The emphasis in past research on the effluent quality probably had an inhibiting effect on the development of the kinetic theory of activated sludge behaviour.

(b) COD:VSS ratio

Mass balances on systems have in the past made use of the COD equivalence of volatile solids, the proportional content ranging between 1,40 and 1,50. In this investigation, with these specific substrates, the COD/VSS ratio differed for every experimental system, ranging from 1,30 to 1,68. Utilizing the relevant ratios in each series, it was possible to obtain good mass balances using VSS. If the "standard" COD/VSS ratio of 1,48 was used, very poor mass balances were obtained in all but one series, the boiled starch experiments. As there is no rule at present whereby a reasonable COD/VSS ratio for a system can be estimated, it is essential that the yield should be expressed in terms of COD instead of volatile solids, and the volatile solids determined from experimental COD/VSS ratios for that system. Generally the yield expressed as COD has a much greater constancy than the yield expressed as volatile solids. Research is needed into the factors that influence the COD/VSS ratio.

3. Growth and solubilization rates

(a) Growth rates:

The maximum specific growth rate constant, μ_H, or equivalently the maximum specific substrate utilization rate constant, K_s, could be determined effectively only for glucose as substrate. Its magnitude was approximately

the same as that accepted normally for municipal wastewater. However Ekama, Dold and Marais (1985) have reported that μ_H (in particular) has a dependency upon the system configuration, being higher when selector reactors are incorporated, or if a feed pattern approaching that of a batch feed is imposed. The glucose feed gave the best fit with a $\hat{\mu}_H$ of 3,0 and the starch and starch-glucose $\hat{\mu}_H$ = 2,5. As the glucose feed period was 8 hours and the others ranged between 12 and 18 hours, the respective $\hat{\mu}_H$ values are in the right relative order of magnitude.

(b) Solubilization/hydrolysis rates:

In this investigation, the maximum specific hydrolysis rate constant, K_H, is defined in terms of the bisubstrate model process for particulate biodegradable COD. The magnitude of the standard solubilization rate constant, K_H, is 2,20 and this is the value that appeared to be applicable to glucose as substrate. Where starch formed a part of the influent, whether boiled or in suspension, the K_H value had to be reduced to 1,80. This reduction appears to be due to the structure of the starch itself in that solubilization is not as rapid as for other particulate substrates, by the observation that the reduced K_H applied to both boiled starch where only 30 percent of the starch was estimated to be in particulate form and substrates which were 100 percent starch in suspension. In estimating the K_H value one should note therefore that other particulate COD compounds may deviate from the mean value found for municipal sewage. At present no positive evidence exists that K_H is affected substantively by the loading pattern or the system configuration.

(c) Yield constant:

Thermodynamically the yield constant for different classes of substrate should vary as it is dependent upon the free energy made available on oxidation. However, the conversions are subject to lower than 100 percent efficiency and as these efficiencies also may differ for different classes of organisms the yields need not be strictly related to the thermodynamic estimates. A number of investigators (Payne 1970) have endeavoured to relate the observed yield to the thermodynamic predicted yield. These would indicate that the yield should be about 0,39 mgVSS/mgCOD. However these estimates are based on COD/VSS ratios of about 1,42 mgCOD/mgVSS and

as we have seen that the COD/VSS ratio can differ appreciably depending upon the system, such experimental estimates of 0,39 may in fact not reflect the corresponding yield value in terms of COD. In this investigation the yield value obtained by curve fitting was 0,666 mgCOD/mgCOD for pure glucose influent and boiled starch in which apparently 70 percent of the starch had been converted to glucose. For the maize starch and 50 percent maize starch/glucose mixture, the yield appeared to be about 0,600 mgCOD/mgCOD, but with the maize starch, to obtain the best fits it was necessary also to assume that it contained an unbiodegradable particulate fraction of 5 percent. As these factors interact, it is not possible to obtain unequivocal estimates of the yield. However for practical purposes the difference in yield would not normally have a significant effect on the estimate of oxygen demand and volatile solids production.

4. General conclusion

The experimental response data of the glucose, starch and glucose starch substrates and the predictions of the response by the bisubstrate model appear to be consistent and in this fashion lend support to the validity of the bisubstrate hypothesis.

4.6 DESIGN IMPLICATIONS FOR AERATED LAGOONS

This investigation has brought one aspect into pertinence which is of importance to design - depending on the division of biodegradable substrate into readily and slowly biodegradable fractions, and the temperature, a process may fail by washout at much higher sludge ages than presumed at present. For example in this investigation at 5,0 days sludge age the process failed when the substrate consisted of 100 percent maize starch, whereas the process achieved dynamic equilibrium when pure glucose served as substrate. Indeed, it is very likely the reason for the success of short sludge age systems (of 2-3 days) is that the influent normally contains a readily biodegradable component. Municipal waste normally has a readily biodegradable fraction of about 25 percent of its biodegradable COD. Using the general model and the standard constants it can be shown that a heterotrophic bacterial population can be maintained readily down to less than two days at 20°C under steady state, see Fig 4.7. If this

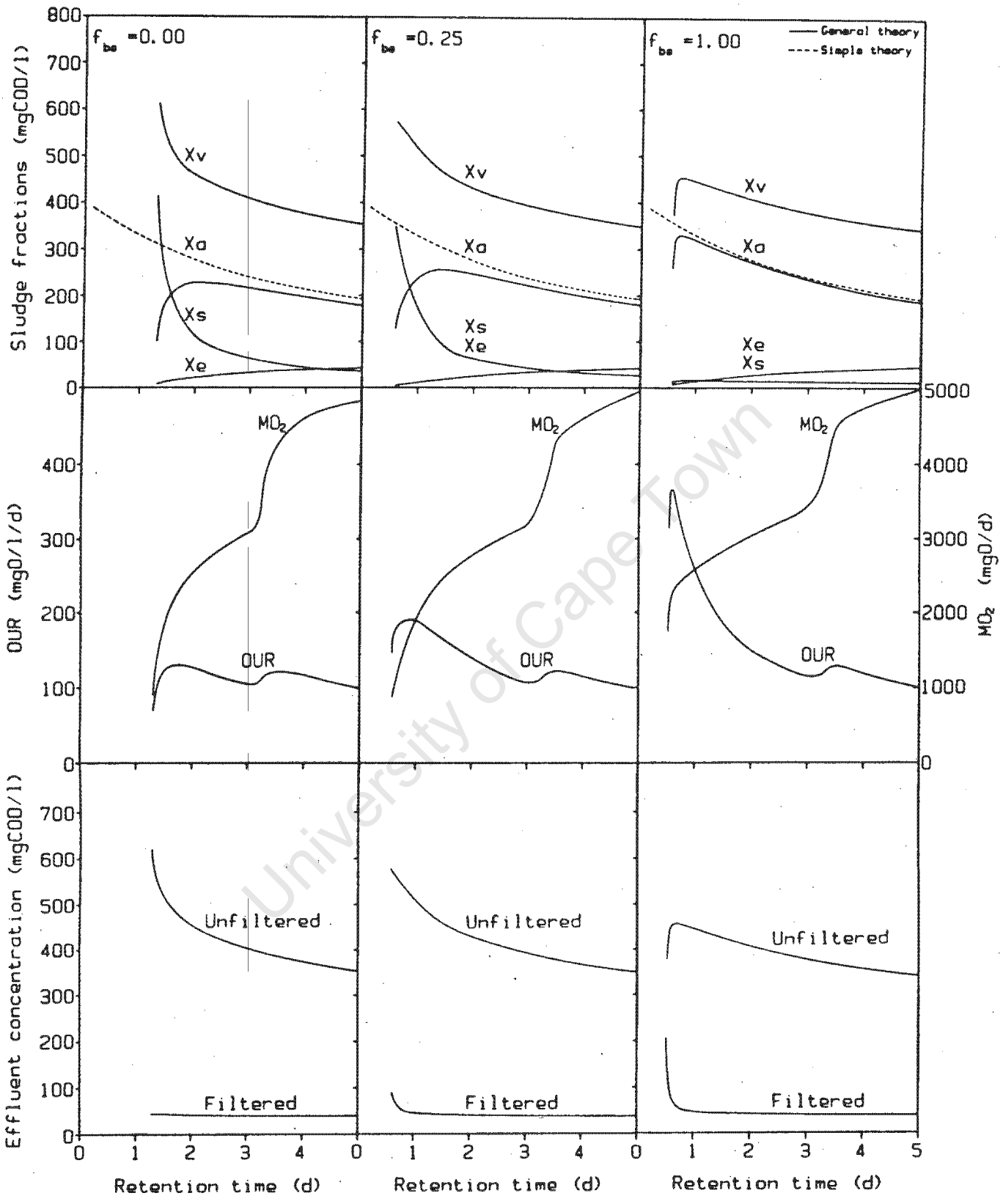

Fig 4.7: Theoretical illustration of the effect of increasing readily biodegradable soluble COD fraction (f_{bs}) on the magnitude of the minimum retention time required for satisfactory operation of a suspension mixed aerated lagoon. (Influent COD = 750 mg/ℓ; influent TKN = 60 mg/ℓ; f_{us} = 0,05 mgCOD/mgCOD; f_{up} = 0,13 mgCOD/mgCOD; T = 20°C.)

waste did not contain any readily biodegradable COD the system would require a minimum sludge age of at least 2,5 days This aspect is illustrated by simulating the heterotrophic growth response of a flow-through activated sludge system a suspension mixed aerated lagoon, under steady state, for a series of retention times, with an influent COD of normal unsettled wastewater, f_{up} = 0,13, f_{us} = 0,05, influent COD of 750 mg/ℓ but with f_{bs} = 100, 25 and 0 percent respectively, see Fig 4.7. It is clear the different lagoon systems cannot metabolize the waste effectively for sludge ages less than 0,75, 1,50 and 2,20 days respectively.

Table 4.6: Minimum retention times for stable operation of suspension mixed aerated lagoons with differing fractions of soluble readily biodegradable COD in the influent wastewater stream.

% f_{bs}	Minimum retention time (days)
100	0,75
25	1,50
0	2,20

From these simulations it is clear that the selection of the retention times of suspension mixed aerated lagoons must take account of the constitution of the waste. As the growth rate is temperature dependent, the rate being reduced at lower temperatures, the nominal retention time should be that adequate at the lowest expected temperature.

Aerated lagoons that are not suspension mixed (facultative lagoons), treating soluble wastes, sometimes have been reported to operate very inefficiently. One reason could be that in the lagoon, not being suspension mixed, the organisms generated tend to settle out continuously in regions distant from the aerators thereby preventing the formation of sufficient sludge mass to metabolize the soluble waste adequately. If biodegradable particulate COD should be present in the influent this also would settle out thereby giving a reduction of COD which is unrelated to the biological action of the organisms.

A final point, suspension mixed aerated lagoons with short sludge ages, of 2 to 5 days, do not cause high oxidation of the organic influent; it principally converts the influent COD to live particulate mass. Consequently the total COD of the effluent still will be in the region of 50-60 percent of that in the influent. However, being particulate, if a facultative lagoon is incorporated receiving the effluent from the suspension mixed one the sludge will settle out and be digested anaerobically; oxygen needs to be supplied only to preserve an aerobic environment in the liquid mass. In this fashion an adequate removal of COD from the wastewater can be accomplished.

CHAPTER FIVE

MEASUREMENT OF THE READILY BIODEGRADABLE COD FRACTION (S_{bs}) IN WASTEWATER

The Chemical Oxygen Demand (COD) of domestic wastewaters can be divided into biodegradable and unbiodegradable fractions; both of these fractions can be subdivided into soluble and particulate portions, as illustrated in Fig 5.1. Estimates of the four COD components are necessary for design of new activated sludge plants and can also prove useful for the purposes of control and performance optimization in existing plants Specifically, the readily biodegradable COD (S_{bs}) fraction is of crucial importance in the design and evaluation of nitrogen and phosphorus removal activated sludge systems. For raw and settled wastewaters in South Africa there is acceptable consistency in the values of the unbiodegradable soluble and particulate fractions; hence it is possible to make rough estimates of these parameters in cases where there is insufficient data. However values for the S_{bs} fraction may vary widely from plant to plant; values between 0,05 and 0,40 mgCOD per mg total influent COD have been reported.

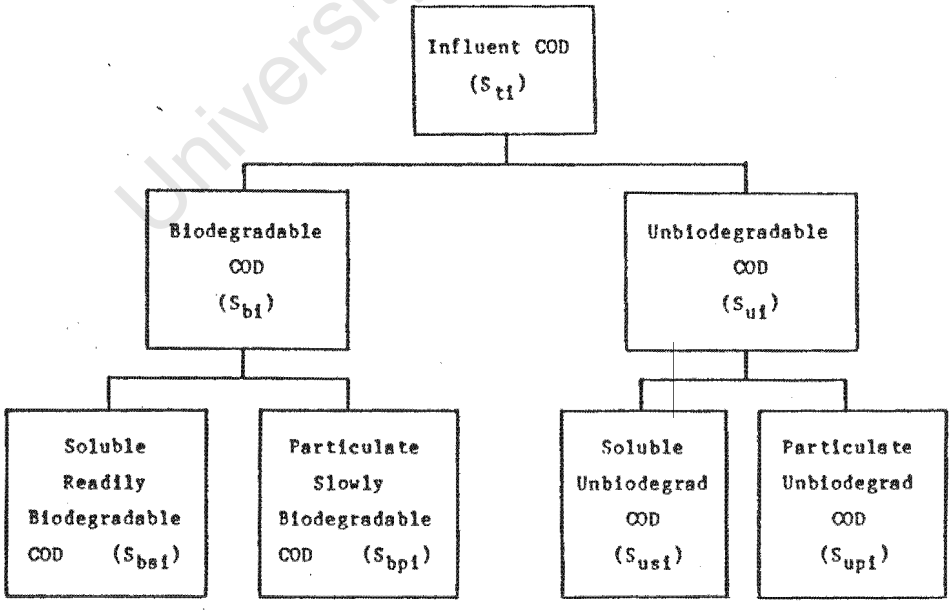

Fig 5.1: Subdivision of influent COD into biodegradable/unbiodegradable and soluble/particulate fractions.

The variability of the S_{bs} fraction and the importance of this parameter in the design and operation of nitrogen and phosphorus removal activated sludge plants highlight the need for reliable and accurate measurement methods. This chapter deals with four methods for measuring the S_{bs} fraction in municipal wastewater:

(1) Through-flow activated sludge process method.
(2) Batch aerobic activated sludge method.
(3) Batch anoxic activated sludge method.
(4) Ultrafiltration method.

The first three methods are based on the different rates of utilization of readily and slowly biodegradable COD by the active organism mass and have been applied successfully in practice. The fourth method involves physical separation of the various wastewater components and is based on the assumption that S_{bs} is comprised of soluble material with a certain (as yet unknown) maximum molecular mass.

In this chapter, the different methods for S_{bs} measurement are compared and evaluated. A brief description will be given for each biological method; detailed descriptions of these methods are provided elsewhere (Dold, Bagg, Ekama and Marais, 1985; Ekama, Dold and Marais, 1985). The ultrafiltration method is discussed in greater detail as development of this method formed a part of the current study. Background to the development of the method and the data gathered during the investigation are included to illustrate pertinent features of the ultrafiltration technique.

5.1 THROUGH-FLOW ACTIVATED SLUDGE PROCESS METHOD

The through-flow activated sludge process method involves monitoring the oxygen utilization rate (OUR) response in a single reactor completely mixed aerobic activated sludge unit (Fig 5.2). The unit is operated at a sludge age of about 2,5 days under daily cyclic square wave loading conditions (i.e. feed on for 12 hours/feed off for 12 hours). At the end of the feed period there is a characteristic precipitous decrease in OUR, followed by a period of a few hours during which the OUR remains near

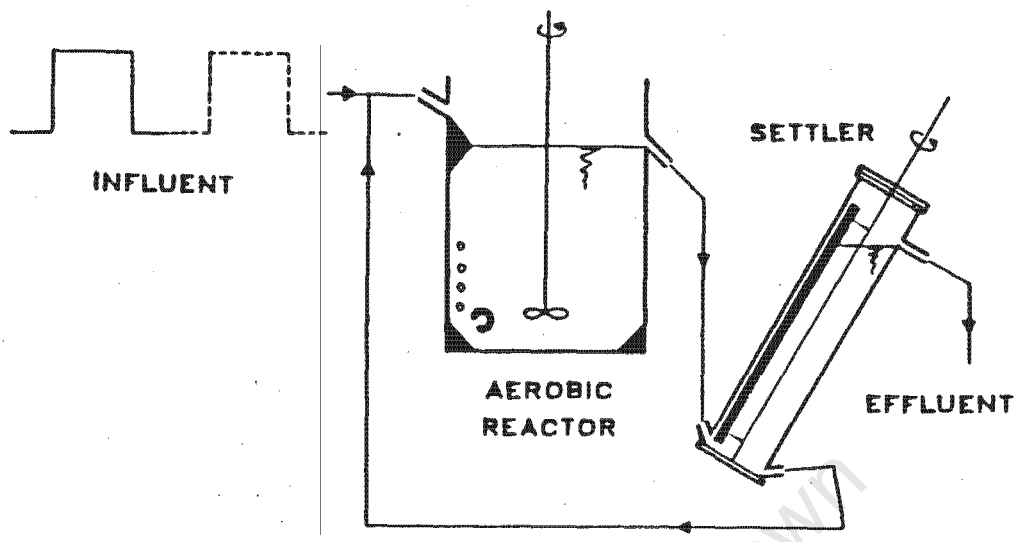

Fig 5.2: Schematic diagram of the single reactor completely mixed aerobic activated sludge system utilized for the square-wave S_{bs} measurement method.

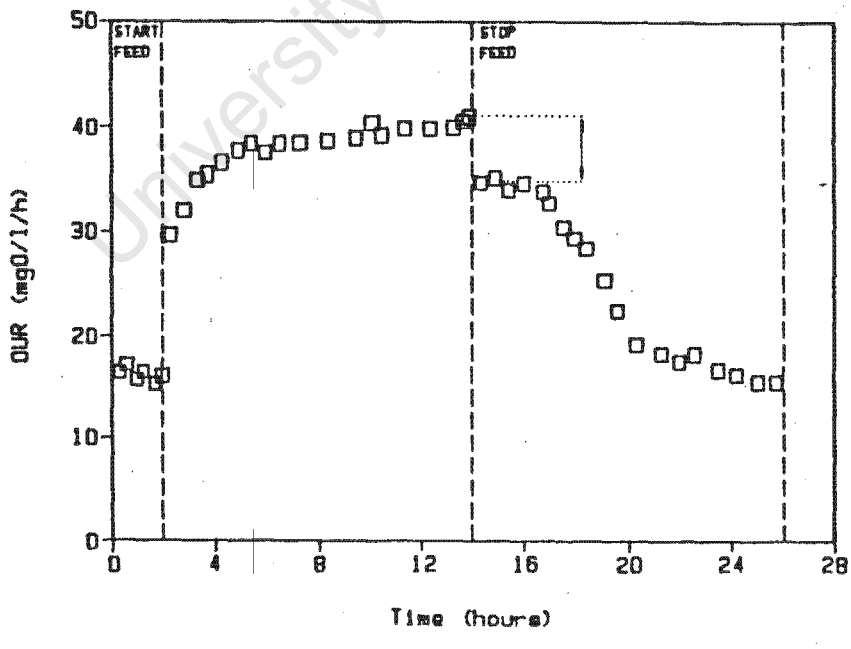

Fig 5.3: Oxygen utilization rate (OUR) response observed over one cycle in the aerobic activated sludge system (Fig 5.2) under daily cyclic square wave loading conditions (12 hours feed/12 hours no feed) with municipal wastewater as substrate. [Sludge age = 2,8 days; T = 20°C: influent COD = 500 mg/ℓ].

constant. The OUR then decreases to the rate associated with "endogenous respiration" (Fig 5.3). The step change in OUR occurs for the following reason: the utilization of S_{bs} for synthesis is so rapid that on termination of the feed. the oxygen requirement for this fraction also ceases virtually instantaneously. Hence there is an immediate drop in OUR from before to just after feed termination. In contrast the slowly biodegradable COD (S_{bp}) is utilized at a rate about an order of magnitude slower than for the S_{bs}. Hence, as a result of the system's short sludge age, there is an accumulation of S_{bp} in the sludge mass until the sludge is saturated and a plateau of OUR is attained i.e. the S_{bp} is utilized at its maximum constant rate. At the end of the feed period this OUR rate is sustained for a few hours, eventually decreasing when sufficient of the accumulated S_{bp} has been degraded. The nett effect is a step change in OUR on feed termination (as shown in Fig 5.3), the magnitude of which can be used to calculate the value of the influent readily biodegradable COD fraction.

The following experimental information is required to calculate the S_{bs} fraction in the influent feed:

Q = feed flow rate over period before feed is stopped (ℓ/d)
V_p = reactor volume (ℓ)
OUR_b = average OUR for period of, say, 1 hour before feed termination (mgO/ℓ/h)
OUR_a = average OUR for period of, say, 1 hour after feed termination (mgO/ℓ/h)
S_{ti} = total COD concentration of feed (mgCOD/ℓ)
ΔOUR = $OUR_b - OUR_a$ (mgO/ℓ/h).

The influent readily biodegradable COD concentration is given by:

$$S_{bsi} = \Delta OUR.V_p.24/[Q.(1-f_{cv}.Y_h)] \qquad (5.1)$$

where
f_{cv} = COD/VSS ratio of volatile settleable solids (1,48 mgCOD/mgVSS)
Y_h = specific heterotrophic organism yield coefficient (0,45 mgVSS/mgCOD)

The influent readily biodegradable COD fraction with respect to the total COD is given by

$$f_{ts} = S_{bsi}/S_{ti} \quad (5.2)$$

5.2 BATCH AEROBIC ACTIVATED SLUDGE METHOD

In this method, appropriate samples of mixed liquor (from the aerobic zone of an activated sludge plant) and wastewater are combined in a batch reactor and the OUR is measured at regular intervals for a few hours after mixing. Initially the OUR remains constant, as both readily and slowly biodegradable COD fractions are utilized at their maximum rate. On depletion of the S_{bs}, the S_{bp} continues to be utilized at its maximum rate and the OUR drops to a new, constant level. The second OUR level is observed because (1) S_{bs} is utilized at a rate about an order of magnitude more rapidly than S_{bp}, and (2) the initial mass of S_{bs} usually is approximately only 20 per cent of the total biodegradable COD, thus ensuring that the concentration of S_{bp} remains sufficiently high to maintain S_{bp} utilization at its maximum (constant) rate over the period that S_{bs} is utilized. The concentration of S_{bs} can be calculated from the measured area under the OUR-time curve (Fig 5.4).

The following experimental information is required to calculate the S_{bs} concentration in the wastewater:

$V_{m\ell}$ = volume of mixed liquor (at concentration X_v mgVSS/ℓ) (ℓ)
V_{ww} = volume of wastewater (ℓ)
S_{ti} = total COD concentration of wastewater sample (mgCOD/ℓ)
ΔMO = mass of oxygen consumed in S_{bs} utilization per litre batch mixture (mgO/ℓ/hr).

The influent readily biodegradable COD concentration is given by:

$$S_{bsi} = \frac{\Delta MO}{(1 - f_{cv}.Y_h)} \cdot \frac{(V_{m\ell} + V_{ww})}{V_{ww}} \quad (5.3)$$

5.3 BATCH ANOXIC ACTIVATED SLUDGE METHOD

The basis for the anoxic batch test is identical to that for the aerobic

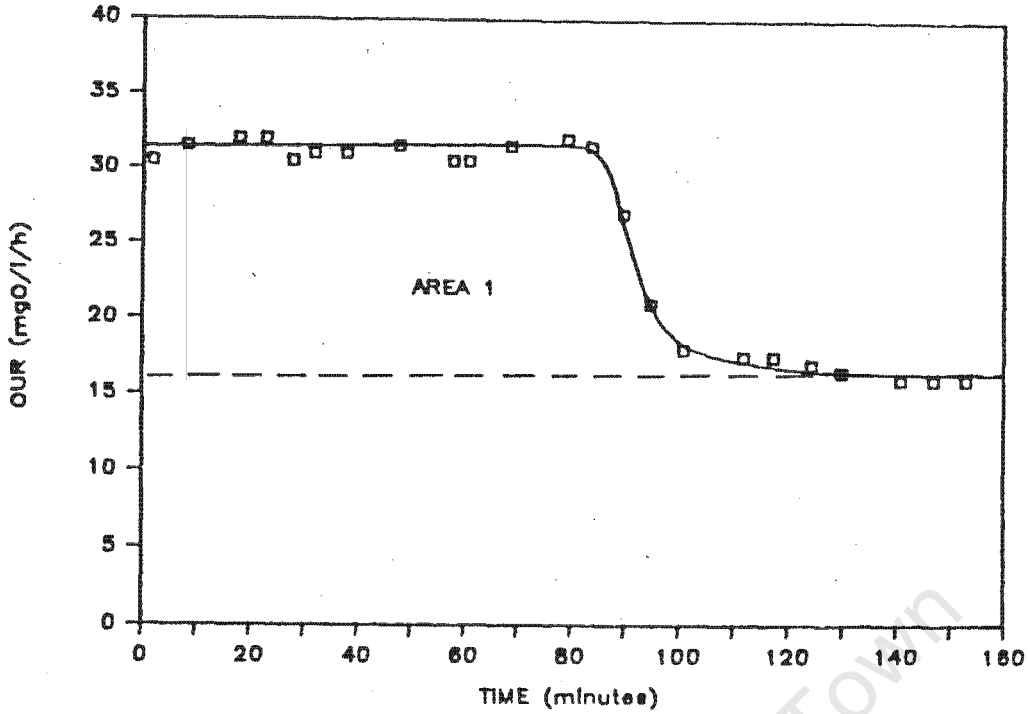

Fig 5.4: Example of the oxygen utilization rate (OUR) response observed in the batch aerobic activated sludge method for measurement of soluble readily biodegradable COD.

instead of oxygen is utilized as the electron acceptor in the metabolism of the S_{bs} and S_{bp}. At the start of the test, nitrate (up to a concentration of about 30 $mgNO_3$-N/ℓ) is added to the batch mixture and its concentration monitored for about three hours. Initially the nitrate concentration decreases at a constant rapid rate, reflecting the sum of the maximum rates of utilization of both S_{bs} and S_{bp}. Once the S_{bs} fraction has been depleted, the rate of decrease of nitrate concentration declines to a less rapid, constant rate reflecting the continued constant rate of utilization of S_{bp} (see Fig 5.5).

The readily biodegradable COD concentration in the wastewater is calculated from the observed nitrate-time data as follows:

(1) the second slow rate of nitrate reduction is extrapolated to intersect the nitrate axis at time zero. The difference between the initial and intercept nitrate concentrations, ΔNO_3, is the concentration of nitrate utilized in the removal of S_{bs}.

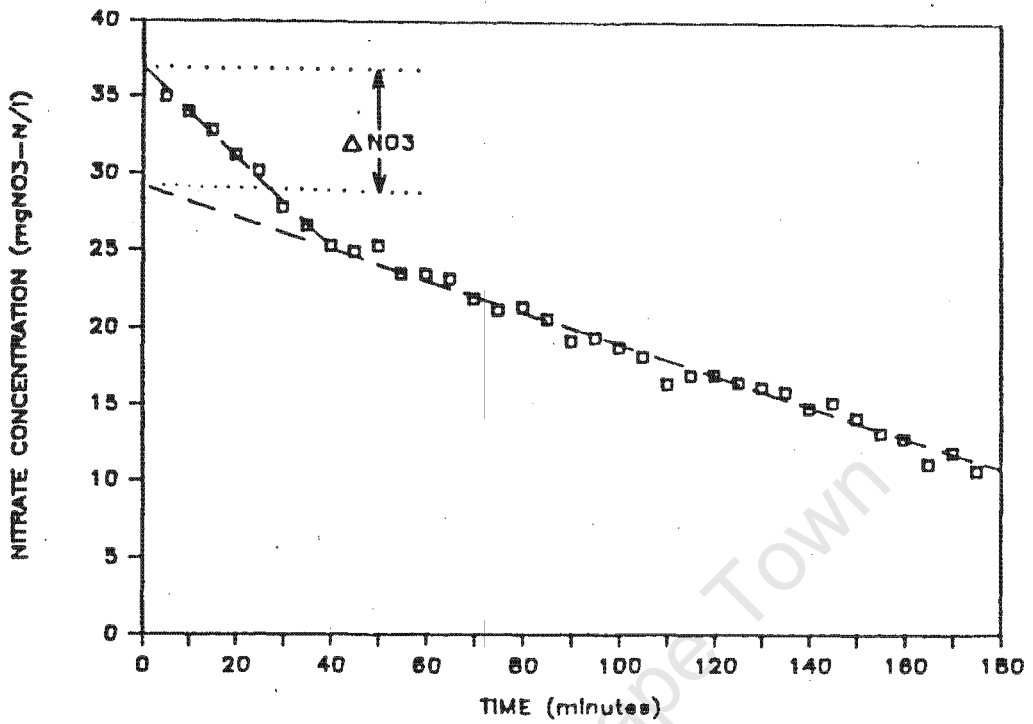

Fig 5.5: Example of the nitrate concentration-time response observed in the batch anoxic activated sludge method for determination of soluble readily biodegradable COD.

(2) The S_{bs} concentration in the wastewater is given by Eq (5.3) with an adjustment for the nitrate/oxygen equivalence (1 $mgNO_3$-N ≡ 2,86 mgO):

$$S_{bs} = \frac{2{,}86 \cdot \Delta NO_3}{(1 - f_{cv} \cdot Y_h)} \cdot \frac{V_{ml} + V_{ww}}{V_{ww}} \qquad (5.4)$$

5.4 ULTRAFILTRATION METHOD

It has been hypothesized that the readily biodegradable COD fraction of wastewater consists of relatively small soluble molecules which are easily transported through the cell wall and therefore are rapidly metabolized. In contrast, the larger molecules require adsorption and extracellular breakdown prior to utilization and hence are slowly biodegradable. If this hypothesis is correct, then it should be possible to estimate the S_{bs} fraction on the basis of physical separation using ultrafiltration; with this filtration medium molecules with molecular mass larger than a certain (approximate) cut-off value are retained. Thus, the difference between the

low molecular weight filtrate COD fraction of the influent (biodegradable and unbiodegradable soluble) and the effluent (unbiodegradable soluble) should reflect the readily biodegradable COD, S_{bs}, i.e.

S_{bs} = Influent filtrate COD - Effluent filtrate COD. (5.5)

5.4.1 Summary of experimental method

Ultrafiltration membranes are very susceptible to blocking ("blinding") by colloidal and particulate matter. Therefore, prior to filtering wastewater samples it is necessary to follow a pre-treatment procedure to remove as much of this material as possible. The following step-wise procedure was found suitable:

(1) Initially the influent wastewater sample is centrifuged at 6500 r.p.m. for a minimum of 30 minutes. This step ensures removal of a large fraction of the particulate matter.

(2) Both influent and effluent samples are subjected to two stages of pre-filtration. First the samples are pre-filtered by vacuum through two successive glass microfibre (Whatman GF/B) membranes; then the samples are passed through a 0,45 µm membrane filter (Millipore). This step ensures removal of (1) residual particulate matter, and (2) colloidal matter which was evidenced by a "milky" colour in certain wastewater samples.

(3) Finally the filtrates are passed through an ultrafiltration membrane in a stirred, static cell (Amicon Corp. 50 mℓ cell) under pressure of nitrogen gas. The difference between the ultrafiltered COD's of the influent and effluent gives the estimate of S_{bsi}. Details of the ultrafiltration procedure are given in Appendix B.

5.4.2 Comments on experimental technique

The objective of the intensive research into the ultrafiltration method was the development of a quick, reliable, non-biological method of S_{bs} measurement which could be used in a plant laboratory equipped with a minimum of basic analytical apparatus. The advantages of a simple filtration and COD analysis in terms of time and man hours saved, compared

with the input required to successfully maintain a square wave continuous flow activated sludge laboratory scale plant are clear. The following points are included, however, to explain the experimental method in greater detail and the reasons for the application of certain techniques.

5.4.2.1 Centrifugation: The purpose of centrifuging the influent wastewater sample is to remove as much of the large-size particulate matter as possible because this material is a possible source of filter "blinding". Without this removal the particulate matter can form a layer on the surface of the filtration membrane which acts as an extra "filter", tending to retain some of the soluble material and leading to an abnormally low filtrate COD value. By centrifuging the influent before filtration, it was intended that the formation of the blinding layer would be prevented.*

Analysis of samples centrifuged at different speeds of rotation indicated that the amount of particulate matter removed increased with increased centrifuge speed. Speeds higher than the current specified value of 6500 r.p.m. have not been tested as higher rates are attained only in special centrifuges not normally available in plant laboratories. However it would appear that this rate of centrifugation is sufficient to remove the large-size particulate matter.

5.4.2.2 Pre-filtration: Centrifugation at rates of approximately 6500 rpm ensures removal of the bulk of the particulate matter in wastewater samples. However, problems of filter blinding nevertheless were encountered if centrifuged samples were used directly in the ultrafiltration stage. Three different indications of filter blinding were apparent: (1) unusually low filtration rates; (2) the formation of a brown

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*An alternative method for removing the bulk of the particulate matter (say, particles > 1μm) would be to filter samples through a glass microfibre filter. However, while this method can be used, it was found that these filters clogged very rapidly when filtering raw wastewater samples and several 47 mm diameter filters were required to process 100 mℓ of sample. Centrifuging thus reduced the cost of the analysis.

slime layer (gel) on the ultrafiltration membrane; and (3) very low filtrate COD values indicating retention of a portion of the soluble COD by the membrane. To avoid filter blinding it was necessary to include a pre-filtration stage.

Initially filtration through a 0,45µm membrane was evaluated for the pre-filtration. However, these membranes also were subject to "blinding". This was evidenced by a sudden drop in filtration rate after approximately 20 mℓ of sample had passed through the filter (47mm diameter filters). In this case the blinding appeared to relate principally to the presence of a "milky" colour in the wastewater samples caused by high molecular weight colloidal organic compounds. In batches of sewage which did not exhibit the milky colour volumes of 100mℓ could be filtered through a single 0,45µm membrane without any apparent decrease in filtration rate, and with minimal deposit of the brown slime on the membrane as encountered with filtration of the discoloured samples.

The organic colour present in wastewater generally consists of the so-called aquatic humus or humic acids. It is not a single definable organic compound; many different organic compounds resulting from the decomposition of carbohydrates, proteins, lignins and tannins in the soil, such as hydroxybenzoquinones, phenolic units, peptides, amino acids and acid radicals can combine to form the humic acid molecules (Christman and Ghassemi, 1966). The possibilities for reactions or combinations are unlimited; every molecule is likely to be different, but most have similar properties, related to the active nature of the carboxyl, phenol and hydroxyl groups which are common to all. A possible structure for humic acid is given in Fig 5.6. The formation of humus is dependent on factors such as vegetation, population and activity of micro-organisms, and on the hydrothermal conditions. The physical and chemical properties of the soil also are of great importance, both with regard to the rate of the humification process and to the composition of the humus products. Thus the humus content of a wastewater will depend largely on the nature of the soil in the areas from which the wastewater originates. A further factor is the ingress of groundwater into the sewer system during the rainy season. This was particularly noticeable with the wastewater used in this investigation (drawn from the Cape Flats); during the winter rainfall

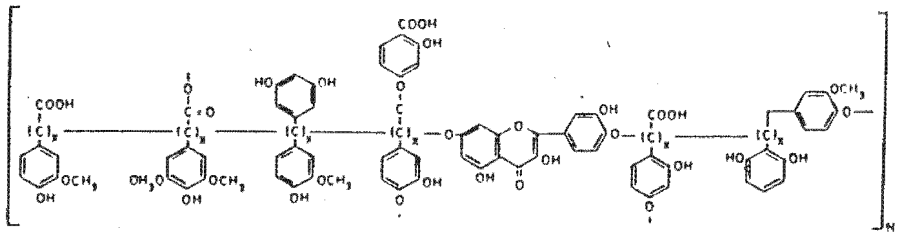

Fig 5.6: Proposed structure of the coloured humus molecule, according to Christman and Ghassemi (1966).

period, the wastewater, although more dilute than normal, had an appreciably higher organic colour content.

Glass micro-fibre filter papers were found to give the best results with respect to retention of the humic compounds. The rate of filtration is extremely fast, but a long filtration path through the criss-cross matrix of glass micro-fibres ensures that a major portion of the colloidal molecules either are adsorbed or entrapped and thus filtered out. By adopting a two-stage pre-filtration (first through the glass fibre filter and then through a 0,45µm membrane) problems of blinding in the ultra-filtration appeared to be obviated.*

5.4.2.3 Range of ultrafiltration membranes tested: The validity of this method is dependent on utilizing an ultrafiltration membrane which provides the appropriate division between large colloidal slowly biodegradable molecules and the soluble readily biodegradable molecules. During the course of the investigation a range of filters with molecular weight (MW) cut-off values between 500 and 100 000 were tested. The initial results were obtained using 500 MW and 5000 MW cut-off filters;

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* Flocculation was considered as an alternative method for the removal of organic colour. However research in Israel (Narkis et al., 1980) has shown that between 30 and 40 per cent of the soluble COD also is removed. Furthermore, the inclusion of flocculation in the pre-treatment of an influent sample was considered to be an unnecessary complication, bearing in mind the ultimate objective of a quick, reliable and simple method.

these are shown plotted against the corresponding S_{bsi} values obtained from the standard square wave activated sludge method (Figs 5.7 and 5.8). The relationship between the measurement pairs is approximately linear. However the overall trend of the data indicated an underprediction of the S_{bs} value. Thus, it was decided to evaluate higher molecular weight cut-off membranes.

In an attempt to define minimum and maximum limits of a molecular weight range within which further investigation could be instigated, parallel tests were carried out using ultrafiltration membranes with molecular weight cut-off's of 10 000 and 100 000 respectively. Data gathered from these experiments are shown plotted against the corresponding standard square wave activated sludge process value in Figs 5.9 and 5.10. [It should be noted that for the data shown here the problems of filter blinding had not been eliminated as yet]. Comparison of Figs 5.9 and 5.10 indicates that the distribution of data was very similar for the two molecular mass cut-off filters. In each case a portion of the data straddled the diagonal but the major portion indicated that ultrafiltration under-predicted the S_{bs} value, irrespective of whether a 10 000 or a 100 000 molecular weight cut-off filter was used. The reasons for the under-prediction relate to filter blinding and problems of filter storage - these are discussed later. However, on the basis of these results it was decided to continue the investigation with only the 100 000 molecular weight cut-off membranes.

5.4.2.4 Storage and re-use of membranes: The determination of S_{bs} requires ultrafiltration of an influent and an effluent sample. In the method proposed here the volume of filtrate collected for each sample is approximately 30 mℓ. Generally only one S_{bs} determination was made per day; however, on certain occasions up to eight analyses were made on a single day. It was found that all eight samples could be filtered through a single membrane sequentially (flushing with distilled water between samples). No problems of filter blinding were encountered provided the pre-filtration was performed satisfactorily; this was evident in that there was not a decrease in the filtrate COD of sequential samples. The

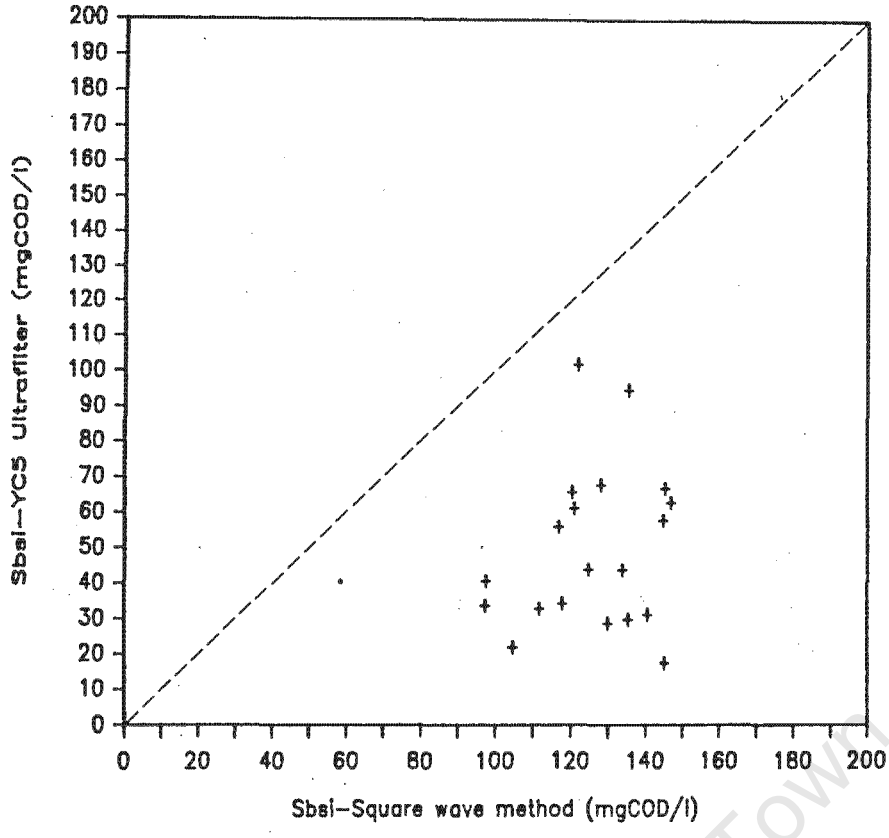

Fig 5.7: Diagram to show relationship between corresponding S_{bs} values measured by square wave biological assay method and 500 MW cut off ultra-filtration membrane respectively.

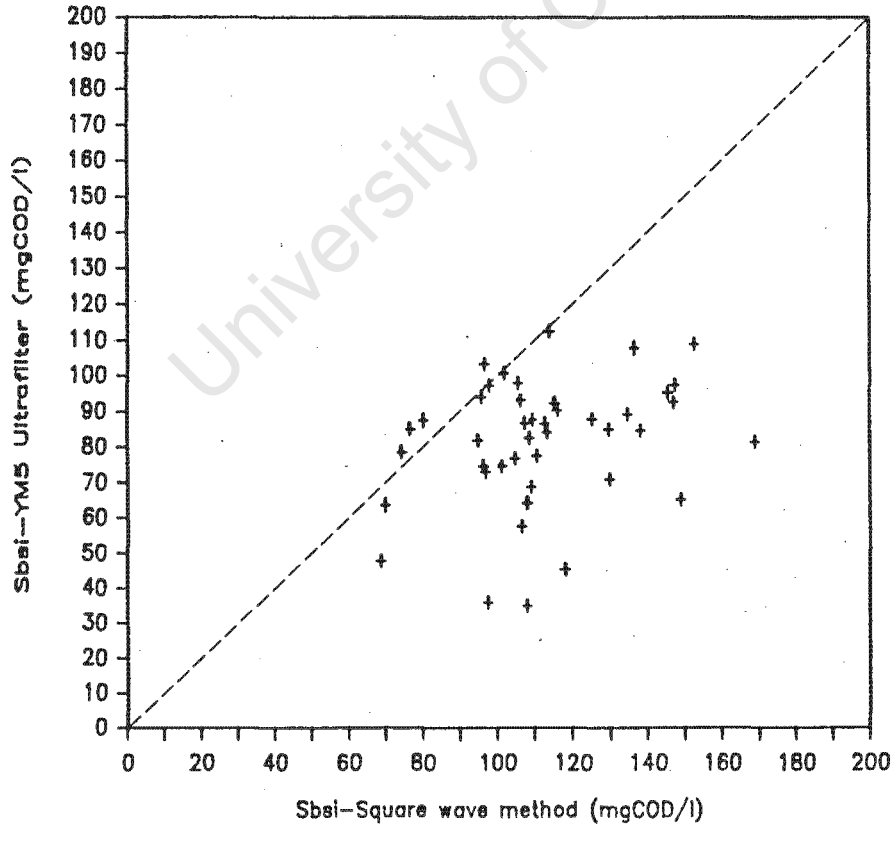

Fig 5.8: Diagram to show relationship between corresponding S_{bs} values measured by square wave biological assay method and 5000 MW cut off ultra-filtration membrane respectively.

fact that a number of samples could be filtered sequentially through the same membrane is not unusual. In fact, the ultrafiltration membranes used in this investigation (Diaflo ultrafilter, Amicon Corp.) reportedly can be re-used after suitable treatment and storage.

The manufacturer recommends storage in a 10 per cent ethanol/water solution at low temperature. However, it was found that membranes stored in this manner could not be re-used successfully. Even with thorough flushing of the membrane before and after use with distilled water, and careful removal of any thin scum layer due to humic compounds, the reproducibility of the results deteriorated with daily use even where only one S_{bs} determination was made per day. This is illustrated in Fig 5.11 which shows results for six different membranes on consecutive days of use (i.e. storage for 24 hours). For the first day of use the ultrafiltration membrane provides a value close to that of the S_{bs} measured by the through-flow square-wave biological method. However on succeeding days there is a continual drop in the value of the influent ultrafiltration value; the effluent ultrafiltration results remain fairly constant, but the end result for each pair of data is a very low estimate of the influent S_{bs} of the wastewater.

It was found by trial that storage of the membrane in deionised water (containing a few drops of mercuric chloride solution), at low temperature (in a fridge at 4°C) for an extended period of <u>at least 72 hours</u> was a more suitable technique. It seems that storing the membrane for up to 3 days before re-use promotes lysing of the filtered substrate out of the membrane, thus restoring the membrane almost to its original condition. Using this method of storage, several sets of influent and effluent samples could be filtered sequentially on a single day with <u>no</u> deterioration in the reproducibility of the results. A minimum of four pairs of influent and effluent samples could be filtered before deterioration of the quality of the results due to gradual blocking of the membrane. The favourable influence of storage on the membranes between periods of use is illustrated in Fig 5.12; for this data the membrane had been stored for a period of at least 72 hours between usage. Apart from days 9, 15 and 22 , where the influent filtrate COD values obtained were lower than the average, the filtrate S_{bs} values were either closely similar to those determined by the square wave method (in the first set of data) or greater than the biological values by an average of 40 mgCOD/ℓ (in the second set of data).

Bearing in mind that storage of the membranes was found virtually to eliminate deterioration in the quality of the results by filter blocking, the low results obtained on days 9, 15 and 22 respectively can be said to be due to the influence of organic colour in the wastewater which was not removed totally in the pre-filtration stage. This is substantiated by the fact that it is only the influent filtrate data which show values markedly less than the average. This can be explained by noting that the "milky" colloidal colouring was present only in the influent; although the humic compounds often are not degradable in the time span of the activated sludge treatment, it appears that the colour is excluded from the effluent through enmeshment and adsorption in the sludge mass once the influent enters the reactor and thus do not appear in the effluent. Both the influent and effluent samples receive the same pre-filtration treatment and are passed through the same grade of ultrafiltration membrane; if organic colour was present in the effluent, the effluent COD value for the sample corresponding to the "affected" influent sample would be lower than the average - this is not the case.

A great deal of spurious data was accumulated prior to finding an effective storage method for the membranes. It is worth considering some of the initial results to illustrate the importance of the storage procedure.

5.4.3 Analysis of ultrafiltration results

In order to assess the validity of an ultrafiltration method for S_{bs} measurement, the results should be compared with data obtained from parallel tests using a recognized standard method. At present the most reliable and general procedure for determining the S_{bs} fraction in an influent wastewater is the short sludge age square wave fed biological (through-flow ASP) method. The two batch test methods, aerobic and anoxic, will also usually produce reliable results, but are not regarded as a general "standard" as is the square wave method. For the purposes of this investigation the daily S_{bs} measurements obtained by ultrafiltration and COD analysis were compared with those provided by a through-flow CMAS system operated in accordance with the procedure outlined in Section 1. The 2,5 day sludge age system was run under square wave conditions i.e. 12 hours feed/12 hours no feed; the influent flow of 36 ℓ/day, at a concentration of 500 mgCOD/ℓ provided a process load of 2760 mgCOD/ℓ reactor/day.

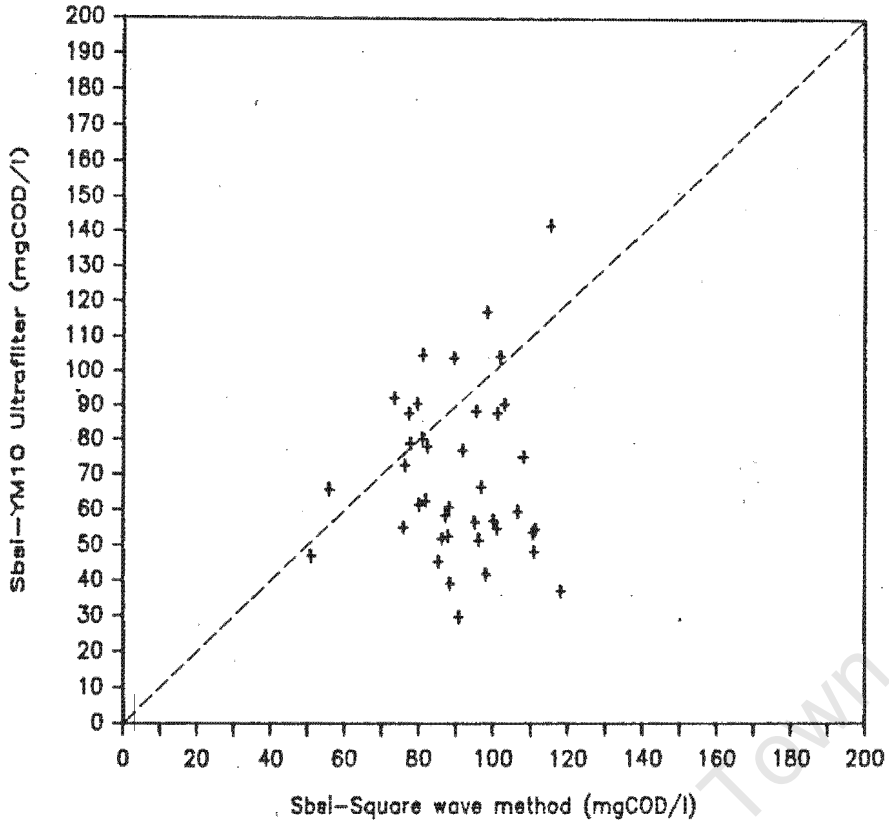

Fig 5.9: Diagram to show relationship between corresponding S_{bs} values measured by square wave biological assay method and 10000 MW cut off ultra-filtration membrane respectively.

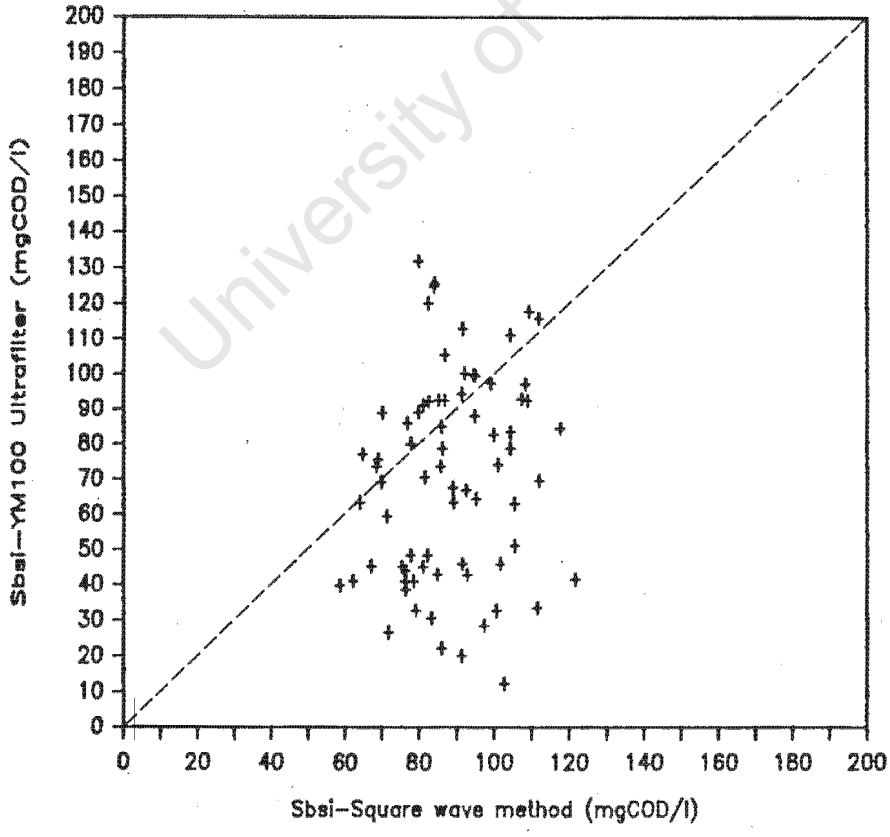

Fig 5.10 Diagram to show relationship between corresponding S_{bs} values measured by square wave biological assay method and by 100 000 MW cut off ultra-filtration membrane respectively. (Unprocessed data).

At the outset, it was realised that analysis of the S_{bs} data involved comparing two sets of experimental data, both of which were influenced by different sources of error - hence one would never be certain which of the values for a particular day's analysis was the "correct" one. Bearing this in mind it was accepted that the through-flow square wave system would provide, under carefully controlled laboratory conditions, the most reliable and accurate estimate of the S_{bs} fraction of the wastewater.

Fig 5.10 shows how the initial ultrafiltration S_{bs} estimates ranged from about 15 mgCOD/ℓ to about 135 mgCOD/ℓ while the S_{bs} estimates from the square wave test only ranged from 65 to 110 mgCOD/ℓ. No attempt has been made to select and remove data points on the basis of the influence of "blinding" by an organic colour layer, or the gradual blocking up of a membrane with use, or that the data points were obtained before it was detected that storage of the membrane had a very favourable effect on the quality of the results.

The data presented in Fig 5.10 has been replotted (Fig 5.13) in terms of the criteria that the data points correspond to the first time that the membrane has been used, or to the first sets of samples filtered after storage of the membrane for more than 72 hours between filtrations. In terms of the second criterion, several of the membranes used during the latter stages of the investigation have provided up to five separate data points. The squares correspond to the first time that a membrane was used; the crosses represent the samples treated after storage of a membrane (Fig 5.13). Regression analysis of the data in Fig 5.13 assuming a linear multiplicative relationship between the two parameters (i.e. a straight line through the origin) gives a relationship where the filtered value is about 102 percent of the biologically determined value. This close correspondence would appear to validate the filtration method. It may be asserted therefore that provided the details of the filtration method technique are carefully adhered to, the filtration method will produce an acceptably accurate estimate of the soluble readily biodegradable substrate fraction of a municipal wastewater.

5.4.4 Statistical assessment of results

It has been noted in Section 4.3 that one of the problems in assessing the validity of the ultrafiltration method for S_{bs} determination is the lack of a common basis for comparing the different methods. Essentially, the

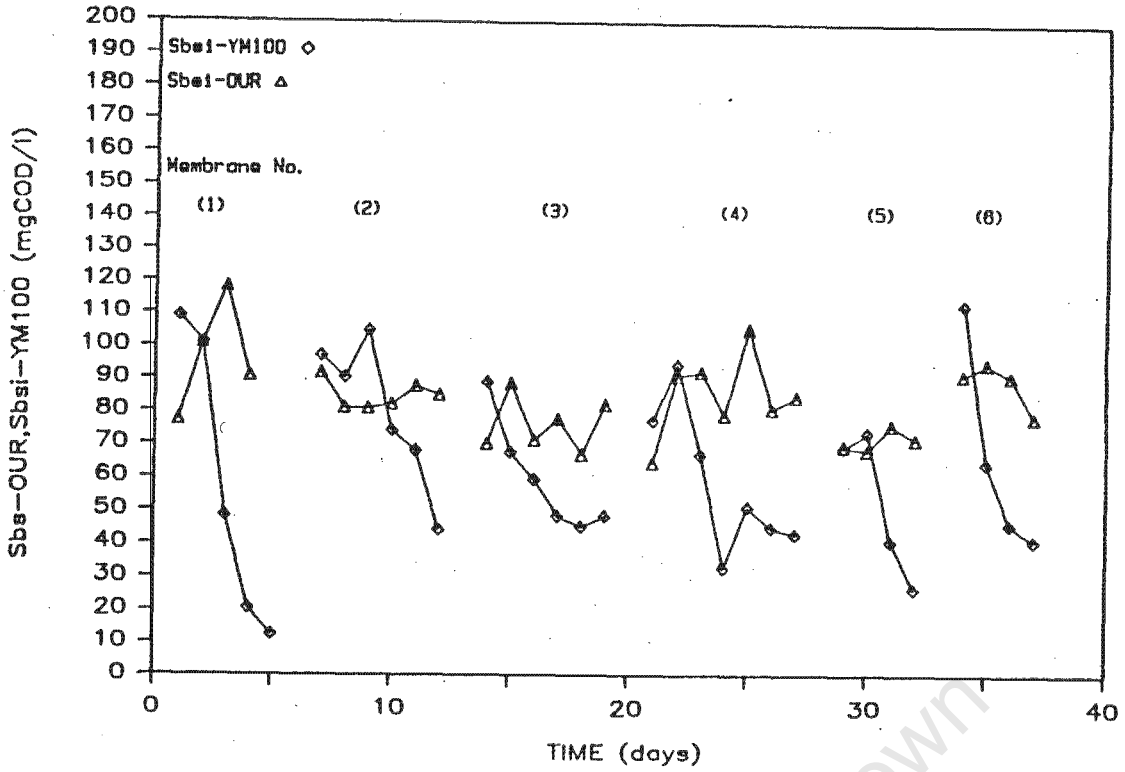

Fig 5.11 Diagram to illustrate the effect of blocking of ultra-filtration membranes on the magnitude of the measured (filtration) S_{bs} value.

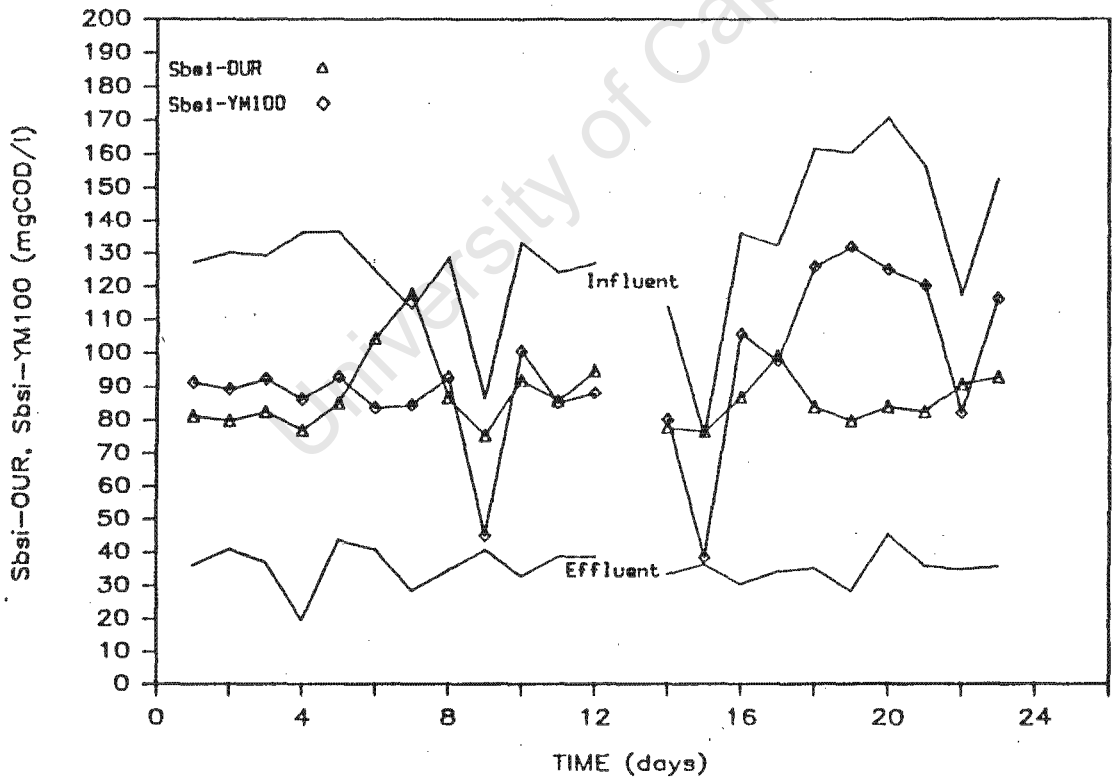

Fig 5.12 Diagram to illustrate the effect of storage of YM100 ultra-filtration membranes on the magnitude of the measured (filtration) S_{bs} value. Low estimates on days 9, 15 and 22 were due to excessive colour in the influent sample.

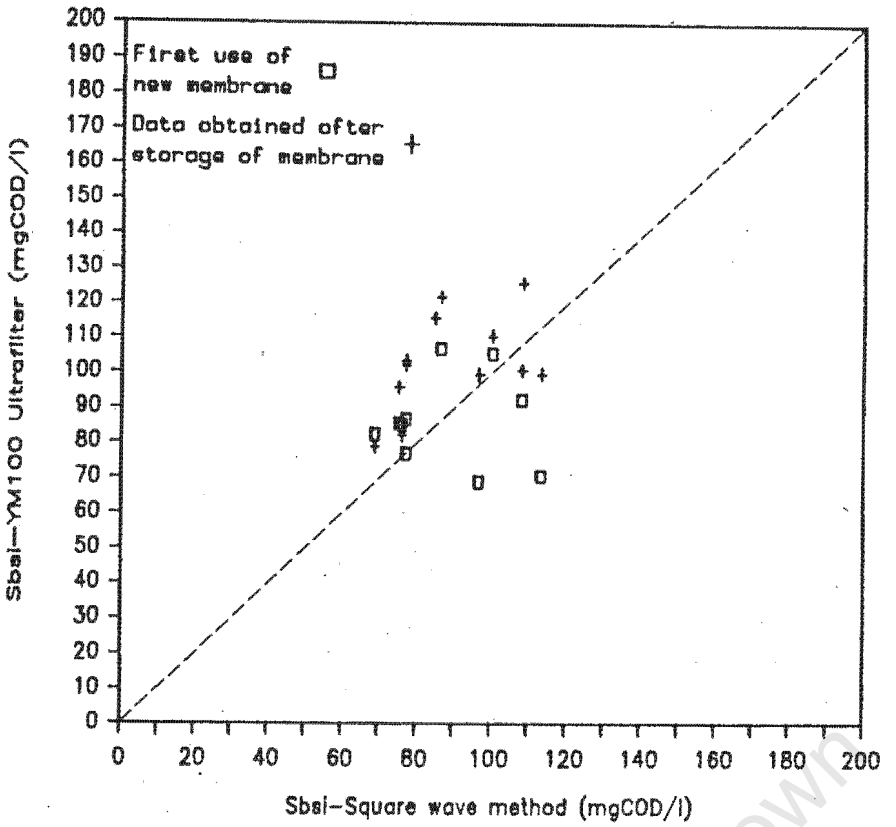

Fig 5.13: Diagram to show relationship between corresponding S_{bs} values measured by the biological assay method and 100 000 MW cut off ultra-filtration membrane (first use of a new membrane, or after 72 hours storage at 4°C) respectively.

only method of assessment is through comparison of two sets of experimental values, each of which is influenced by a different set of experimental conditions. So far the assessment has been through comparison of sets of daily data pairs, with values of S_{bs} determined by ultrafiltration plotted versus values from the square wave OUR method with the latter method being the reference. It is instructive, however, to consider each method separately on the basis of the statistical distribution of results yielded by each.

In the operation of the laboratory where this investigation was undertaken the influent to experimental activated sludge systems is taken each day from a batch collected from a Cape Town treatment plant. The batches are stored at 4°C and utilised for periods of between 7 and 14 days. Each day a routine determination of the S_{bs} is made via the square wave OUR method. A typical set of results from one batch of wastewater is shown in a percentage probability statistical plot [Fig 5.14(a)]. In this case eleven daily determinations of S_{bs} were performed. The fact that the data points lie close to a straight line indicates that the data is normally distributed. The mean S_{bs} value from the plot is 102 mgCOD/ℓ with a standard deviation of 9 mgCOD/ℓ.

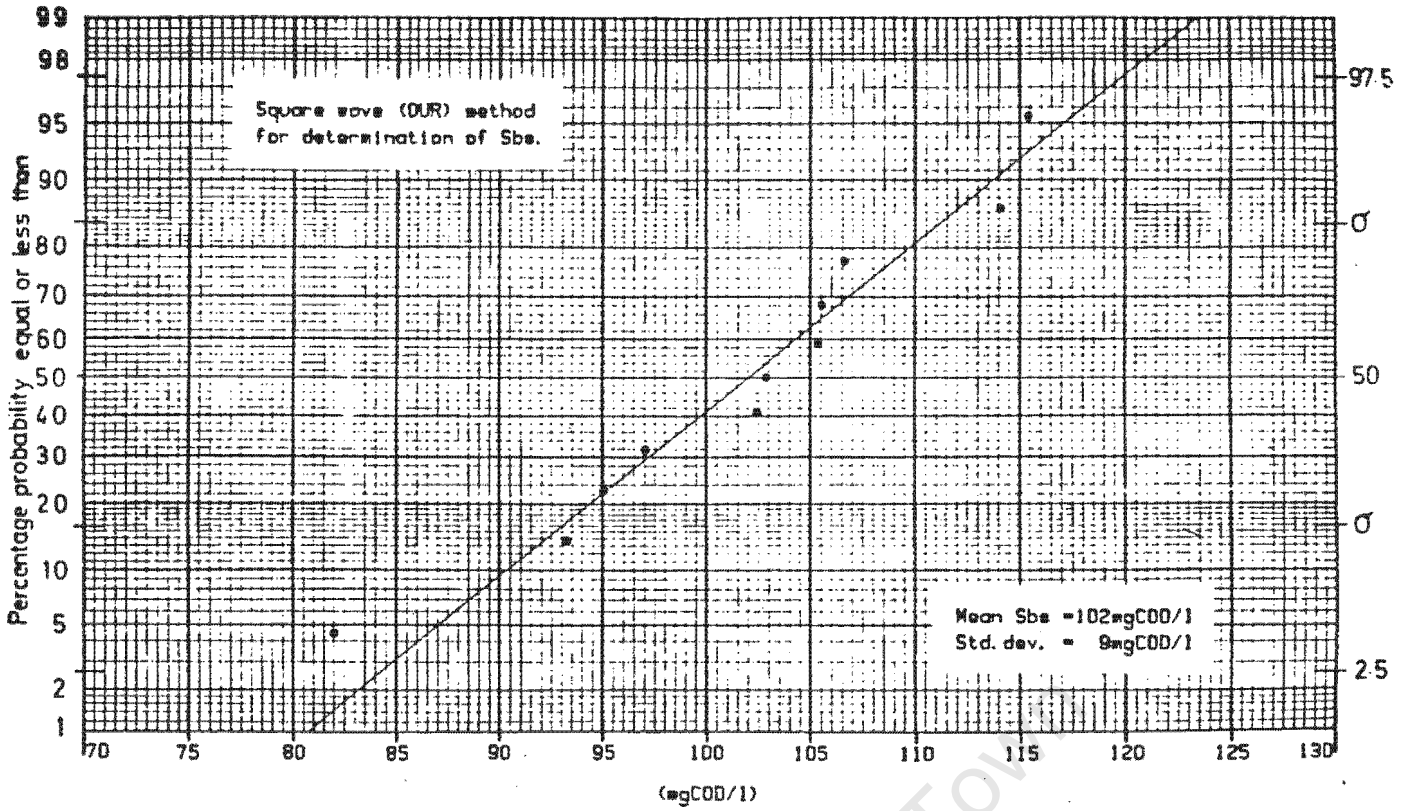

Fig 5.14(a): Statistical percentage probability plot of daily S_{bs} (square wave OUR method) measurements for a single batch of raw influent wastewater.

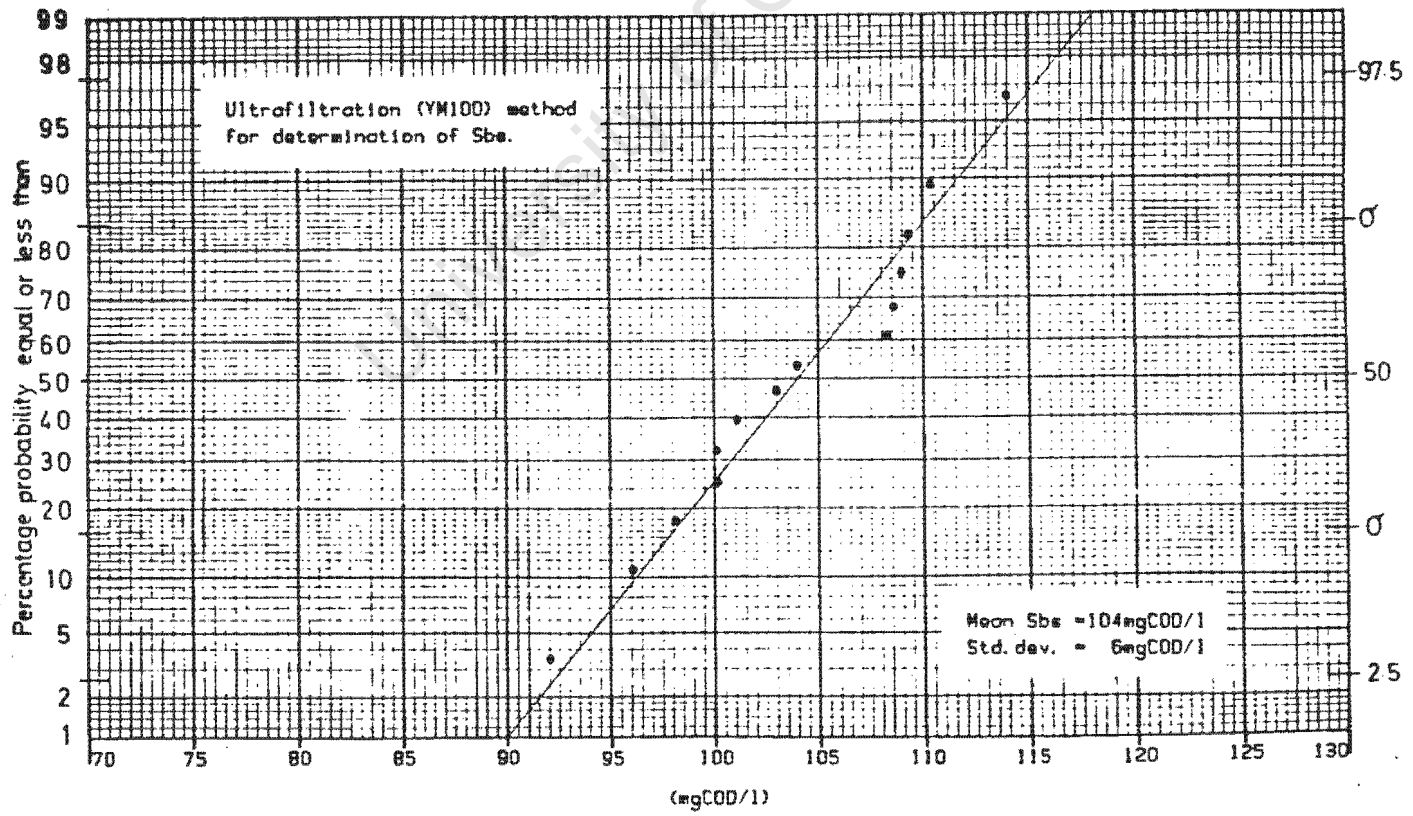

Fig 5.14(b): Statistical percentage probability plot of S_{bs} measurements (by ultra-filtration using 100 000 MW cut off membranes) for the same batch of influent wastewater [cf. Fig 5.14(a)].

Ultrafiltration S_{bs} determinations were performed on the same batch of wastewater as considered above. The percentage probability plot of the data obtained from 14 sequential daily determinations is shown in Fig 5.14(b). Again the data is normally distributed, with a mean of 104 mgCOD/ℓ and a standard deviation of 6 mgCOD/ℓ. Comparison with the values from Fig 5.14(a) indicates that the two methods yield very similar estimates of the S_{bs}, with the ultrafiltration results showing a slightly narrower distribution.

5.4.5 0,45 µm Filtration Measurement

The application of the ultrafiltration method of S_{bs} measurement requires the use of relatively expensive and sophisticated laboratory equipment. The availability of this equipment at small activated sludge treatment plants is unlikely; also the plant operators may only require an approximate estimate of the S_{bs} concentration in the wastewater. For this reason it was decided to evaluate the data from the pre-filtration stage (glass fibre plus 0,45 µm filtration); perhaps these values would provide estimates of S_{bs} sufficiently accurate for general plant operating purposes. The data obtained are plotted against OUR S_{bs} in Fig 5.15.

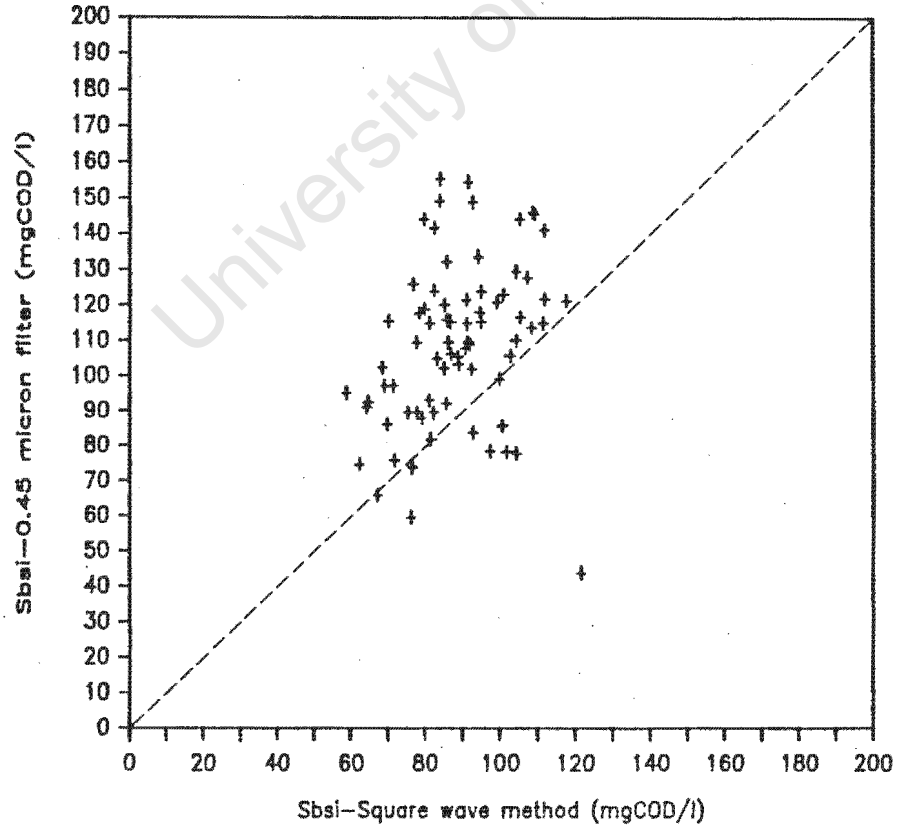

Fig 5.15: Diagram to show relationship between corresponding S_{bs} values measured by square wave biological assay method and 0,45 µm Millipore membrane respectively.

Linear regression analysis of the data in Fig 5.14 between 0,45 µm filtrate COD and the S_{bs} concentration, indicates that the filtered value is approximately 25 percent greater than the biologically determined value. Alternatively, the S_{bs} value appears to be approximately 80 percent of the 0,45µm filtrate value. It seems that filtration of influent and effluent wastewater samples with 0,45 µm membranes will yield an approximate estimate of the readily biodegradable soluble substrate concentration by difference, and in some cases this may be of sufficient accuracy for plant operation and control. However if a more accurate estimate of S_{bs} is required, 0,45 µm filtration will not be a satisfactory measurement technique. It should be stressed that prior to 0,45 µm filtration the samples should be filtered through a glass-fibre microfilter; this reduces problems of blinding with the 0,45 µm filters due to colours in the influent wastewater.

5.5 CONCLUSIONS

The most reliable and general procedure for determining the readily biodegradable COD fraction of an influent wastewater seems to be the short sludge age square wave fed biological method (through-flow ASP method). The aerobic and anoxic batch biological methods will usually provide reliable results but are not as general as the square wave method. The physical separation method has developed to the point where an experimental technique has been defined and tested, and found to provide suitably accurate results by ultrafiltration. Further research is necessary on wastewaters of different origin and composition in order to test the range of applicability of the method. Thus far, however, the advantages of the ultrafiltration method lie in the simplicity of the method, the speed of operation and the accuracy of the results obtained.

CHAPTER SIX

CONCLUSIONS

This investigation was undertaken to:

(1) verify the bisubstrate hypothesis in the activated sludge process by (a) monitoring the response of systems to the feeding of selected substrates apparently characteristic of the bisubstrate fractions, and (b) checking in what measure the general activated sludge model, based on the bisubstrate hypothesis, simulates the observed bisubstrates. The artificial substrates selected were glucose and starch, representative of soluble readily biodegradable and particulate slowly biodegradable substrate fractions respectively;

(2) evaluate a physical-chemical method i.e. ultrafiltration, as a possible suitable alternative to the standard (at present) biological assay method for determining the readily biodegradable COD fraction of municipal wastewater.

1. BISUBSTRATE HYPOTHESIS INVESTIGATION

Systems were run under steady state and square wave cyclic state with glucose only, starch only and glucose/starch mixtures. From the steady state response the specific yield values were determined and the reliability of the data checked by doing mass balances on the COD. From the cyclic response the specific rate constants for growth and solubilization respectively were determined by simulation using the general model and specifying the concentrations of readily and slowly biodegradable fractions equal to the stoichiometric concentrations of glucose and starch in the feed. The constants thus determined were compared with the "standard" constants for municipal wastewaters.

With a purely readily biodegradable substrate (glucose) it was necessary to increase only the maximum specific growth rate constant for heterotrophs, $\hat{\mu}_H$ from 2,50 to 3,0/day and the half saturation coefficient, K_S, from 5,0 to 10,0 mgCOD/ℓ; the specific yield constant, Y_H, remained as before, Y_H = 0,666 mg cell COD yield/mg COD utilized. For a purely

particulate slowly biodegradable substrate (maize starch) and a mixture of glucose and maize starch, the growth rate constant, $\hat{\mu}_H$, remained at 2,5/d the solubilization rate for particulate substrate, K_h, had to be reduced from 2,20 to 1,80 mgCOD/mg cell COD/day and the specific yield, Y_H, reduced from 0,666 to 0,592 mg cell COD yield/mg COD utilized. With these constants reasonably good simulated fits to the observed data were obtained. Compared to the standard constants the deviations of the constants for these specific substrates were small and very likely are due to the specificness of the substrates - it is to be expected that both the readily and slowly biodegradable COD fractions would be influenced in some degree by the chemical structure or the organic material in each fraction.

The conclusion formed from this investigation is that the observed response data of the glucose, starch and glucose/starch substrates and the response predicted by the bisubstrate model are consistent to such a degree that the bisubstrate hypothesis is strongly supported, i.e. that it has a physical basis in the chemical structure of the organic materials in the feed.

2. MEASUREMENT OF READILY BIODEGRADABLE COD

The objective of this part of the investigation was to develop and evaluate a physico-chemical method of determining the readily biodegradable COD fraction of municipal wastewater. It was hypothesized that readily biodegradable COD consists of relatively small soluble molecules which are easily transported through the cell wall. If this hypothesis is correct then it should be possible to form an estimate of the readily biodegradable fraction by physical separation using, for example, ultra-filtration.

The basic method whereby the readily biodegradable COD has been identified and estimated is from the oxygen utilization rate response of a short sludge age system operated on a square wave feed pattern. In this investigation this biological method was used as the reference against which the ultra-filtration method was compared.

Considerable effort had to be expended to develop the technique; problems with blinding of the ultra-filters had to be resolved and procedures for

cleaning the membrane for re-use had to be developed. To limit the number of extraneous variables to a minimum the investigation was restricted to one wastewater only, that from Mitchell's Plain, Cape Town.

It was found that filters with molecular weight cut-off values of 500, 5000 and 10000 significantly underestimated the readily biodegradable COD concentration, but it did so in a consistent manner in that the values were proportional to those of the biological method. The 100000 molecular weight cut-off filter gave values reasonably well correlated with the values determined by the biological method. It was found further that 0,45µm filters also gave rise to consistent results but overestimated the biologically derived value by approximately 20 percent.

From this investigation it was concluded that both the molecular weight cut-off filters and the < 0,45 µm filter can be used to estimate the readily biodegradable COD fraction. However before this method is proposed for general use it needs to be checked on a number of waste flows.

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APPENDIX A1

EXPERIMENT No.1: CYCLIC FEED WITH GLUCOSE AS SUBSTRATE
(SLUDGE AGE = 2.50 days).

TABLE 1.1: Data extracted from daily experimental results to determine mean influent COD value for simulation purposes.

Date	Influent (mgCOD/l)	Reactor (mgCOD/l)	Waste (mgCOD/l)	Reactor (mgVSS/l)	Waste (mgVSS/l)	Reactor (fcv)	Waste (fcv)
31/3	1406	811	805	490	440	1.54	1.70
1/4	1426	847	800	486	452	1.62	1.64
2	1575	893	821	512	454	1.63	1.68
3	1524	845	711	504	484	1.56	1.35
4	1452	840	747	548	440	1.43	1.57
5	1375	937	783	560	516	1.57	1.41
6	1355	927	762	(738)	666	1.18	1.06
7	1432	876	845	614	(796)	1.33	(0.99)
8	1406	778	664	480	NA	1.50	NA
9	1391	886	726	554	492	1.49	1.36
10	1483	901	865	512	580	1.65	1.39
11*	1488	881	943	534	590	1.54	1.50
12*	1411	865	834	444	436	1.82	1.78
13*	1452	855	845	600	570	1.33	1.38
14	1468	834	886	568	560	1.37	1.48
15	1400	859	807	560	560	1.43	1.34
16	1548	925	833	580	560	1.49	1.38
Mean	1447	867	804	535	520	1.50	1.47
Std.dev	120	82	144	91	136	0.29	0.36

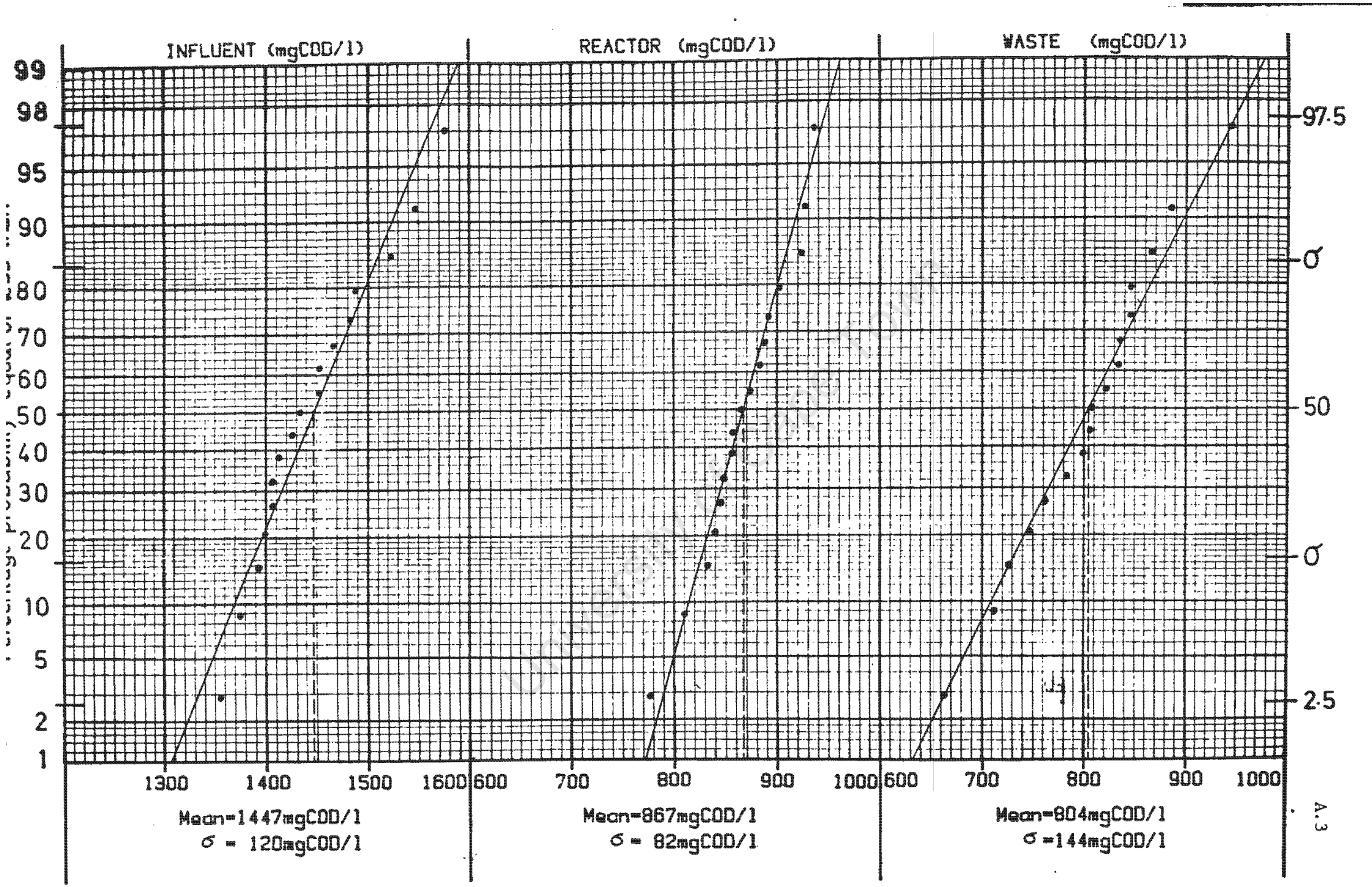
Note: (i) (_) values discarded as statistical outliers.
(ii) no filtered effluent COD values due to unavailability of 0.45 m filters. Data from period 30/4-7/5 utilised: Mean=58 (Std.dev=8).

TABLE 1.2: Observed experimental data for Glucose Experiments 1.1,1.2 and 1.3 (11-13/4/84).

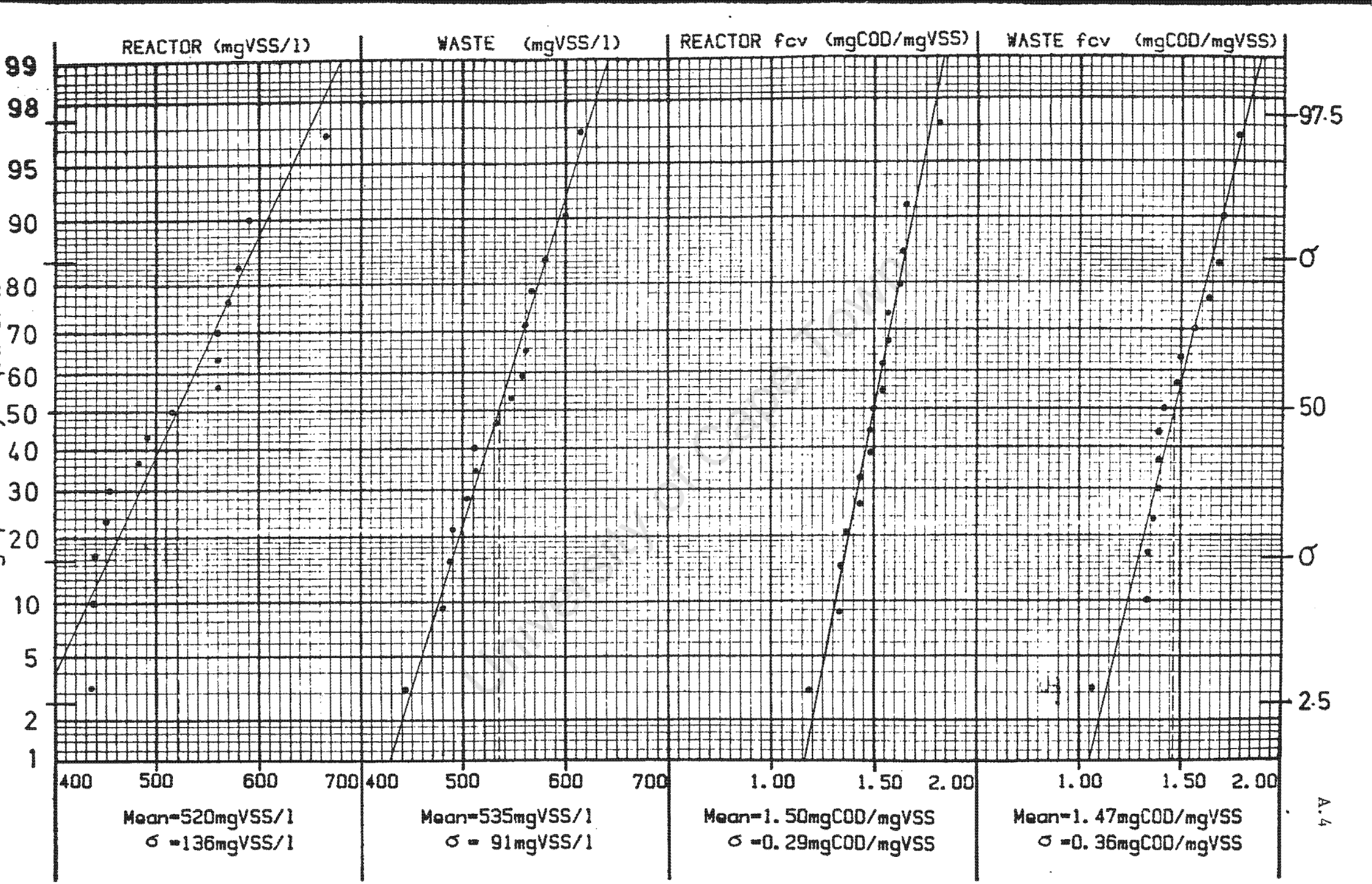
Parameter		Experiment No. 1.1	1.2	1.3
Influent COD	(mgCOD/l)	1488	1411	1452
Reactor COD	(mgCOD/l)	881	865	855
Waste COD	(mgCOD/l)	943	834	845
Reactor VSS	(mgVSS/l)	534	444	600
Waste VSS	(mgVSS/l)	590	436	570
Reactor COD/VSS	(mgCOD/mgVSS)	1.54	1.82	1.33
Waste COD/VSS	(mgCOD/mgVSS)	1.50	1.28	1.38
Reactor pH		6.53	6.57	6.61
OUR	(mgo/l/h)	(See TABLE 1.3)		
Start feed		0745h	0745h	0745h
Stop feed		1545h	1545h	1530h
COD Mass balance		1.159	1.059	1.099

TABLE 1.3: Observed OUR data for Glucose Experiments 1.1,1.2 and 1.3.

Experiment 1.1		Experiment 1.2		Experiment 1.3	
Time	OUR	Time	OUR	Time	OUR
0745	5.0	0745	3.5	0745	3.0
0810	26.5	0810	27.0	0815	25.7
0840	26.5	0840	25.0	0840	23.7
0910	27.0	0915	23.5	0910	25.5
0945	26.3	0945	24.0	0945	26.0
1015	26.5	1015	24.0	1015	26.7
1045	27.4	1045	24.0	1045	27.5
1115	27.0	1115	24.0	1115	27.7
1200	26.5	1145	24.0	1145	28.0
1215	28.0	1215	24.0	1215	29.0
1300	27.7	1245	24.5	1245	29.0
1415	27.5	1315	23.5	1315	29.3
1445	26.0	1345	23.5	1345	29.7
1515	26.0	1415	23.5	1415	29.3
1540	28.0	1445	24.0	1445	29.3
1550	14.5	1515	25.3	1515	29.7
1600	12.7	1525	25.5	1525	29.3
1615	9.3	1535	10.7	1535	13.3
1645	7.5	1545	9.3	1545	10.5
1715	7.5	1555	7.5	1555	10.3
1915	5.3	1615	7.0	1605	8.3
2015	5.3	1630	6.0	1615	8.5
2115	5.5	1645	5.5	1625	8.3
2215	5.7	1700	5.7	1635	7.5
		1715	6.2	1645	7.5
		1730	5.5	1700	6.7
		2045	3.7		
		2245	3.5		



SUBSTRATE: GLUCOSE (Rs=2.50days)



SUBSTRATE: GLUCOSE (Rs=2.50days)

EXPERIMENT No.2: CYCLIC FEED WITH MAIZE STARCH AS SUBSTRATE
(SLUDGE AGE = 5.0 days).

TABLE 2.1: Data extracted from daily experimental results to determine mean influent COD value for simulation purposes.

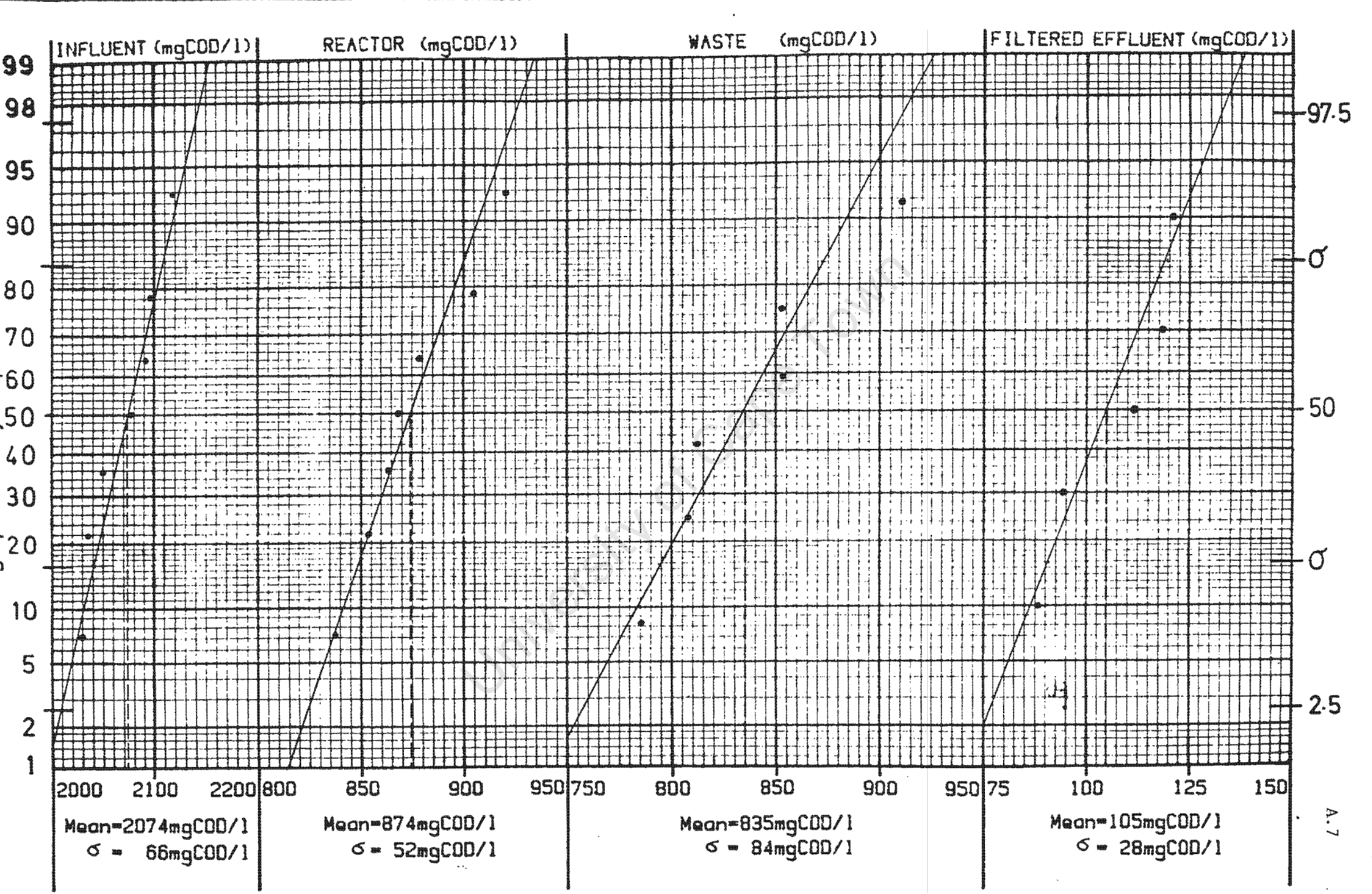
Date	Influent (mgCOD/l)	Reactor (mgCOD/l)	Waste (mgCOD/l)	Filtered effluent (mgCOD/l)	Reactor (mgVSS/l)	Waste (mgVSS/l)	Reactor (fcv)	Waste (fcv)	OUR (mgO/l/h)
18/7	2097	905	910	118	482	514	1.63	1.54	11.2
19	2051	(781)	853	112	454	456	1.47	1.63	12.8
20	2030	838	786	121	404	462	1.77	1.44	8.8
21	2077	879	812	NA	454	426	1.70	1.66	12.1
22	2118	920	(709)	NA	404	480	2.02	1.26	9.7
23	2035	853	853	88	446	416	1.72	1.84	10.0
24	2092	858	807	NA	424	394	1.78	1.78	12.2
25	(1897)	869	(699)	94	416	540	1.38	1.12	12.7
Mean	2074	874	835	105	434	459	1.68	1.53	11.1
Std.dev	66	52	84	28	54	102	0.40	0.52	3.0

LE 2.2: Observed experimental data for 24h test (1-2/8/84).

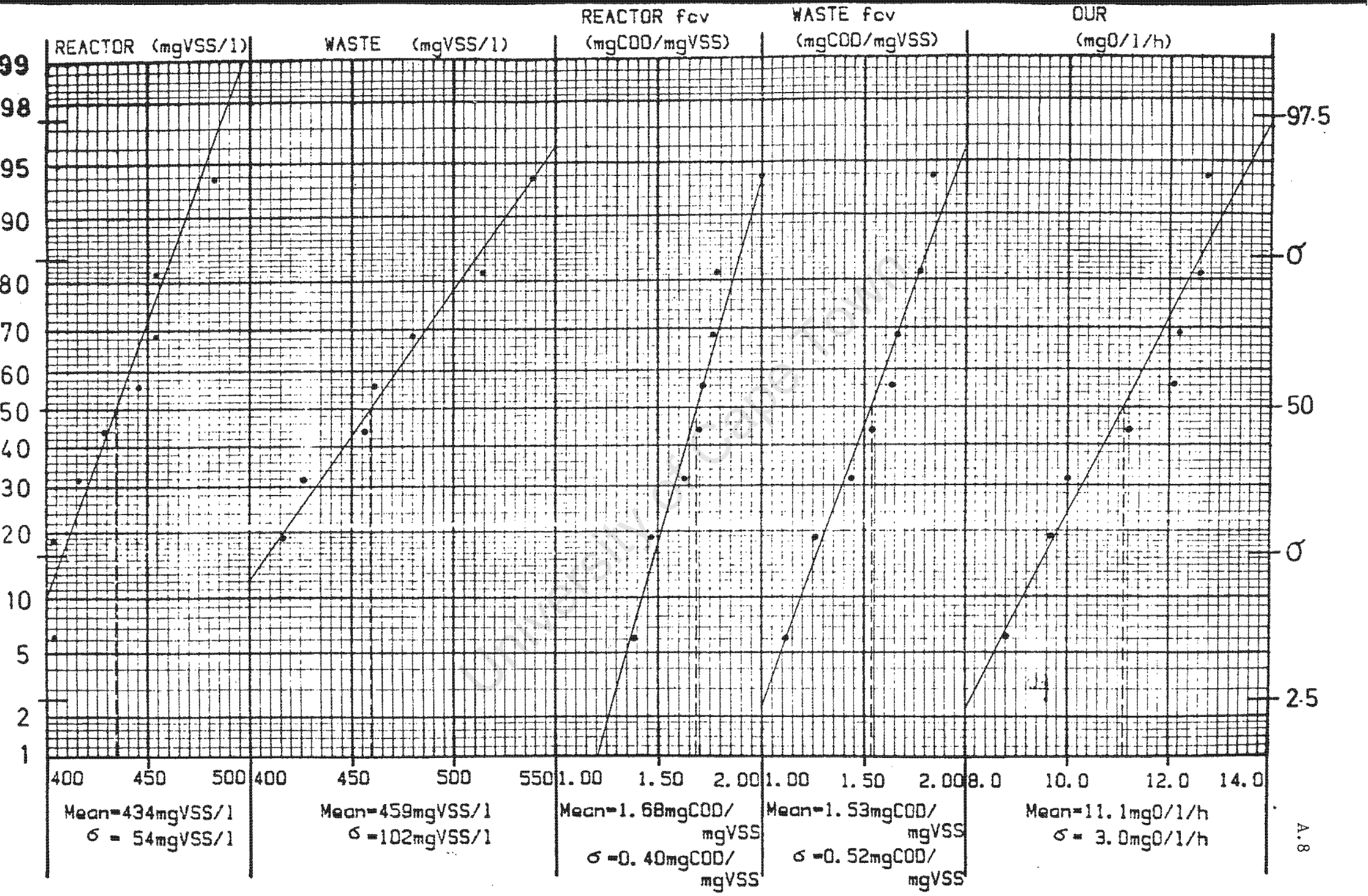
ameter		Time											
		2200	0100	0400	0700	1000	1300	1600	1700	1800	1900	2000	2100	2200
luent COD	(mgCOD/l)	2008												2075
ctor COD	(mgCOD/l)	772	833	792	746	807	853	767		807		848		756
ste COD	(mgCOD/l)	761	772	772	797	700	772	792						
. effluent COD	(mgCOD/l)	77	83	94	65	95	89	77	71	87	78	84	91	75
ctor VSS	(mgVSS/l)	478			550			550						516
ste VSS	(mgVSS/l)				552			534						
ctor COD/VSS	(mgCOD/mgVSS)	1.45			1.24			1.25						1.32
ste COD/VSS	(mgCOD/mgVSS)				1.33			1.34						
	(mgO/l/h)						(See TABLE 2.3)							
d period		(Start)						(Stop)						(Start

TABLE 2.3: Experimental OUR data for 24h test (1/8/84-2/8/84)

Time	OUR	Time	OUR
2100	11.7	1000	10.7
2130	11.5	1030	10.5
2200	11.3	1100	10.5
2230	11.3	1130	11.0
2300	11.0	1200	10,5
2330	11.3	1230	10.0
2400	11.3	1300	10.7
0030	10.3	1330	11.3
0100	10.3	1400	10.0
0130	10.5	1430	10.7
0200	10.5	1500	10.7
0230	10.0	1530	10.5
0300	10.3	1600	11.7
0330	10.3	1630	11.5
0400	10.3	1700	11.5
0430	10.0	1730	12.0
0500	10.3	1800	13.0
0530	9.7	1830	12.0
0600	9.7	1900	12.3
0630	9.7	1930	12.3
0700	9.7	2000	12.7
0730	9.7	2030	12.5
0800	9.7	2100	12.5
0830	10.0	2130	12.0
0900	10.0	2200	12.7
0930	10.3	2230	13.3



SUBSTRATE: MAIZE STARCH (Rs=5.0days)



SUBSTRATE: MAIZE STARCH (Rs=5.0days)

PENDIX A3

FERIMENT No.2: CYCLIC FEED WITH MAIZE STARCH AS SUBSTRATE
(SLUDGE AGE = 10.0 days).

BLE 3.1: Data extracted from daily experimental results to determine mean influent COD value for simulation purposes (Test 1).

Date	Influent (mgCOD/l)	Reactor (mgCOD/l)	Waste (mgCOD/l)	Filtered effluent (mgCOD/l)	Reactor (mgVSS/l)	Waste (mgVSS/l)	Reactor (fcv)	Waste (fcv)
14/8	4163	1002	930	118	674	734	1.31	1.11
15	3906	977	1019	128	734	NA	1.16	NA
16	4050	946	1028	92	630	716	1.36	1.31
17	3886	987	NA	94	776	NA	1.25	NA
18	4050	977	NA	NA	864	710	1.01	NA
19	4277	977	987	NA	736	(772)	1.18	1.14
20	4004	1087	1097	99	728	702	1.36	1.43
21	3890	1097	1087	104	754	(830)	1.32	1.18
22	4295	1087	1217	117	774	NA	1.25	NA
23*	4347	1163	1120	110	(921)	NA	1.44	NA
ean	4075	1030	1060	108	741	716	1.23	1.23
d.dev	410	107	120	27	136	24	0.17	0.19

Note: (i) (_) values discarded as statistical outliers.

BLE 3.2: Data extracted from daily experimental results to determine mean influent COD value for simulation purposes (Test 2).

Date	Influent (mgCOD/l)	Reactor (mgCOD/l)	Waste (mgCOD/l)	Filtered effluent (mgCOD/l)	Reactor (mgVSS/l)	Waste (mgVSS/l)	Reactor (fcv)	Waste (fcv)
2/9	3944	(1438)	(1432)	(255)	890	990	1.33	1.19
3	3758	(1422)	(1848)	(290)	904	864	1.25	1.80
4	4277	(1469)	1557	(295)	818	820	1.44	1.54
5	4598	(1412)	(1412)	438	NA	794	NA	1.23
6	(4899)	1557	1640	NA	772	724	NA	1.71
7	NA	(1422)	(1407)	375	724	794	1.45	1.30
8	4224	1593	1635	381	790	842	1.53	1.49
9	3999	1588	1542	408	800	884	1.48	1.28
0	3855	1573	1645	395	794	NA	1.48	NA
1*	4210	1559	1662	421	901	914	1.26	1.36
an	4108	1574	1645	403	821	830	1.40	1.43
.dev	386	18	10	32	91	90	0.16	0.30

Note: (i) (_) values discarded as statistical outliers.

ABLE 3.3: Observed experimental data for 24h test (No.1) (22-23/8/84).

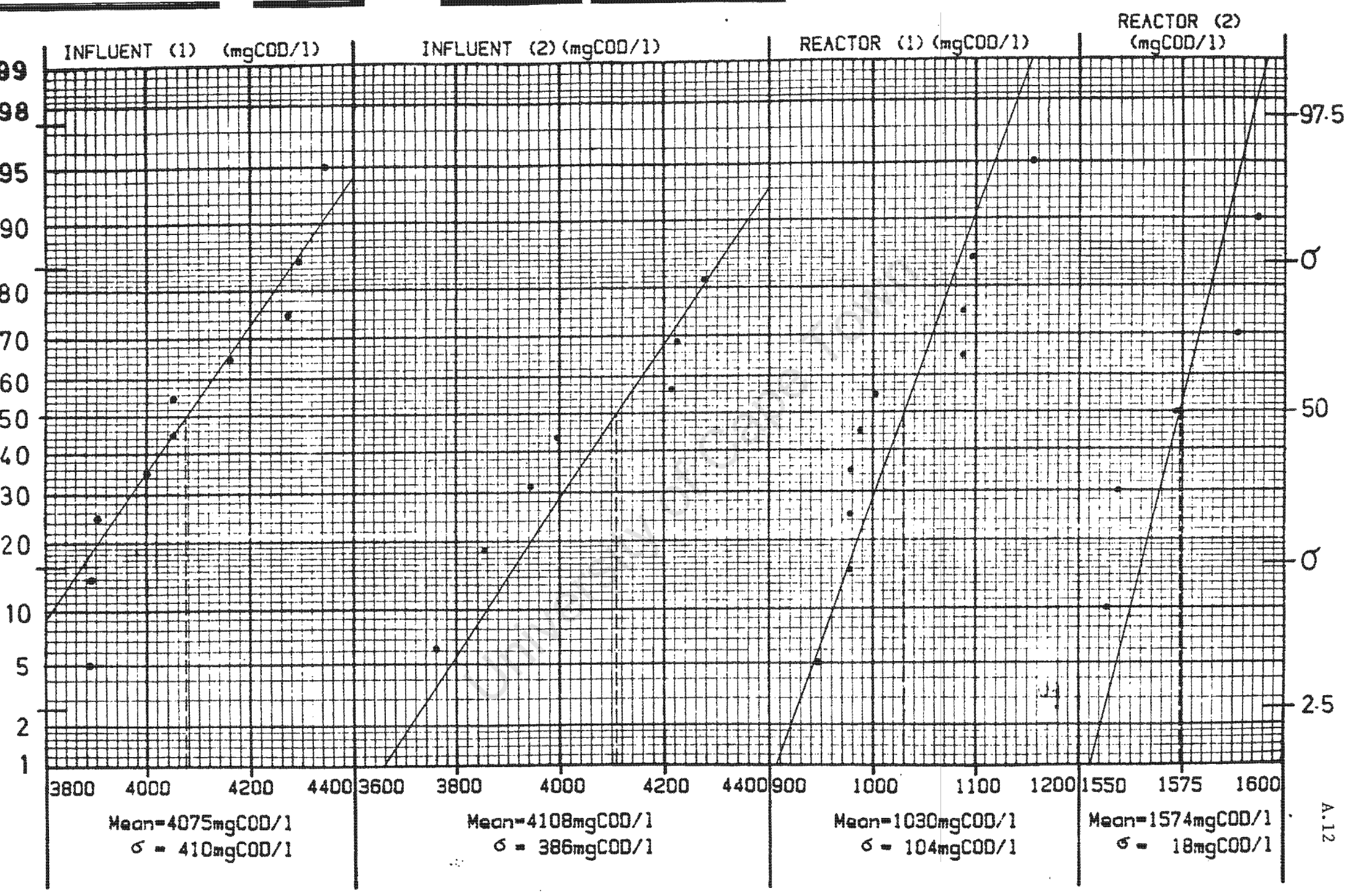
arameter		Time											
		2100	2400	0300	0600	0900	1200	1500	1600	1700	1800	1900	2100
nfluent COD	(mgCOD/l)	4347											
eactor COD	(mgCOD/l)	1144	1170	1092	1134	1175	1180	1222		1165		1210	1136
aste COD	(mgCOD/l)		1123	1102	1082	1087	1232	1092					
il. effluent COD	(mgCOD/l)	112	108	106	120	94	111	98	122	108		99	102
eactor VSS	(mgVSS/l)	866			884			1012					
aste VSS	(mgVSS/l)				826								
eactor COD/VSS	(mgCOD/mgVSS)	1.19			1.15								
aste COD/VSS	(mgCOD/mgVSS)				1.16			1.11					
eed period		(Start)						(Stop)					(Start)
UR							(See TABLE 3.5)						
est pH		7.00											

ABLE 3.4: Observed experimental data for 24h test (No.2) (20-21/9/84).

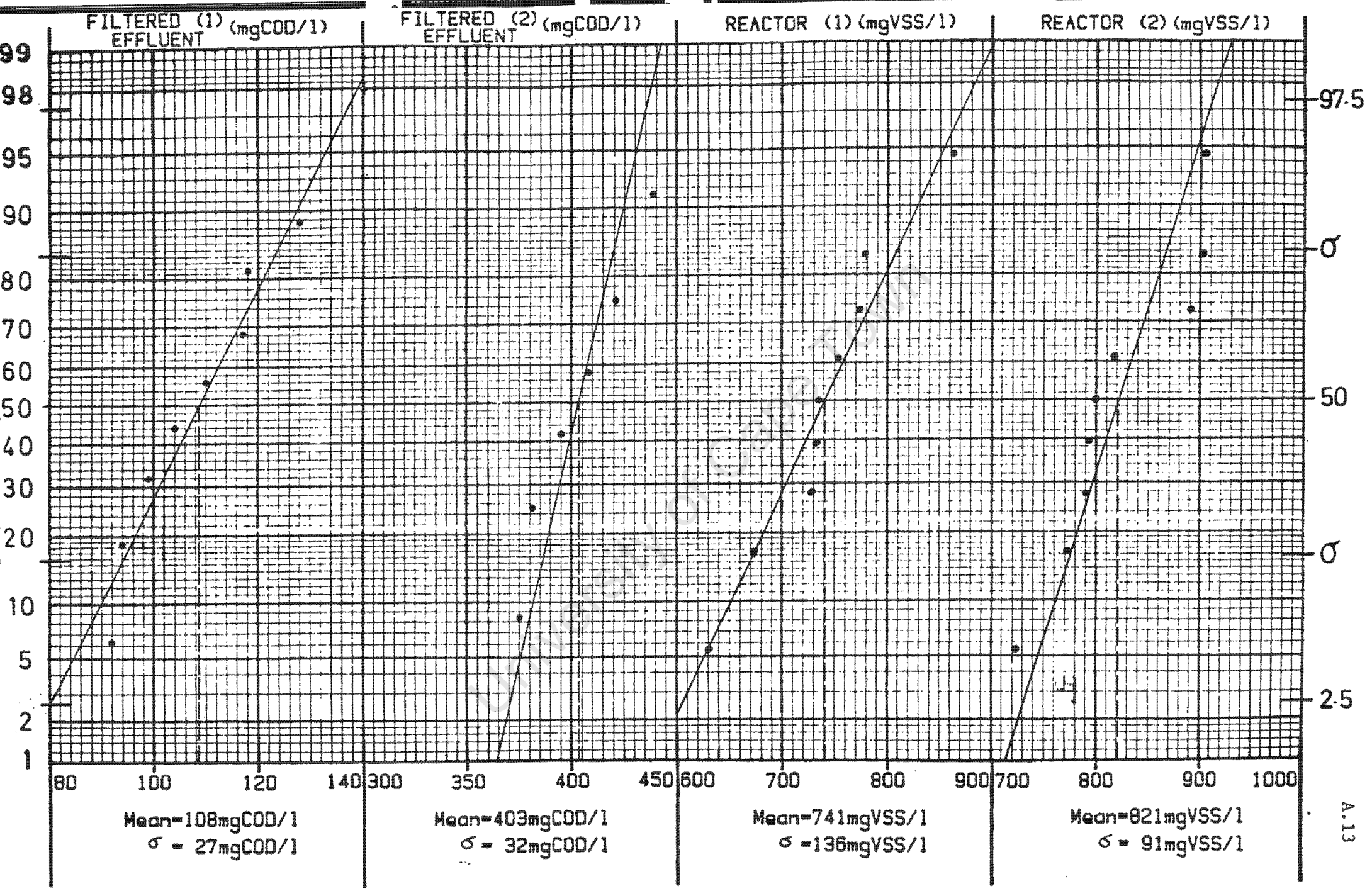
arameter		Time											
		2100	2400	0300	0600	0900	1200	1500	1600	1700	1800	1900	2100
nfluent COD	(mgCOD/l)	4210											3773
eactor COD	(mgCOD/l)	1501	1563	1599	1609	1578	1614	1557		1501		1506	1557
aste COD	(mgCOD/l)		1645	1681	1660	1686	1660	1640					
il. effluent COD	(mgCOD/l)	439	436	411	444	417	417	412	430	391	413	418	423
eactor VSS	(mgVSS/l)	820			922			960					
aste VSS	(mgVSS/l)				914								
eactor COD/VSS	(mgCOD/mgVSS)	1.30			1.26			1.19					
aste COD/VSS	(mgCOD/mgVSS)				1.27								
eed period		(Start)						(Stop)					(Start)
UR	(mgO/l/h)						(See TABLE 3.5)						
est pH		7.01											

TABLE 3.5: Experimental OUR data for 24h tests
No.1 (22-23/8/84); No.2 (20-21/9/84)

Time	OUR(1)	OUR(2)	Time	OUR(1)	OUR(2)
2030	12.0	11.0	1000	11.3	11.3
2100	11.3	10.7	1030	11.7	11.2
2130	11.7	10.5	1100	11.7	12.0
2200	11.0	10.7	1130	11.5	11.8
2230	11.5	1.0	1200	12.0	12.0
2300	11.0	11.0	1230	12.3	12.3
2330	NA	10.8	1300	11.7	12.7
2400	10.7	10.3	1330	11.7	12.8
0030	10.0	10.3	1400	11.7	13.0
0100	9.5	10.0	1430	12.0	13.3
0130	10.0	10.0	1500	12.3	13.8
0200	10.0	10.0	1530	12.5	12.2
0230	9.5	10.2	1600	12.7	11.7
0300	9.7	10.0	1630	12.5	11.7
0330	10.0	10.2	1700	12.3	12.0
0400	9.7	10.7	1730	12.0	11.4
0430	10.0	10.5	1800	12.3	11.5
0500	10.0	10.3	1830	11.7	11.7
0530	10.7	10.0	1900	12.3	11.7
0600	10.0	10.5	1930	11.7	10.8
0630	10.0	10.5	2000	11.5	10.3
0700	10.3	10.5	2030	11.5	10.3
0730	10.0	10.7	2100	11.0	10.3
0800	10.3	11.0	2130	11.5	10.3
0830	11.0	10.9	2200	11.3	10.0
0900	10.7	11.3	2230	10.7	NA
0930	11.0	10.5			

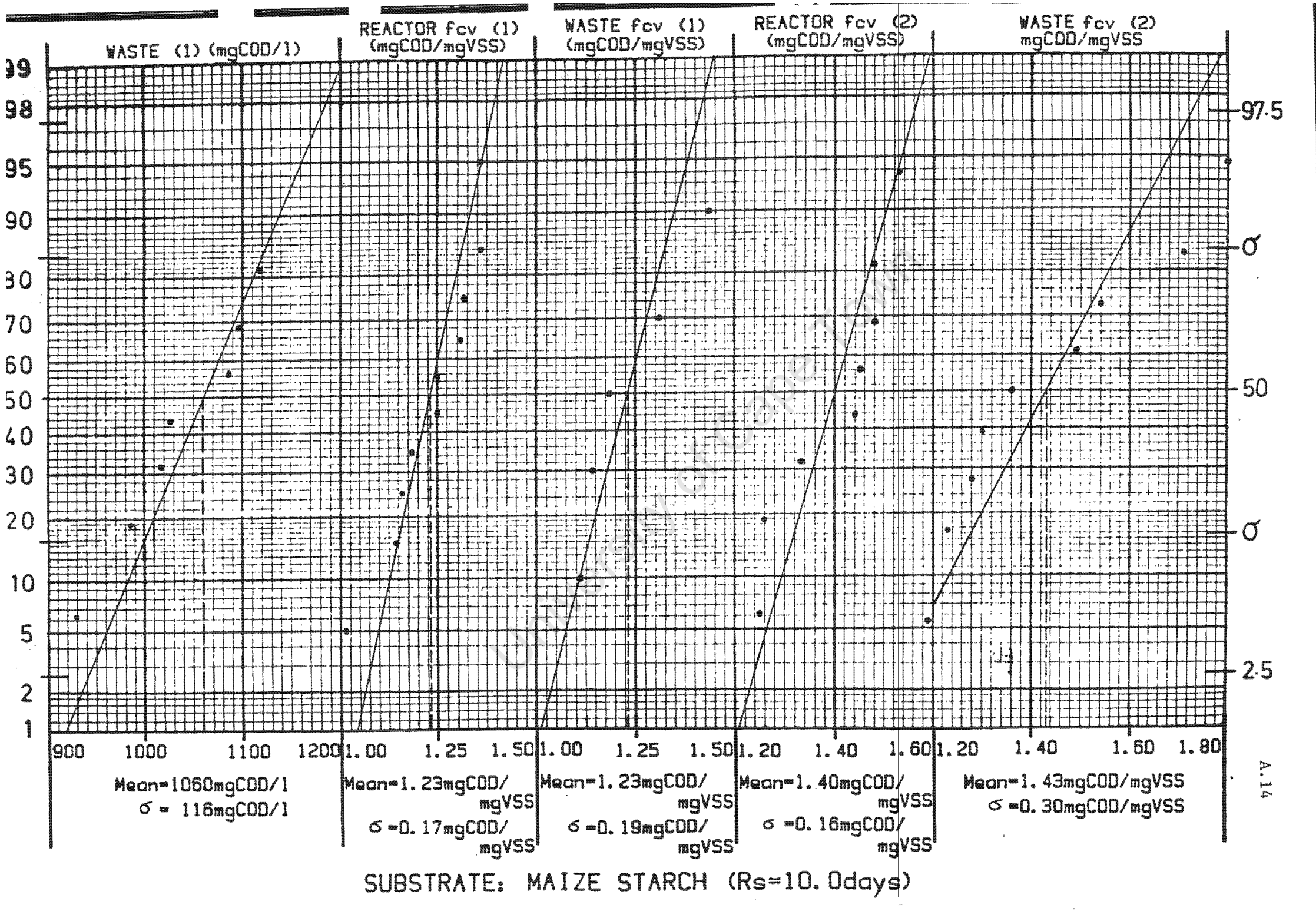


SUBSTRATE: MAIZE STARCH (Rs=10.0days)



A.13

SUBSTRATE: MAIZE STARCH (Rs=10.0days)



SUBSTRATE: MAIZE STARCH (Rs=10.0days)

ENDIX A4

ERIMENT No.3: CYCLIC FEED WITH GLUCOSE AND MAIZE STARCH AS SUBSTRATE (SLUDGE AGE = 10.0days).

LE 4.1: Data extracted from daily experimental results to determine mean influent COD value for simulation purposes (Test 1).

te	Influent (mgCOD/l)	Reactor (mgCOD/l)	Waste (mgCOD/l)	Filtered effluent (mgCOD/l)	Reactor (mgVSS/l)	Waste (mgVSS/l)	Reactor (fcv)	Waste (fcv)
/11	4069	1524	NA	NA	874	968	NA	NA
	4099	1494	1406	205	918	926	1.40	1.30
	3934	1406	1360	194	920	NA	1.32	NA
	4069	1442	1355	177	904	992	1.40	1.19
	3996	1458	1344	166	924	1012	1.41	1.16
	3924	1462	1411	150	946	1068	1.39	1.18
	3966	1447	1349	140	1078	NA	(1.21)	NA
	3924	1540	1308	122	1010	NA	1.40	NA
	NA	NA	NA	NA	NA	950	NA	NA
	3930	1626	NA	132	976	NA	(1.53)	NA
*	4125	1589	(1578)	134	1001	996	1.45	1.45
n	4004	1500	1362	157	953	987	1.40	1.25
dev	116	140	70	58	116	106	0.08	0.22

E 4.2: Data extracted from daily experimental results to determine mean influent COD value for simulation purposes (Tests 2&3).

te	Influent (mgCOD/l)	Reactor (mgCOD/l)	Waste (mgCOD/l)	Filtered effluent (mgCOD/l)	Reactor (mgVSS/l)	Waste (mgVSS/l)	Reactor (fcv)	Waste (fcv)
/12	3937	1347	1362	153	892	780	1.34	1.55
	4020	1429	1419	146	848	956	1.51	1.33
	4091	1511	(1552)	142	908	916	1.51	1.54
	4194	1388	1434	128	924	922	1.35	1.42
	3989	1403	1460	119	914	NA	1.40	NA
	NA	NA	NA	NA	NA	890	NA	NA
	4258	1409	1409	154	842	986	1.49	1.27
	3999	1388	(1264)	112	1010	888	1.26	1.30
	4061	1404	1331	132	968	802	1.31	1.50
*	4196	1554	1362	148	928	848	1.52	1.43
	4072	1477	1357	132	884	NA	1.52	NA
	4134	NA	NA	NA	NA	848	NA	NA
*	4040	1513	1383	148	1030	NA	1.33	NA
n	4078	1438	1392	137	920	882	1.41	1.42
dev	184	122	80	26	112	130	0.16	0.18

te: (i) Overall mean influent COD = 4047 mgCOD/l (std.dev=94).
(ii) (_) values discarded as statistical outliers.

BLE 4.3 Observed experimental data for
24h test (No.1) (27/11/84).

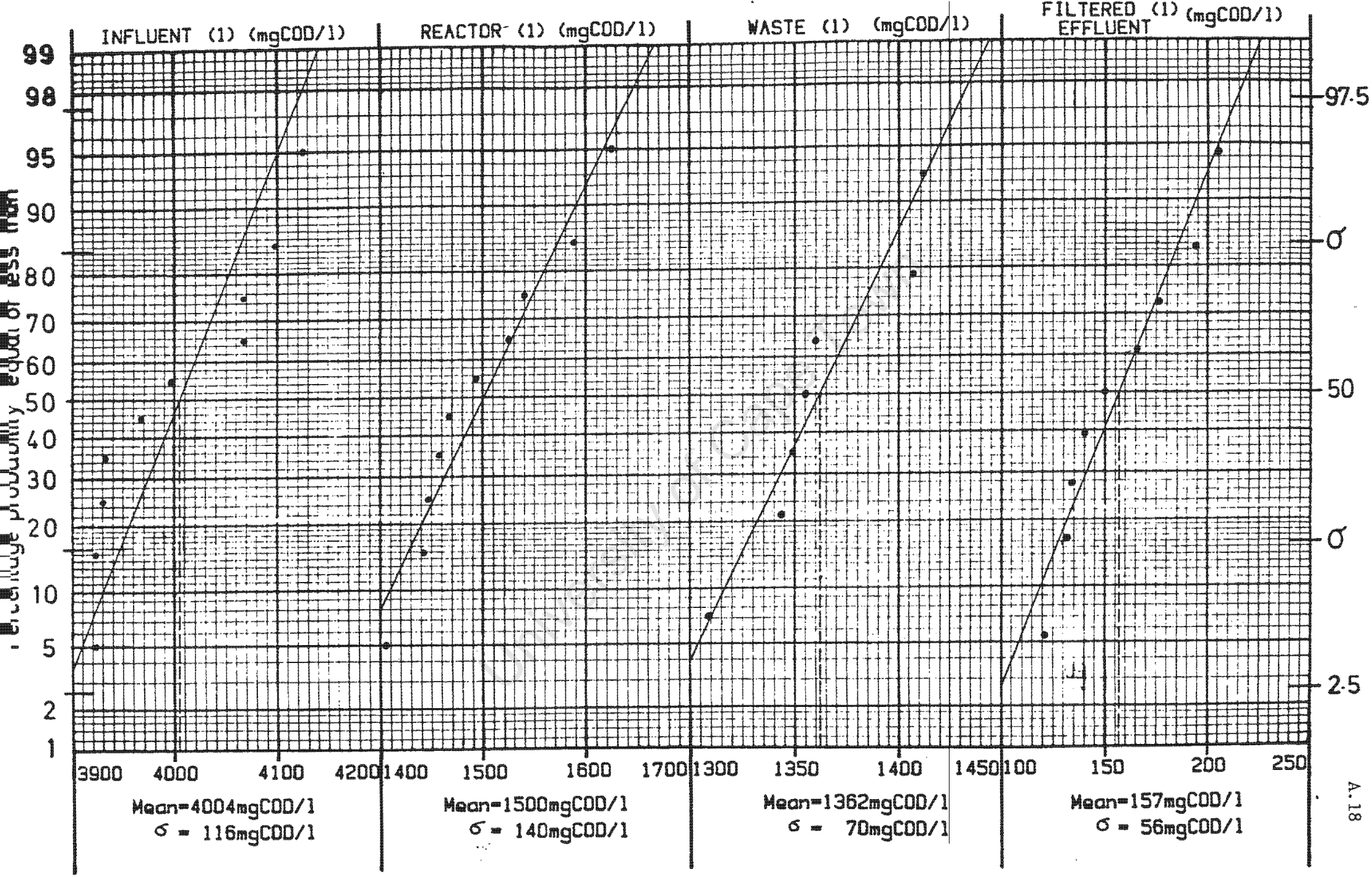
rameter		Time												
		0700	0900	1100	1300	1500	1700	1900	2100	2300	0100	0300	0500	0700
fluent COD	(mgCOD/l)	4125												4042
actor COD	(mgCOD/l)	1590	1565	1657	1580	1652	1560	1585	1595	1580	1611	1457	1508	1570
ste COD	(mgCOD/l)			1544			1616	1575						
l. effluent COD	(mgCOD/l)	131	138	170	152	131	144	123	132	128	123	135	142	125
actor VSS	(mgVSS/l)	990						1066						948
ste VSS	(mgVSS/l)							996						
actor COD/VSS	(mgCOD/mgVSS)	1.47						1.37						1.52
ste COD/VSS	(mgCOD/mgVSS)							1.46						
R	(mgO/l/h)							(See TABLE 4.5)						
ed period		(Start)						(Stop)						(Start
st pH		7.00												

TABLE 4.4: Observed experimental data for 24h tests,
Test 2 (13/12/84) and Test 3 (16/12/84).

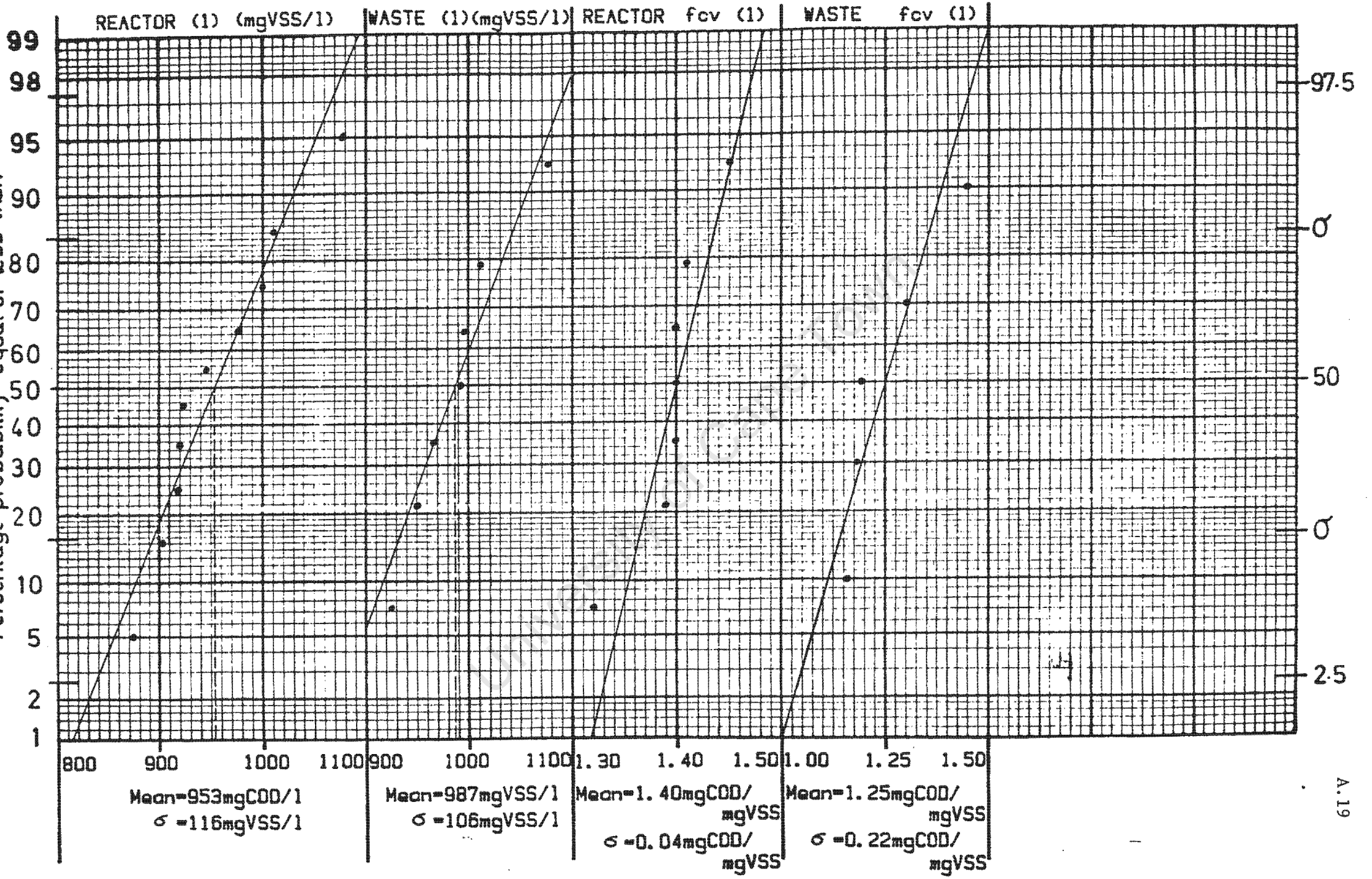
Parameter		Test 2	Test 3
Influent COD	(mgCOD/l)	4196	4040
Reactor COD	(mgCOD/l)	1554	1513
Waste COD	(mgCOD/l)	1362	1383
Fil. effluent COD	(mgCOD/l)	148	148
Reactor VSS	(mgVSS/l)	928	1030
Waste VSS	(mgVSS/l)	802	848
Reactor COD/VSS	(mgCOD/mgVSS)	1.52	1.32
Waste COD/VSS	(mgCOD/mgVSS)	1.51	1.46
OUR	(mgO/l/h)	(See TABLE 4.5)	
Test pH		7.06	NA

TABLE 4.5: Experimental OUR data for 24h tests,
No.1 (27/11),No.2 (13/12),No.3 (16/12/84).

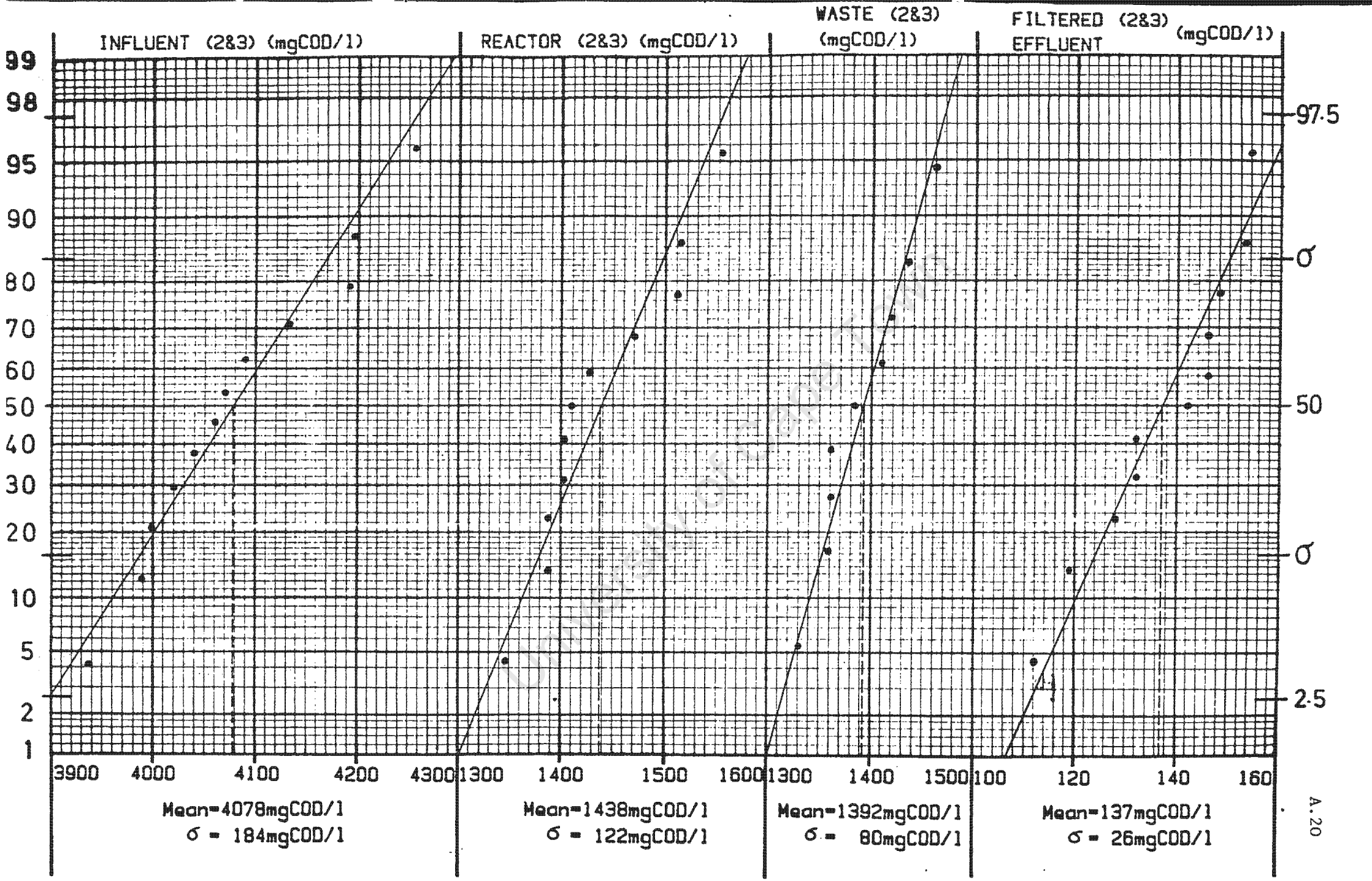
Time	OUR(1)	OUR(2)	OUR(3)	Time	OUR(1)	OUR(2)	OUR(3)
0600	6.1	NA	NA	1945	8.3	NA	NA
0630	6.2	NA	NA	2000	8.0	9.8	10.3
0650	6.2	NA	7.7	2030	7.7	NA	NA
0700	NA	NA	NA	2100	8.0	9.0	9.4
0710	9.4	NA	NA	2130	7.3	NA	NA
0730	11.5	11.4	12.0	2200	7.4	9.8	10.6
0800	11.2	12.3	13.0	2230	6.8	NA	NA
0830	11.5	NA	NA	2300	6.7	9.8	10.1
0900	11.5	NA	NA	2330	7.0	NA	NA
0930	11.3	NA	NA	2400	6.7	9.0	10.3
1000	11.3	12.7	14.6	0030	6.8	NA	NA
1030	11.1	NA	NA	0100	6.8	9.8	10.2
1100	10.8	14.1	16.0	0130	6.6	NA	NA
1130	11.0	NA	NA	0200	6.4	9.8	9.4
1200	10.8	14.5	15.7	0230	6.6	NA	NA
1230	11.1	NA	NA	0300	6.6	9.0	9.8
1300	11.0	14.9	15.3	0330	6.1	NA	NA
1330	11.1	NA	NA	0400	6.2	7.7	9.3
1400	11.0	15.0	15.5	0430	5.9	NA	NA
1430	11.4	NA	NA	0500	5.8	7.5	9.0
1500	10.5	15.0	15.7	0530	6.2	NA	NA
1530	11.0	NA	NA	0600	5.4	8.0	8.9
1600	10.9	14.8	16.0	0630	5.4	NA	NA
1630	10.5	NA	NA	0700	5.3	10.9	8.9
1700	10.4	15.4	15.9	0705	9.4	NA	NA
1730	10.7	NA	NA	0720	10.1	NA	NA
1800	10.2	15.2	15.5	0730	NA	10.8	11.3
1830	9.7	NA	NA	0740	10.0	NA	NA
1840	8.0	NA	NA	0800	10.3	11.9	12.0
1850	8.0	NA	NA	0830	10.3	NA	NA
1900	8.5	11.7	17.0	0900	10.3	NA	11.9
1915	8.0	NA	NA	0930	NA	12.2	NA
1930	7.9	NA	NA	1000	NA	NA	12.5



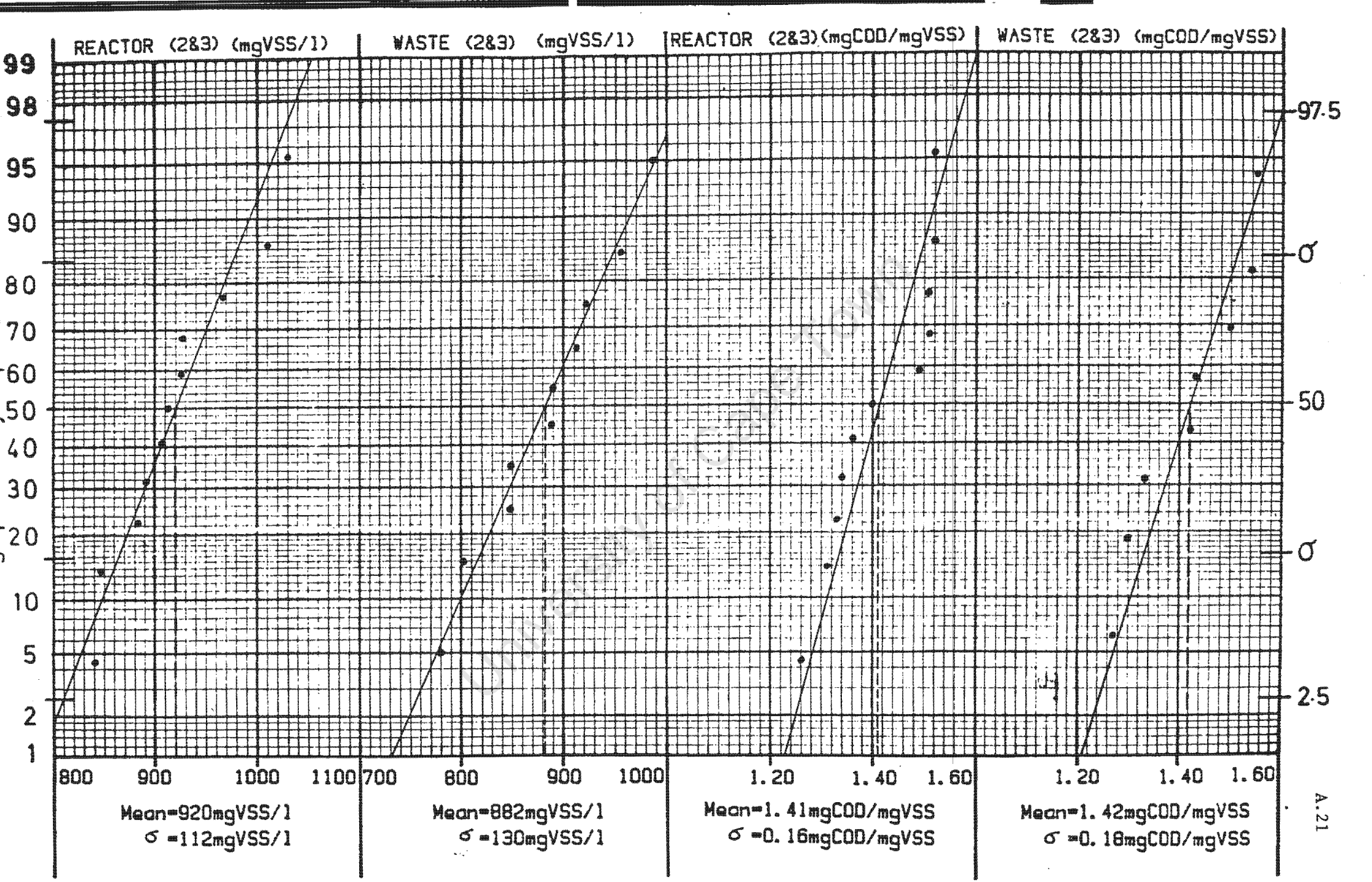
SUBSTRATE: GLUCOSE/MAIZE STARCH (Rs=10.0days)



SUBSTRATE: GLUCOSE/MAIZE STARCH (Rs=10.0days)



SUBSTRATE: GLUCOSE/MAIZE STARCH (Rs=10.0days)



SUBSTRATE: GLUCOSE/MAIZE STARCH (Rs=10.0days)

A.21

APPENDIX A5

EXPERIMENT No.4: CYCLIC FEED WITH SOLUBLE STARCH AS SUBSTRATE
(SLUDGE AGE = 2.50 days)

TABLE 5.1: Data extracted from daily experimental results to determine mean influent COD value for simulation purposes (steady state conditions).

Date	Influent (mgCOD/l)	Reactor (mgCOD/l)	Filtered effluent (mgCOD/l)	Reactor (mgVSS/l)	Reactor (fcv)	OUR (mgO/l/h)
23/5	1498	779	67	386	1.84	11.8
24	1599	810	87	450	1.61	11.1
25	1538	724	77	548	1.18	12.3
26	1574	693	71	522	1.19	17.3
27	1493	658	71	474	1.24	14.8
28	1591	760	82	492	1.38	13.5
29	1622	796	87	622	1.14	11.5
30	1668	806	58	660	1.13	12.8
31	1662	969	78	574	1.55	10.3
1/6	1489	989	(182)	508	1.59	9.2
2	1499	847	108	492	1.50	9.5
3	1448	913	78	474	1.76	9.9
4	1515	959	117	416	2.02	9.8
Mean	1554	820	82	510	1.47	11.7
Std.dev	136	190	30	154	0.56	4.0

TABLE 5.2: Data extracted from daily experimental results to determine mean influent COD values for simulation purposes (dynamic steady state).

Date	Influent (mgCOD/l)	Reactor (mgCOD/l)	Filtered effluent (mgCOD/l)	Reactor (mgVSS/l)	Reactor (fcv)
5/6	1515	887	(226)	380	1.74
6	1568	918	(212)	314	2.25
7	1520	912	(163)	216	3.47
8	1563	868	(142)	262	2.77
9	1547	891	78	214	3.80
10	1491	891	74	230	3.55
11	1624	(774)	86	216	3.19
12	1532	(774)	94	370	1.84
13	1532	(646)	96	316	1.74
14*	1461	(681)	82	408	1.47
Mean	1535	893	85	291	2.60
Std.dev	94	34	16	70	1.50

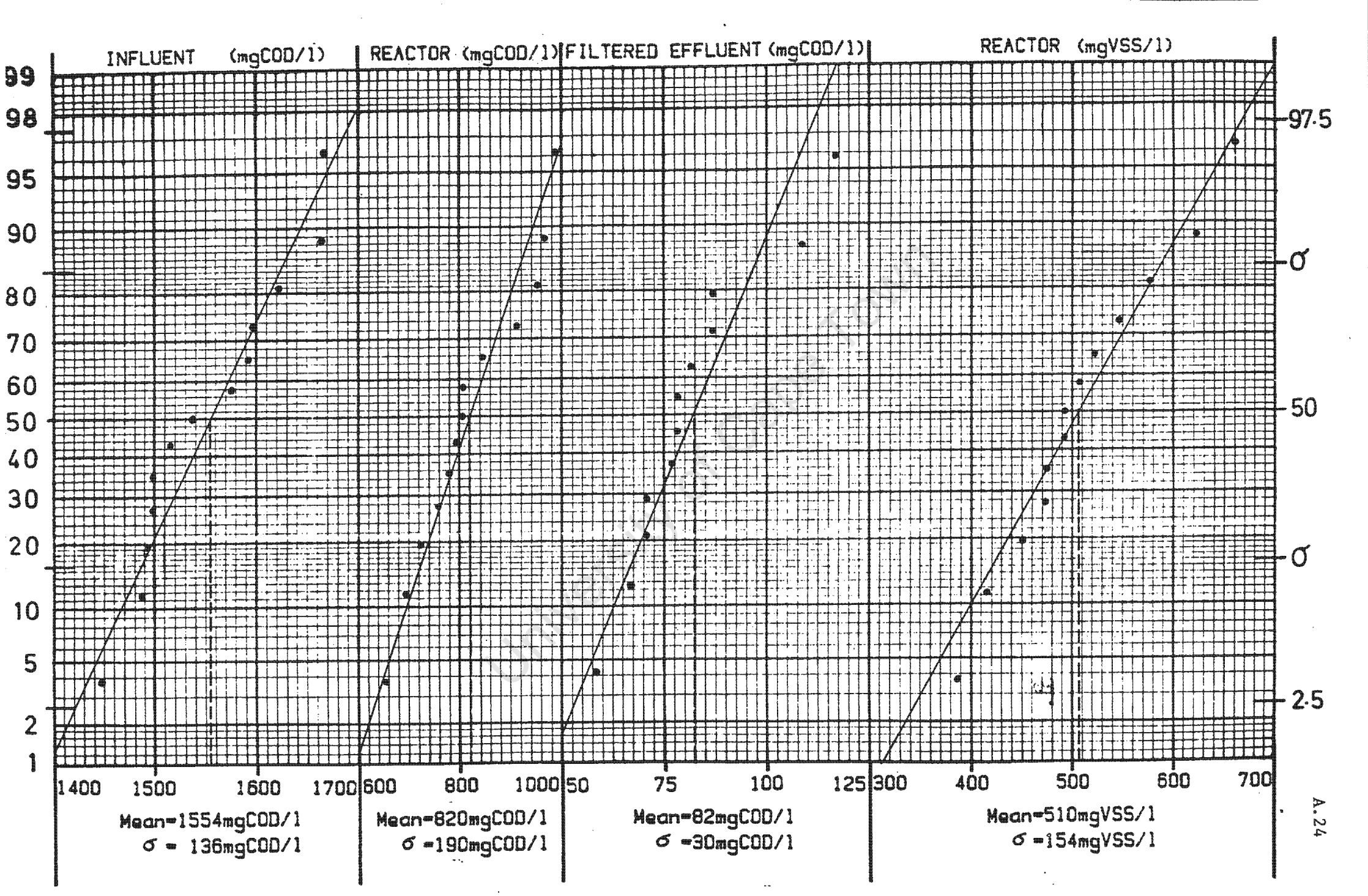
Note: (i) (_) values discarded as statistical outliers.

TABLE 5.3: Observed experimental data for intensive test on 14/6/84.

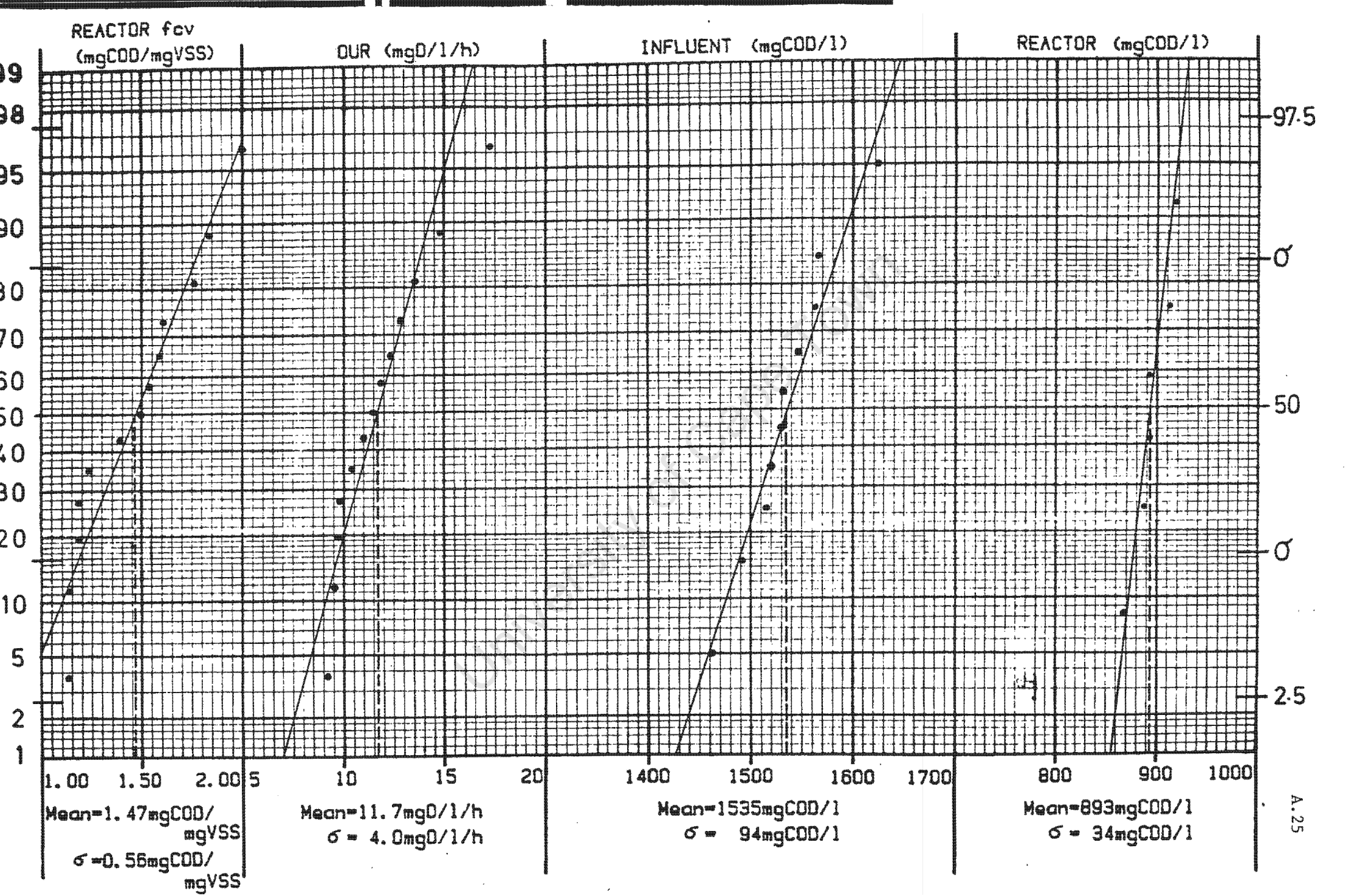
Parameter		Time								
		0730	0930	1200	1430	1700	1930	2100	2230	0730
Influent COD	(mgCOD/l)	1461								1517
Reactor COD	(mgCOD/l)	657	692	728	657	886	692	692	652	655
Fil. effluent COD	(mgCOD/l)	104	89	95	58	83	86	92	54	67
Reactor VSS	(mgVSS/l)	408								
Reactor COD/VSS	(mgCOD/mgVSS)	1.35								
Feed period		(Start)					(Stop)			(Start)
OUR	(mgO/l/h)					(See TABLE 5.4)				
Test pH		6.78								

TABLE 5.4 Experimental OUR data for intensive test on 14/6/84.

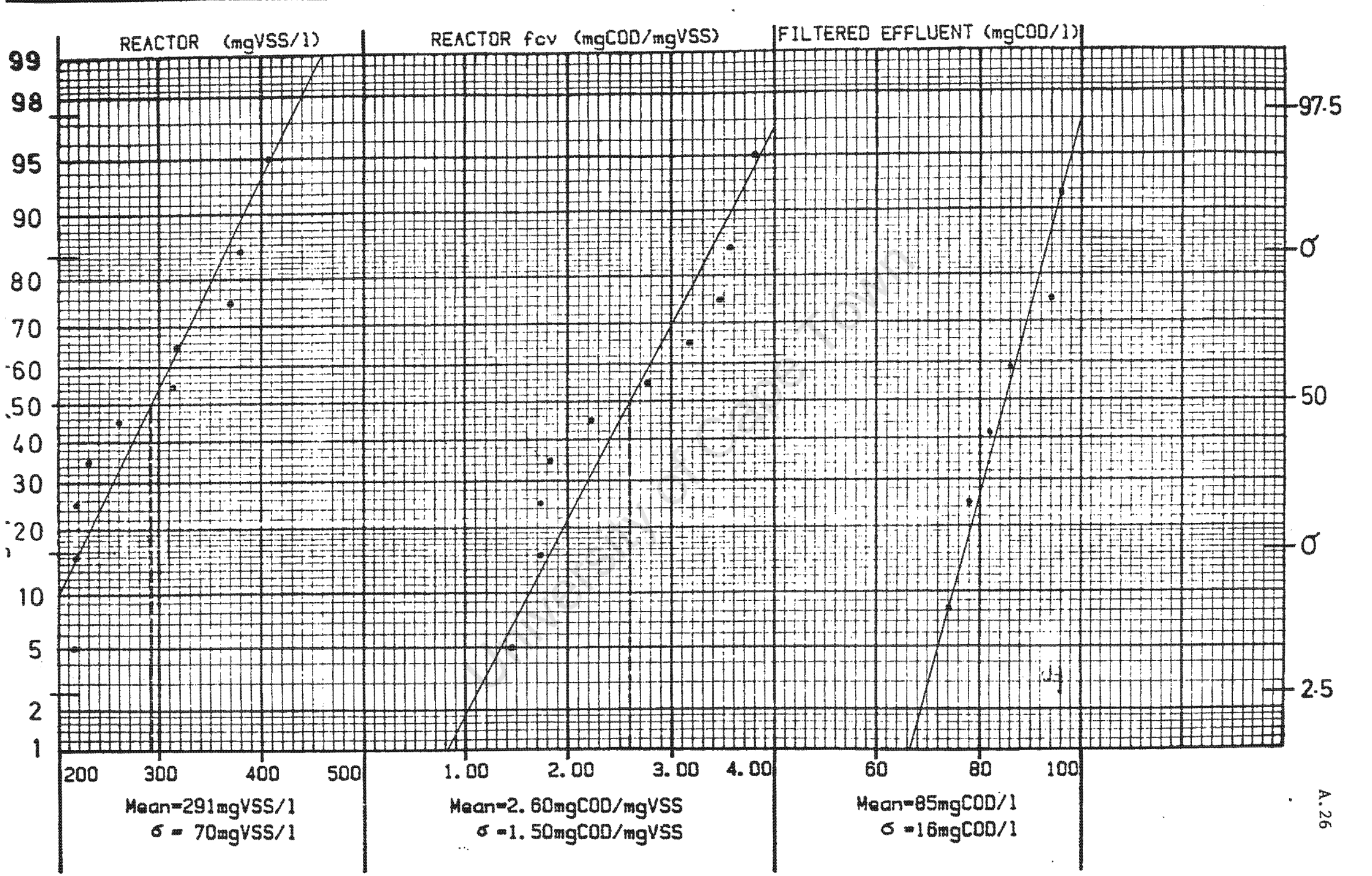
Time	OUR	Time	OUR
0715	3.7	1700	19.7
0820	15.7	1730	19.0
0900	16.0	1800	19.0
0930	16.7	1830	19.5
1000	17.5	1900	19.7
1030	18.5	1920	20.3
1100	18.3	1930	10.0
1130	18.3	2030	8.7
1200	18.3	2100	8.0
1230	18.3	2130	7.7
1300	18.5	2200	6.7
1330	18.0	2230	7.3
1400	18.0	2300	6.0
1430	18.7	2330	6.7
1500	18.3	0710	5.0
1530	18.3	0900	17.3
1600	18.7	0930	17.7
1630	19.0		



SUBSTRATE: SOLUBLE STARCH (Rs=2.50days)



SUBSTRATE: SOLUBLE STARCH (Rs=2.50days)



SUBSTRATE: SOLUBLE STARCH (Rs=2.50days)

APPENDIX B

DETAILED EXPERIMENTAL TECHNIQUE OF ULTRAFILTRATION METHOD

The experimental method for the determination of the soluble readily bidoegradable substrate (S_{bs}) fraction of influent wastewater was developed using municipal wastewater from the Cape Flats WWTP as the test influent. The method is not intended to be specific to this wastewater; however the characteristics of other wastewaters. for example, the quantity of organic humic compounds present may necessitate slight modifications to the basic experimental techniques.

The details of the method are as follows:

(1) Extract a representative sample of about 150 mℓ from both the influent and effluent streams of the activated sludge plant. Preserve each sample with a few drops of mercury (11) chloride solution (Concentration = 8,62 g $HgCℓ_2/ℓ$) and store at 4°C.

(2) Remove as much as possible of the particulate material in the influent sample by centrifuging at 6500 r.p.m. for 30 minutes. Decant and retain the supernatant liquid.

(3) Pre-filter both the influent and effluent samples twice under vacuum through a glass microfibre filter membrane (Whatman's GF/C, 55 mmØ). The glass microfibre filter is extremely delicate and easily compacted if subjected to a strong vacuum. A mechanical vacuum pump is unsuitable; however a standard water vacuum device run off a laboratory tap will provide sufficient suction for reasonably quick filtration without crushing the filter membrane. Compaction of the glass microfibre filter reduces its effectiveness in removing long chain humic compounds, by reducing the overall length of the filtration path through the membrane.

(4) A 0,45µm filter membrane is used to fractionate further the pre-filtered samples of influent and effluent. After pre-filtration the

samples should be relatively clear of colour; however experience has shown that in some cases, a significant quantity of humic compounds are still present, usually in the influent sample. This manifests itself in the form of a thin brownish layer on the surface of the 0,45 µm membrane during filtration, and has the effect of acting as an extra "blinding" layer. Usually about 50 mℓ of the sample can be successfully filtered before the extent of this "blinding" layer is such that the rate of filtration is drastically reduced. Filtration must be stopped at this stage, to prevent dilution of the filtrate already collected. What remains of the sample will eventually filter through if filtration is allowed to continue, but the blinding layer of organic colour is likely to remove some of the smaller molecules in solution, thus reducing the concentration of the sample volume already filtered. Significant differences in the values obtained from COD analysis of 0,45 µm filtrate from samples for which filtration was allowed to proceed to completion and for which filtration was terminated as soon as a blinding layer began to form have been recorded.

(5) The influent and effluent samples are now ready for treatment with the ultrafiltration membrane.

Membrane preparation. New membranes are supplied in a protective envelope. The membranes should be handled by the edge only and care must be taken not to scratch the glossy surface. Sodium azide is added to the membranes (YM series) as a preservative and they are pre-treated with glycerine to prevent drying. To remove these materials before use the membrane should be floated skin (glossy surface) side down in a beaker of distilled water for at least one hour, changing the water three or four times. After soaking, the membrane should be placed skin (glossy surface) side up i.e. toward the solution in the ultrafiltration device and rinsed with four or five 50 mℓ aliquots of distilled water.

Membranes which are to be re-used should be stored at 4°C in de-ionised water treated with a few drops of mercury (11) chloride solution.

Ultrafiltration device: The ultrafiltration device is a stirred ultrafiltration cell (Amicon Corp., Model 8050) with a volume of 50 mℓ. The cell is shown in exploded detail in Fig (B.1) below. The maximum recommended operating pressure (nitrogen gas) is 470kPa; for the YM membranes, however, the optimum operating pressure lies between 70 and 120 kPa. A 50 mℓ volume of distilled water will pass through a YM100 membrane in approximately 3 minutes under a pressure of 120 kPa.

Ultrafiltration procedure:

(i) Place the membrane in the membrane holder, glossy surface up; handle the membrane by the edges only.

(ii) Place the O-ring on top of the membrane. Gently push the O-ring down so that it contacts and seats the membrane evenly in the bottom of the holder.

(iii) Fit the membrane holder into the cell body, aligning the tabs on the sides of the holder with the slots in the base of the cell body.

(iv) Invert the cell body (with membrane holder) and screw the base firmly into the bottom of the cell body. A definite stop will be felt when completely engaged, and the tops of the membrane holder tabs will be flush with the bottom of the slots in the cell body.

(v) Place the stirrer assembly into the cell body. When properly inserted, the arms of the stirrer assembly sit on a small ridge inside the top of the cell body.

(vi) Introduce the sample directly into the cell; a maximum volume of 30 mℓ is recommended.

(vii) Using a twisting motion, push the cap assembly on as far as it will go. If the cap assembly does not slide down easily, lubricate the O-ring sparingly with vaseline. Align the gas inlet port on the cap directly opposite the filtrate exit port in the membrane holder.

(viii) Turn the pressure relief valve to the horizontal (open) position.

(ix) Slide the cell into the retaining stand. The ring on the cell base must be placed in the hole in the stand base. Flattened edges on the bottom edge of the cap ensure that the cell is inserted properly, and prevent rotation of the cell in the stand.

(x) Turn the pressure relief valve to the vertical (closed) position.

(xi) Place the assembled cell on the magnetic stirring table, and pressurize the system to the recommended nitrogen pressure. When the cell is pressurized, the cap assembly moves up by itself, forming a secure lock with the retaining stand.

(xii) Turn on the stirring table. Adjust the stirring rate so that the vortex is no more than one-third the depth of the liquid volume.

(xiii) To vent pressure after filtration is complete, slowly turn the pressure relief valve to the horizontal position (rapid depressurization may result in buckling and rupture of the membrane). Push the cap down, slide the cell out of the retaining stand, and remove the cap with a twisting motion.

(xiv) Flush the membrane with a minimum of 200 mℓ distilled water. After use store the membrane in deionised water. Disassemble the cell and rinse the components first with hot water, then

distilled water. A mild detergent may be used, but rinsing must be extremely thorough.

(6) Analyse the influent and effluent ultrafiltrate samples for COD concentration in accordance with the procedure laid down in "Standard Methods for the Examination of Water and Wastewater", 13th Ed (1971). It is also useful to analyse samples of influent and effluent collected after filtration through a 0,45 μm membrane.

(7) The soluble readily biodegradable substrate (S_{bs}) concentration of influent municipal wastewater is given by the difference between the COD values obtained for ultrafiltered influent and effluent samples i.e. S_{bs} = Influent COD - Effluent COD (mgCOD/ℓ).

NOTES:

(i) Care should be taken to ensure that the cell assembly is placed in the centre of the stirring table. This will enable smooth rotation of the stirrer blade; if the cell assembly is positioned off-centre, rotation of the stirrer blade is unstable and the surface of the membrane may be scored by the sharp leading edge. If the surface of the membrane is damaged, further stirring is likely to result in the stripping of the surface layer, and destruction of the membrane.

(ii) The cell body should be periodically examined for any cracks or crazing in the polysulfone compound. The most susceptible place for cracks is the threaded cell body base, since this is stressed each time the cell is assembled. As mentioned in part (iv) of the ultrafiltration procedure, a definite stop will be felt when the cell body is completely engaged in the base, and it should not be overtightened. If any cracks or extensive crazing are found the cell body must be replaced, to eliminate the possibility of disintegration whilst under pressure.

(iii) The cell assembly should <u>never</u> be pressurized without being positioned in the retaining stand.

REFERENCE

Amicon Corp. (1984). Operating instructions for stirred ultra-filtration cells. Publication No. 1-228. Amicon Corporation Scientific Systems Division, Danvers, Mass.