Effects of Sulfate and Sulfide on Methanogenic and Sulfate Reducing Activity during Degradation of Simple Organics: Role of Propionate and Acclimation

by

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EFFECTS OF SULFATE AND SULFIDE ON METHANOGENIC AND SULFATE REDUCING ACTIVITY DURING DEGRADATION OF SIMPLE ORGANICS: ROLE OF PROPIONATE AND ACCLIMATION

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

The effect of elevated sulfate and sulfide levels on the anaerobic utilization of lactate, butyrate, propionate and acetate was investigated in this thesis. The primary objective was to determine the substrate utilization pathway which was the most sensitive to elevated sulfide concentrations. Four experimental phases were completed, utilizing ten semi-continuous breeder reactors and one hundred and fifty single-run batch reactors. The COD to sulfate ratios investigated ranged from 0.8 to 3.7 g g^{-1}.

Acetate removal was facilitated by methanogens, not sulfate reducers at the COD to sulfate ratios investigated (3.7 to 0.8 g g^{-1}). Acetate, propionate, and butyrate utilizations were more sensitive to aqueous hydrogen sulfide rather than total dissolved sulfide. The aqueous hydrogen sulfide concentration, which resulted in fifty percent inhibition of propionate, acetate, and butyrate utilization were 2.5, 3.4, and 7.3 mM (85, 115, and 250 mg L^{-1}).

The lactate degradation pathway was dependent upon the feed COD:sulfate ratio. A ratio of 3.7 g g^{-1} resulted in a non-sulfate reducing pathway producing propionate and acetate as products, while a ratio of $\leq$ 1.6 g g^{-1} resulted in a sulfate reducing pathway producing only acetate as an organic product. The lactate utilization pathways were by far the least sensitive to sulfide. The fifty percent inhibition thresholds were 11.1 mM (377 mg L^{-1}) aqueous hydrogen sulfide and 65.6 mM (2100 mg S L^{-1}) total dissolved sulfide for the former and latter pathways,

respectively.

The utilization of propionate was correlated to sulfate reduction. This sulfate reduction pathway was slightly more sulfide sensitive than acetoclastic methanogenesis. The sulfate reduction pathway responsible for lactate degradation, however, was much less sulfide sensitive than acetoclastic methanogenesis.

Hydrogen did not build up during inhibition of propionate degradation, suggesting that the inhibition of obligate hydrogen forming acetogens was not the cause of propionate inhibition.

An inoculum acclimated to high lactate and high sulfate conditions developed a completely new lactate degradation pathway when compared to an inoculum taken from an unacclimated biomass source. This demonstrated the importance of acclimation when using biological processes.

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NOMENCLATURE AND ABBREVIATIONS

a	-	species activity (moles L^{-1})
Acetoclast	-	bacteria degrading acetate
Acetogen	-	bacteria producing acetate
atm	-	atmosphere
AUR	-	acetate utilization rate (mM d^{-1}; mg L^{-1} d^{-1})
C	-	analytical concentration (mM; mg L^{-1})
COD	-	chemical oxygen demand (mM; mg L^{-1})
c_s	-	saturation concentration of H_2S at P_{H_2S} = 1 (mole)
$\Delta G^{\circ\prime}$	-	free energy change at defined conditions (kJ $mole^{-1}$)
d	-	day
D	-	dielectric constant for water (78.3)
Dehydrogenation	-	reaction which results in hydrogen-formation or electron donation
E	-	multiply by 10 to the power of (ex. 4.2E3 = 4.2×10^3)
Endogonic	-	reaction that requires energy
Exogonic	-	reaction that releases energy
f	-	free H_2S fraction of TS (fraction)
g	-	gram
γ	-	activity coefficient
h	-	hour
H	-	Henry's gas law proportionality constant (atm $mole^{-1}$ solute mole solvent)
HAc	-	acetate
HLa	-	lactate
HPr	-	propionate
H_2	-	hydrogen
H_2S	-	un-ionized hydrogen sulfide
HRT	-	hydraulic retention time

Hydrogenotroph	-	hydrogen consuming bacteria
Hydrogenation	-	reaction which results in hydrogen consumption or electron-acceptance
I	-	ionic strength (moles L^{-1})
IHT	-	interspecies hydrogen transfer
k	-	reaction constant
K	-	exponential inhibition constant (mM^{-1})
K_H	-	Henry's law absorption coefficient (atm mM^{-1})
K_m	-	half velocity coefficient (mM; mg L^{-1})
L	-	litre
mg	-	milligram (E-3 gram)
MPB	-	methane producing bacteria
mM	-	milli molar (E-3 molar)
m mol	-	milli mole (E-3 mole)
µ mol	-	micro mole (E-6 mole)
n	-	COD removal efficiency (fraction)
n_s	-	sulfate reduction efficiency (fraction)
N	-	newton (m kg sec^{-2})
NRB	-	nitrogen reducing bacteria
OLR	-	organic loading rate (g $L^{-1}d^{-1}$)
P	-	pressure (atm)
Pa	-	pascal (N/m^2)
ppm	-	parts per million (volumes per million volumes)
PUR	-	propionate utilization rate (mM d^{-1}, mg L^{-1} d^{-1})
Px	-	partial pressure of gas x (CH_4, CO_2, H_2S; atm)
R	-	gas constant (1.987 calories $mole^{-1}$ $°K^{-1}$)
R1	-	serum bottle set inoculated from breeder 1
R2	-	serum bottle set inoculated from breeder 2
R3	-	serum bottle set inoculated from breeder 3
R4	-	serum bottle set inoculated from breeder 4
S	-	substrate (mM; mg L^{-1})

S_A	-	acetate serum bottle set
S_L	-	lactate serum bottle set
S_M	-	1:1 mixture (lactate:acetate) serum bottle set
S_o	-	molar COD influent concentration (mole L^{-1})
S_s	-	molar sulfate influent concentration (mole L^{-1})
SRB	-	sulfate reducing bacteria
SRB_c	-	complete oxidizing SRB
SRB_i	-	incomplete oxidizing SRB
T	-	temperature
TS	-	total dissolved sulfide (mM; mg L^{-1})
TS_a	-	average total dissolved sulfide (mM; mg L^{-1})
UIS	-	un-ionized sulfide or hydrogen sulfide (mM; mg L^{-1})
UIS_a	-	average un-ionized sulfide or hydrogen sulfide (mM;mg L^{-1})
V	-	specific reaction velocity or consumption rate (mg L^{-1} h^{-1} g^{-1})
V_m	-	maximum specific reaction velocity (mg L^{-1} h^{-1} g^{-1})
x	-	mole fraction of solute in liquid phase (mole $mole^{-1}$)
Z	-	oxidation number of chemical species

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To life,
and all who endeavour
to expose her secrets

Chapter 1

INTRODUCTION

Anaerobic biological processes are now widely used in the wastewater treatment field. The process has moved from the cesspools and septic tanks of the past, into the sludge digesters of the present and now is being called upon to treat a variety of waste streams for industry, solid waste leachates and for site remediation projects. These new demands have caused a greater need to understand the process, in order to facilitate its role in the global clean-up of our environment.

It has long been recognized that the presence of large concentrations of sulfate ions (SO_4^{2-}) in the wastewater may interfere with the methane producing bacteria (MPB). The past two decades have seen the role of the organisms responsible for sulfate reduction, the sulfate reducing bacteria (SRB), more clearly defined. What was originally thought to be SO_4^{2-} toxicity to MPB is now discussed as the competition between MPB and SRB for available H_2 and acetate, as well as inhibition of both groups by the product of sulfate reduction, sulfide. It is now known that a high concentration of sulfate, sulfide or organic sulfur in the wastewater may lead to less energy recovery from biogass per unit substrate treated, due to these competition and inhibition mechanisms.

Wastewater variability and operational conditions used, make the number of treatment process trains virtually limitless. How sulfate and other forms of oxidized and reduced sulfur affect these processes has brought another order of complexity to the topic. Many researchers have preceded this thesis investigation, resulting in

a large amount of literature available on the topic. The wide range of observations made, which at times appear to be contradictory, have pointed to the difficulty if not impossibility of coming up with general guidelines for treating these types of wastes.

This thesis investigation strives for more details on the effects of the competition and inhibition mechanisms brought on by sulfate and sulfide in low-rate suspended growth anaerobic reactors.

Chapter 2

ANAEROBIC BIOLOGICAL PROCESSES

This chapter reviews the degradation pathways and ultimate fate of energy flow in anaerobic biological treatment processes. Particular emphasis is given to hydrogen, both as an intermediate and as a mediator of degradation reactions. The focus of the chapter is on the interactions between SRB and MPB, and how the product of sulfate reduction sulfide may affect this interaction.

2.1 SUBSTRATE DEGRADATION PATHWAYS AND INTERMEDIATES

Anaerobic biological waste treatment processes are widely used despite the lack of a complete understanding of the fundamental organic degradation pathways. Elucidating these pathways is a difficult task due to the diversity of the types and the roles of the various microflora. In general, the overall process of methane (CH_4) and carbon dioxide (CO_2) formation from complex organic molecules has been broken down into the following nine recognizable steps, each mediated by a specific group of microorganisms (Harper and Pohland, 1986):

1) hydrolysis of organic polymers to organic monomers such as sugars, organic acids and amino acids;

2) fermentation of organic monomers to hydrogen, bicarbonate, acetic, propionic, and butyric acids and other organic products such as ethanol and lactic acid;

3) oxidation of reduced organic products to hydrogen, bicarbonate, and acetic acid by obligate hydrogen forming acetogens;

4) acetogenic respiration of bicarbonate by homoacetogens;

5) oxidation of reduced organic products to bicarbonate and acetate by nitrate reducing bacteria (NRB) and SRB;

6) oxidation of acetate to bicarbonate by NRB and SRB;

7) oxidation of hydrogen by NRB and SRB;

8) aceticlastic methane fermentation; and

9) methanogenic respiration of bicarbonate.

The dynamic balance of each of these steps must be maintained for methanogenesis to continue at a maximum rate. As shown in Figure 2-1, the conversion steps involve direct and indirect symbiotic associations between several groups of bacteria (Harper and Pohland, 1986).

Complex organics are hydrolysed by nonmethanogenic bacteria to three general groups of organic compounds which are: proteins, carbohydrates, and lipids. Simply speaking, hydrolysis is the breakdown of certain organic bonds which result in the addition of a water molecule. The organic polymers are then hydrolysed to their respective monomers which are: amino acids, sugars, and fatty acids. Alcohols are also formed from carbohydrate and lipid hydrolysis. Little energy is removed during these steps (Parkin and Owen, 1986). However, the relatively insoluble

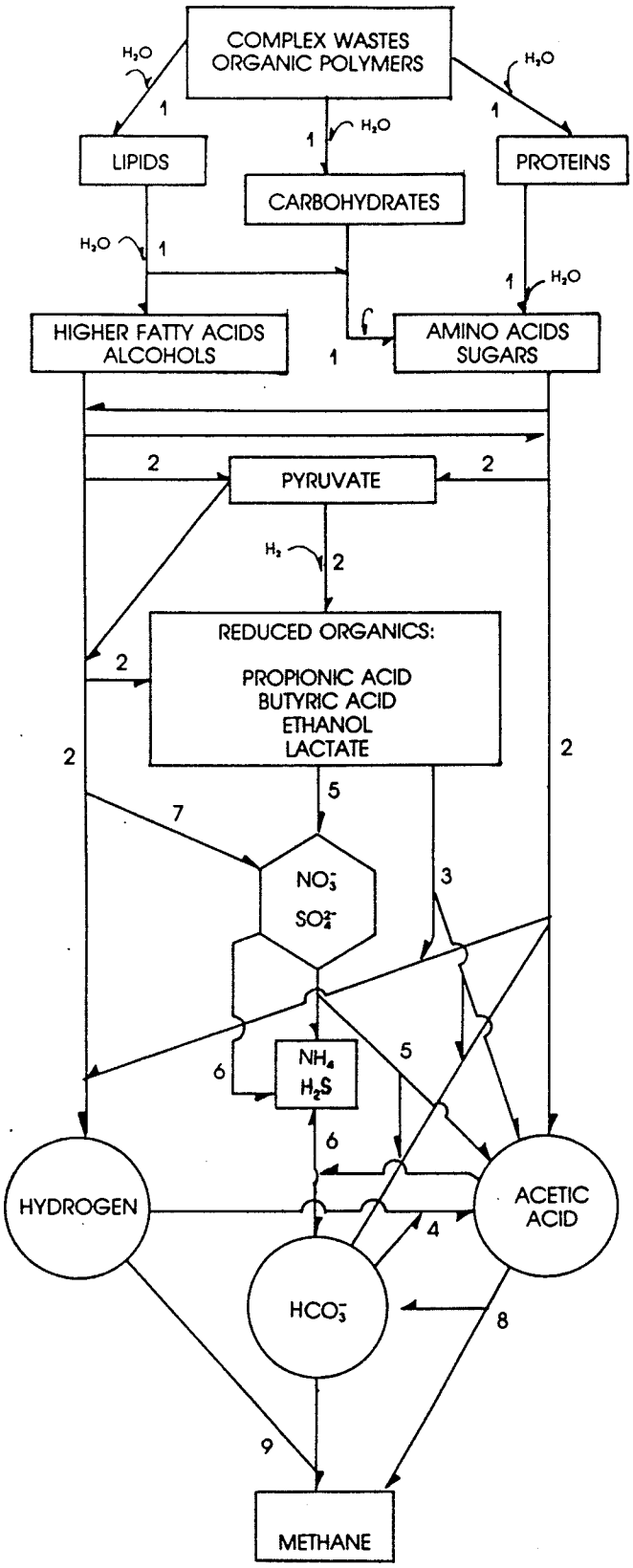


Figure 2-1 Schematic representation of the substrate-linked redox processes operating during the microbially mediated conversion of organic wastes to methane (after Stronach, et al. 1986; Harper and Pohland, 1986).

complex organic molecules are converted into soluble forms which can be utilized further by the bacteria and so it contributes to the overall reduction and stabilization of the waste (Hobson et al. 1974). These hydrolysis steps, which are accomplished by extracellular bacterial enzymes, are considered to be the overall rate-limiting steps of anaerobic digestion processes treating complex wastes (Stronach et al. 1986).

The products of hydrolysis are then fermented or anaerobically oxidized, resulting principally in acetic acid as an end product (Figure 2-1). Acetogenic bacteria are the predominant microflora and this step has been traditionally referred to as the acid-forming step (Parkin and Owen, 1986). Although acetate production is the principal result, other acids such as iso-valerate, valerate, iso-butyrate, butyrate, and propionate may also be formed (Stronach et al. 1986). Hydrogen (H_2) is produced during this stage from both the fermentation and oxidation processes. In addition, H_2 is consumed by hydrogen-consuming, acetogenic bacteria. Hydrogen is now known to play a key role in regulating organic acid production and consumption (Parkin and Owen, 1986; Stronach et al. 1986; Harper and Pohland, 1986). The hydrogen generated in glycolysis during acid-forming metabolism can be used directly by methanogenic bacteria via the mechanism of interspecies H_2 transfer. Some reactions are very sensitive to elevated partial pressures of H_2, so if H_2 is not consumed inhibition may result (Stronach et al. 1986).

Methane is generated from either acetate or from carbon dioxide and hydrogen. It is commonly believed that approximately 70% of the CH_4 produced in an anaerobic digester originates from acetate and the remaining 30% from the reduction of carbon dioxide (Figure 2-2). Methane formation from the oxidation of

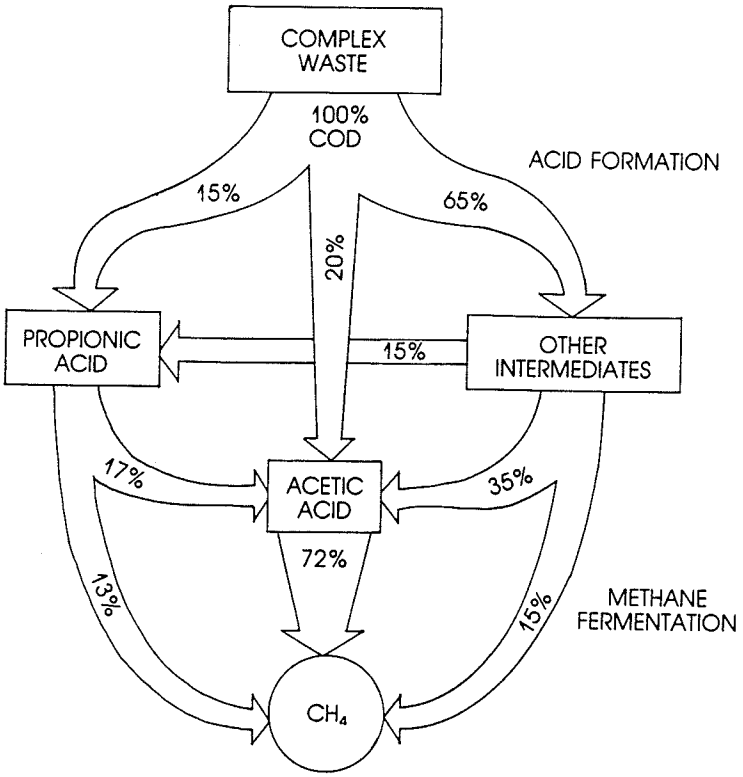


Figure 2-2 Pathways for methane fermentation of complex waste (after McCarty, 1964).

H_2 and reduction of CO_2 is the theoretical metabolic feature that unites the diverse species of methanogenic bacteria. However, as of yet, some species (ex. *Methanothix*) have not been able to use H_2 in combination with CO_2 to form CH_4 (Harper and Pohland, 1986).

The NRB and SRB can play a large role in the final steps preceding methane formation if conditions for their growth exist. The SRB can compete with MPB for free H_2 and also acetate, resulting in lower CH_4 yields per unit COD removal. According to references cited by Harper and Pohland (1986), SRB can also compete

with MPB for organic electron donors such as formate, acetate, propionate, butyrate, lactate, and other organic acids. Hydrogen sulfide, an end product of sulfate reduction, can also inhibit methanogenesis, which may result in the buildup of short-chain volatile acids.

2.2 THERMODYNAMIC CONSIDERATIONS

Methane can only be formed from substrates which have a larger free energy of combustion per equivalent of available electrons. Selected data from Erickson (1988) presented in Table 2-1, gives the free energy of combustion per equivalent of available electrons for the compounds of importance in this thesis investigation. Methane has the smallest free energy per equivalent of available electrons of those in the table. Therefore, the free energy change is negative for the conversion of any

Table 2-1. Free energy of combustion per equivalent of available electrons at standard-state conditions (unit molality for solutions, 25°C, 1 atm, pH 7)

Compound	kJ per equivalent
Methane	102.3
Butyric acid	108.7
Propionic acid	109.4
Acetic acid	111.8
Lactic acid	114.8
Hydrogen	119.0
Glucose	119.7

Source: From Erickson (1988).

of the other substances to methane, and this fundamental property of methane is the thermodynamic basis for the anaerobic digestion process (Erickson, 1988). Hydrogen has the biggest value in Table 2-1 even though it is formed from some of the compounds (Figure 2-1) listed in the table. Therefore, the partial pressure of hydrogen must be relatively low for hydrogen to be an intermediate in the pathways presented in Figure 2-1 (Erickson, 1988; Thauer et al. 1977; Stronach et al. 1986; Speece, 1983; McInerney et al. 1979; Parkin and Owen, 1986). In order to understand this better, it is best to present brief discussions on the free energy change associated with some of the reactions of importance in anaerobic digestion.

The reactions, believed to be the most relevant to this investigation, are presented in Table 2-2. The standard states for the free energy change ($\Delta G^{\circ\prime}$) reported in this table are: aqueous solutions; unit molality; water is a pure liquid; temperature, 25°C; pressure, 1 atmosphere; pH, 7; and methane and hydrogen are in the gaseous state. The free energy change is the free energy of the products minus that of the reactants. As shown in this table, the dehydrogenations (hydrogen-forming reactions) have high free energies of formation. Therefore, the concentrations of one or more of the products of these reactions must be kept low in order for the reaction to occur. When correcting for concentrations the actual free energy change $\Delta G^{\circ\prime}$ can be calculated using the following equation:

$$\Delta G_{reaction} = \Delta G^{\circ} + RT \ln \frac{[(C)^c(D)^d]}{[(A)^a(B)^b]} \qquad 2\text{-}1$$

where ( ) = reaction species activities;

R = gas constant (1.987 calories mole $^{-1}\circ K^{-1}$); and

T = temperature (°K).

for the reaction:

$$aA + bB \rightarrow cC + dD \qquad 2\text{-}2$$

where the quantities in brackets are the activities of the species.

Activities serve to correct the analytical concentrations of ionic species to the thermodynamically correct activity term. By working with activities it is possible to consider nonideal solutions as being ideal solutions (Benefield et al. 1982). The equation for the relationship between activity and concentration is:

$$a = \gamma C \qquad 2\text{-}3$$

where a = activity (moles L^{-1})

γ = activity coefficient; and

C = analytical concentration (moles kg^{-1}) for concentrated solutions (Stumm and Morgan, 1981), ($I > 10^{-2}M$).

I = ionic strength (moles L^{-1}).

Ionic strength (I) is determined by the following equation:

$$I = \frac{1}{2}\sum C_i Z_i^2 \qquad 2\text{-}4$$

where C_i = analytical concentration of the ith species (moles kg^{-1}); and

Z_i = oxidation number of the ith species.

Table 2-2. Standard-state free energy change for reactions of importance in this investigation (Unit molality for solutions, 25∘C, 1 atm, pH 7).

No.	REACTANTS	PRODUCTS	FREE ENERGY CHANGE $\Delta G^{\circ\prime}$(kJ per reaction)
DEHYDROGENATIONS: Electron-donating, formally hydrogen-forming reactions of energy metabolism.			
1	$CH_3CH_2CH_2COO^-+2H_2O$	$2CH_3COO^-+H^++2H_2$	+ 48.1
2	$CH_3CHOHCOO^-+2H_2O$	$CH_3COO^-+H^++2H_2+HCO_3$	- 4.2
3	$CH_3CH_2COO^-+3H_2O$	$CH_3COO^-+3H_2+H^++HCO_3$	+ 76.1
4	$CH_3CH_2COO^-+7H_2O$	$7H_2+2H^++3HCO_3^-$	+181.1
5	$CH_3COO^-+4H_2$	$4H_2+H^++2HCO_3^-$	+104.6
6	$CH_3COO^-+H_2O$	$CH_4+HCO_3^-$	- 31.0
HYDROGENATIONS: Electron-accepting, formally hydrogen consuming reactions of energy metabolism.			
7	$CH_3COO^-+2H_2+H^+$	$CH_3CH_2OH+H_2O$	- 9.6
8	$2CH_3COO^-+2H_2+H^+$	$CH_3CH_2CH_2COO^-+2H_2O$	- 48.1
9	$CH_3COO^-+CH_3CH_2COO^-+H^++2H_2$	$CH_3CH_2CH_2CH_2COO^-+2H_2O$	- 48.1
10	$CH_3COO^-+SO_4^{2-}+H^+$	$2HCO_3^-+H_2S$	- 59.9
11	$4CH_3CH_2COO^-+3SO_4^{2-}$	$4CH_3COO^-+4HCO_3^-+3HS^-+H^+$	-151.0
11a	$2CH_3CHOHCOO^-+3SO_4^{2-}$	$6HCO_3^-+3HS^-+H^+$	-255.3
12	$CH_3CHOHCOO^-+H_2$	$CH_3CH_2COO^-+H_2O$	- 79.9
13	$4H_2+2HCO_3^-+H^+$	$CH_3COO^-+4H_2O$	-104.6
14	$4H_2+HCO_3^-+H^+$	CH_4+3H_2O	-135.6
15	$4HCO_2^-+H_2O+H^+$	$CH_4+3HCO_3^-$	-130.0
16	$4H_2+SO_4^{2-}+H^+$	HS^-+4H_2O	-151.9

Source: From Thauer et al. 1979; Harper and Pohland, 1986; Widdel and Pfennig, 1982; Thiele and Zeikus, 1988; Widdel,1988.)

Various equations are presented in Benefield et al. (1982), which relate the activity coefficient to I at various concentrations (values) of I. Of these equations, the Davies relationship is the most appropriate to this work, as it can be used for solutions whose I's do not exceed 0.5 moles kg^{-1}:

$$\log \gamma = -AZ^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.3I\right) \qquad 2\text{-}5$$

where

γ = activity coefficient, with the subscripts M, D, and T representing the absolute value of the oxidation number of the chemical species in question (ex. γ_m represents the activity coefficient for monovalent ions);

Z = oxidation number of the chemical species of interest;

A = $1.82 \times 10^6 (DT)^{-3/2}$;

T = temperature of the solution (°K); and

D = dielectric constant for water, which is generally taken to have a value of 78.3.

Therefore, the activity of a given species can be calculated from I and analytical concentrations, using equations 2-3, 2-4, and 2-5.

In anaerobic digestion the actual partial pressure of hydrogen is considerably less than 1 atm. A study of twenty municipal digesters reported the ranges from 15 to 199 ppm (Collins et al., 1987). Other researchers have reported concentrations ranging from 10 to 282 ppm, depending on the environmental conditions (Boone et al., 1989; Boone and Xun, 1987; Mosey and Fernandes, 1989). Figure 2-3 shows the effect of H_2 partial pressure on the free energy change of some of the reactions in

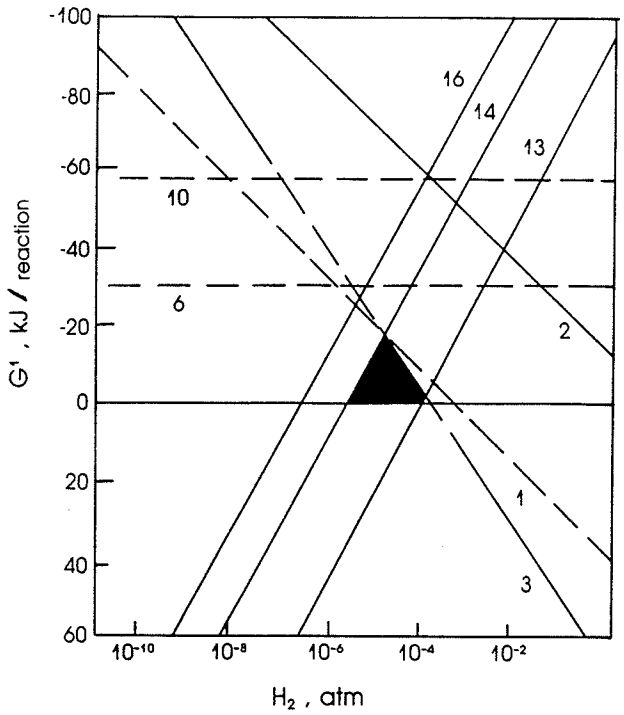


Figure 2-3 Effect of partial pressure of hydrogen on the Gibb's free energy change per reaction. $\Delta G'$ for acetic acid = 25 mM; propionic acid, butyric acid and lactic acid = 10 mM; sulfate = 5 mM; bicarbonate = 20 mM; and methane partial pressure of 0.7 atm. The numbers refer to the reactions in Table 2-2 (1) butyric acid to H2 and acetic acid; (2) lactic acid to H_2 and acetic acid; (3) propionic acid to H_2 and acetic acid; (6) acetic acid to CH_4; (10) acetic acid and sulfate to sulfide and bicarbonate; (13) H_2 and bicarbonate to acetic acid; (14) H_2 and bicarbonate to methane; and (16) H_2 and sulfate to sulfide (Harper and Pohland, 1986).

Table 2-2 (Erickson, 1988; Harper and Pohland, 1986). As shown in Figure 2-1, hydrogen is an intermediate product of the digestion process and as shown in Figure 2-3 the concentration of hydrogen must take a value that allows production and consumption to be in a reasonable balance. Therefore, if the free energy change is negative for both its production and consumption, the concentration of hydrogen must be low (Erickson, 1988; Harper and Pohland, 1986). In this thesis, the partial

pressures of hydrogen were reported in metric units of partial pressure (Pascal, Pa) and volumes of H_2 per million volumes of atmosphere (ppm).

Figure 2-3 indicates that propionic acid oxidation to acetate (line 3) becomes favorable only at hydrogen partial pressures below 10.1 Pa (100 ppm) while butyric acid oxidation (line 1) becomes favorable below 101.3 Pa (1000 ppm). Lactate oxidation (line 2) is only inhibited when hydrogen partial pressures approach 1 atm (Harper and Pohland, 1986). In addition, inorganic hydrogen acceptors such as bicarbonate and sulfate (lines 14 and 16) are thermodynamically favored at 0.1 Pa (1 ppm) H_2 or above. Also indicated is the favorability of bicarbonate respiration to methane (line14) over acetate cleavage to methane(line 6) at hydrogen partial pressures above 10.1 Pa (100 ppm) (Harper and Pohland, 1986). Comparing bicarbonate respiration (line 14) and sulfate reduction (line 16) it can be seen that the latter is favored at all hydrogen partial pressures; similarly, the favorability of acetate cleavage by SRB (line 10) over methanogens (line 6).

A close look at Figure 2-3 shows that a window exists, which is bound by the lines defined by the zero free energy change and reactions 3 and 14 (shaded area of Figure 2-3). Outside this window, either the hydrogen-forming or the hydrogen-consuming reactions become endogenic. If SO_4^{2-} is present (Reaction 16) the window widens and theoretically the H_2 concentration will be lowered. This makes it possible for hydrogenotrophic SRB to make more energy available for the syntrophic organisms, thereby allowing higher growth rates and larger growth yields (Dolfing, 1988). Table 2-3 shows the results of three experiments, as summarized by

Dolfing (1988), which indeed show this to be the case. The syntrophs exhibited higher growth rates in the presence of SO_4^{2-}.

Table 2-3 Growth rates of obligate hydrogen-forming bacteria (after, Dolfing, 1988).

ORGANISM	SUBSTRATE	GROWTH RATE (d^{-1}) with SO_4^{2-}	without SO_4^{2-}
Syntrophobacter wolinii	Propionate	0.192	0.103
**Syntrophomonas wolfei*	Butyrate	0.307	0.185
Syntrophus buswellii	Benzoate	0.127	0.101

* The growth rates on valerate and caproate with *M. hungatei* as hydrogen consumer were 0.185 and 0.092 d^{-1}, respectively.

2.3 ACETOGENESIS AND INTERSPECIES ELECTRON TRANSFER

Culture conditions may greatly influence microbial catabolism, therefore nomenclature for naming reactions rather than for specifying organisms has been advocated by Dolfing, (1988). Fermentations are frequently identified by the major types of end products formed. Consequently, within the diverse group of anaerobic acidogens, acetate-forming organisms have been called acetogens (Dolfing, 1988). Two different types of acetogenic mechanisms can be distinguished: acetogenic dehydrogenations and acetogenic hydrogenations (Table 2-2). Acetogenic dehydrogenations can be further divided into two sub-groups, the obligate hydrogen-

forming acetogens (OHFA) and the fermentative bacteria. Note that acetogenic sulfate reduction (Reaction 11, Table 2-2) is not included in this definition and that the obligate hydrogen-formers can utilize only protons as electron acceptors (Dolfing, 1988).

The degradation steps outlined in Section 2.1 and Figure 2-1 have been demonstrated by recorded observations of organic acid and alcohol accumulations during periods of overloading or other process stress. It has only been recently recognized that the accumulation of these intermediates is due to hydrogen sensitivities moderated by syntrophic associations between OHFA and hydrogen-consuming bacteria (methanogens, SRB, NRB). Thiele and Zeikus (1988) refer to syntrophic OHFA as those species of acetogens that require their reaction products (H_2 and acetate) to be removed by other organisms to prevent product inhibition. However, the full process engineering consequences of these relationships essentially remain unresolved (Harper and Pohland, 1986).

As illustrated in Figure 2-4 for the catabolism of glucose, hydrogen in relatively low concentrations appears to regulate the overall conversion process. In order for catabolism to proceed smoothly the NAD^+ reduced during glycolysis (Figure 2-4; I and II) must be regenerated quickly since the pool of NAD^+ can essentially be considered constant. Hydratase enzymes accomplish this by oxidizing the $NADH + H^+$ into NAD^+ and H_2. The rapid removal of H_2 from the system is usually accomplished by methanogens, SRB or NRB. However, if H_2 is allowed to buildup, propionic and butyric acid will be formed (Figure 2-4; III and IV). These intermediates can't be used directly by methanogens; therefore accumulation ensues,

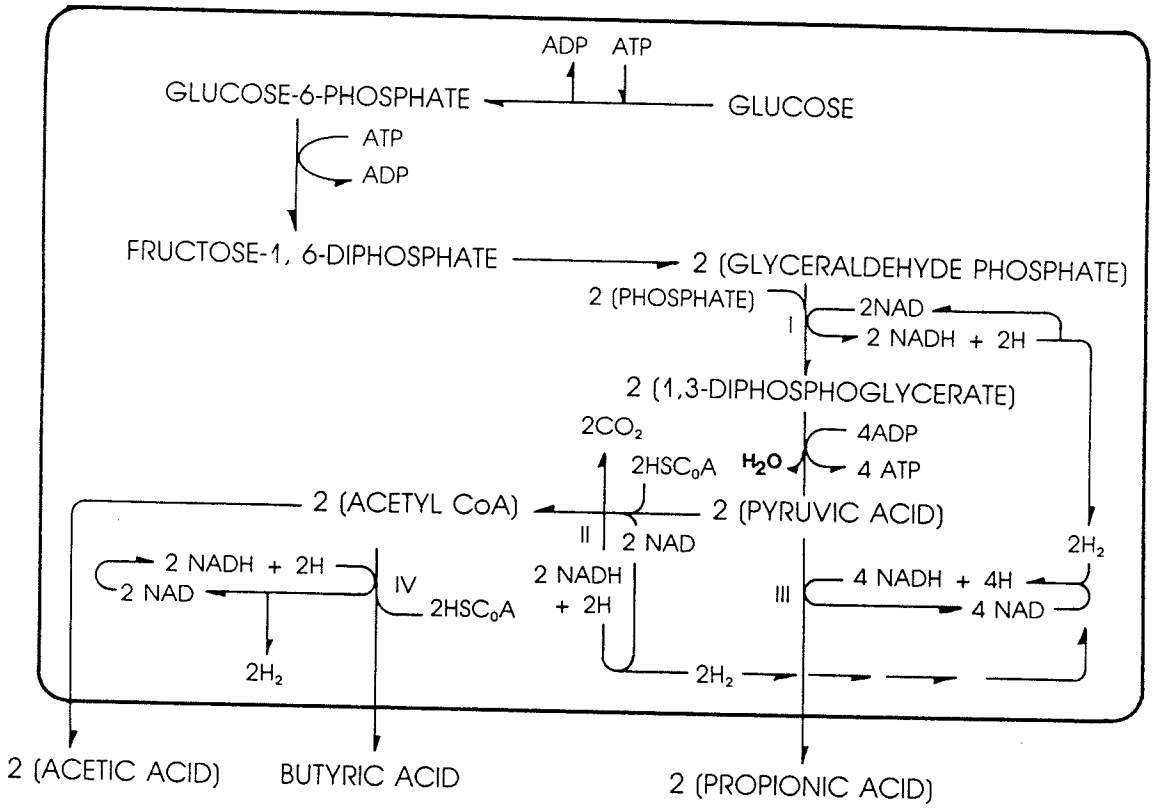


Figure 2-4 Hydrogen-regulated catabolic pathways possible for the conversion of glucose in anaerobic wastewater treatment systems (after Mosey, 1983).

which often leads to a problematic depression of the reactor pH (Harper and Pohland, 1986). This may then lead to process upsets if not mitigated.

The degradations of propionic and butyric acid are also H_2 dependent. The OHFA are closely linked to the H_2 utilizers, and if the H_2 concentration remains high, these organic acids will not be degraded (Figure 2-3). This dependency of the OHFA on the hydrogen-consumers is regarded as "the key to stabilizing and improving anaerobic treatment" (Harper and Pohland, 1986).

This symbiotic link between the OHFA and hydrogen-consumers is called interspecies hydrogen transfer (IHT). Dolfing (1988) recognized the first experiments

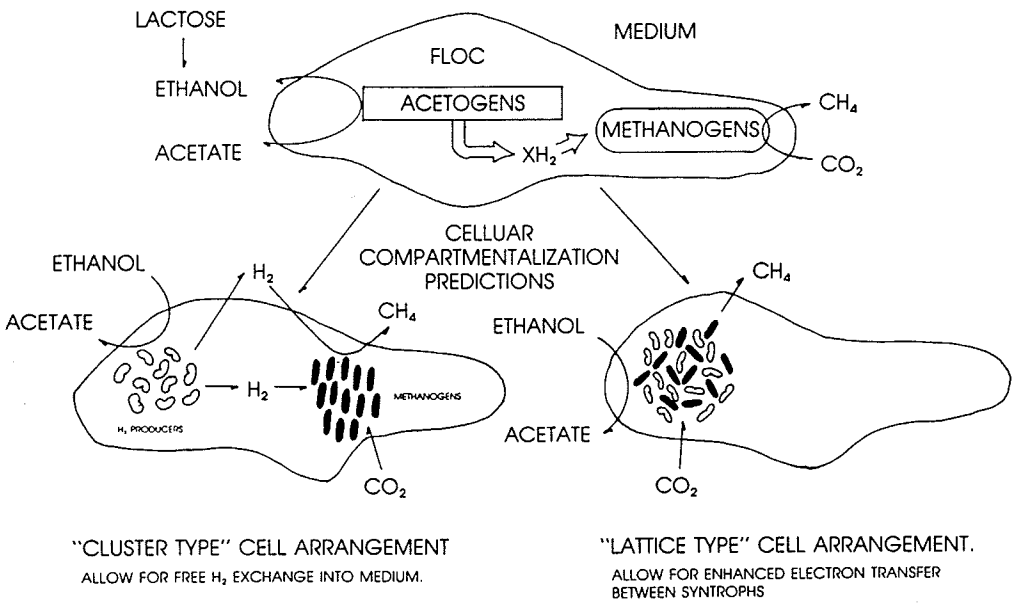


Figure 2-5 Predictive model for species compartmentalization and electron flow coupling during syntrophic metabolism (after Theile et al. 1988).

to illustrate this principle as being reported by Lanott et al. (1973). Direct experimental evidence (Thiele et al. 1988) has shown that CO_2-dependent methanogenesis and IHT were predominantly located in an ecological compartment within bacterial flocs and are independent of the dissolved hydrogen pool in the bulk aqueous phase (Figure 2-5). These same researchers also showed that only ≤ 5% of the ethanol to methane conversion could be accounted for by IHT, while the balance (≥95%) depended on some other mechanism for control of interspecies flow (Thiele et al. 1988). Another study has demonstrated that formate was responsible for the electron flow in the floc ecosystem (Thiele and Zeikus, 1988). Thiele and Zeikus (1988) presented a hypothetical model illustrating the principles of the bicarbonate-formate electron shuttle mechanism (Figure 2-6). The model accounts for the

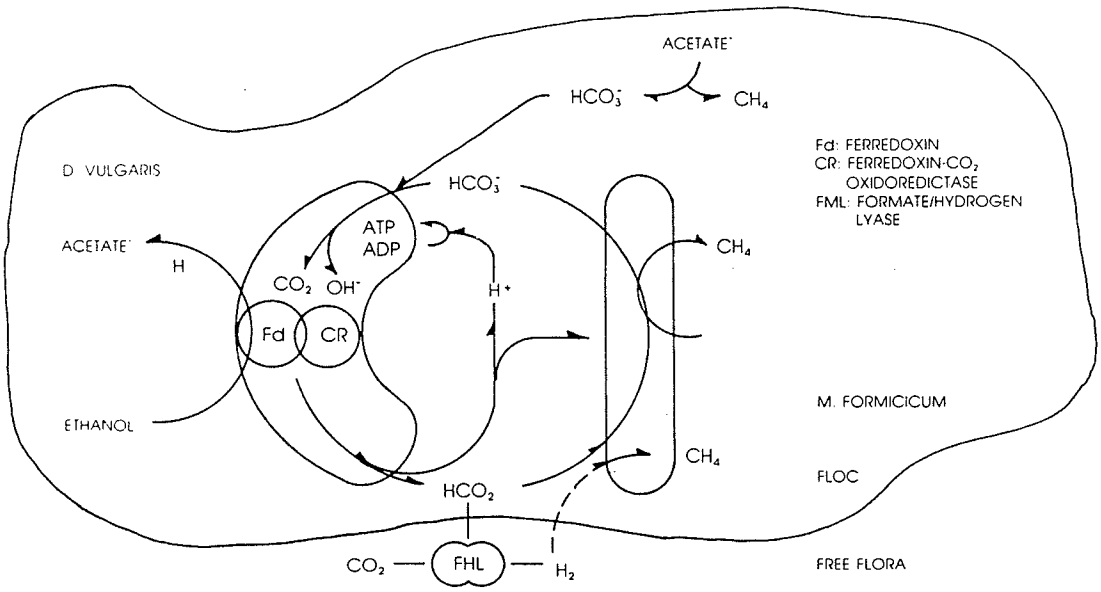


Figure 2-6 A hypothetical model illustrating the principles of the bicarbonate-formate electron shuttle methanism for explaining both the control of interspecies electron flow during syntrophic ethanol conversion to methane by anaerobic digester flocs and energy conservation by electron transport-mediated phosphorylation in syntrophic acetogens (after Thiele and Zeikus, 1988).

coupling of electron flow during syntrophic lactate conversion to CH_4 in the absence of sulfate and for energy conservation for both species (Thiele and Zeikus, 1988). The juxtaposition of *D. vulgaris* and *M. formicicum* in the floc enables methanogenic CO_2 regeneration to be coupled with the acetogenic step of CO_2 reduction to formate. Note that formate hydrogen lyase converts formate which has been released from the floc into hydrogen (Thiele and Zeikus, 1988).

No matter what the exact mechanism is, the most important implication of the electron transport system, is that the hydrogen utilizers can create conditions that allow OHFA to perform catabolic oxidations which would not have been energy-yielding in the absence of hydrogen utilizers (Dolfing, 1988). These syntrophic

reactions are of tremendous importance in controlling the rate-limiting metabolic steps in anaerobic digesters (Thiele and Zeikus, 1988).

The early work of McCarty (1964) suggested that 30% of the methane formed was via propionate (Figure 2-2). In anaerobic sludge digesters, Kaspar and Wuhrman (1978a), showed that 15% of the methane formed was from propionate via acetate and H_2. This value agrees with the value achieved by Mackie and Bryant (1981), where about 23% of the methane formed comes from propionate (15%) and butyrate (8%) in anaerobic cattle waste digesters. Thus IHT plays a dual role by preventing the formation of propionate and butyrate, and by making syntrophic oxidation of these acids possible (Dolfing, 1988). In excess sulfate conditions, propionate can be metabolized by incomplete oxidizing SRB to acetate and also by completely oxidizing SRB (Widdel, 1988). Heyes and Hall (1983) demonstrated that various propionate-degrading sub-populations can be distinguished. The important role of sulfate in propionate degradation was demonstrated in a study by Ueki et al. (1986). The effect of sulfate addition to the cattle waste digester is shown in Figure 2-7. After 12 days without sulfate the residual propionate concentration was quickly utilized when sulfate was added.

2.4 METHANOGENESIS

The MPB belong to the primary kingdom of the archaebacteria on the basis of a number of characteristics (Balch et al. 1979; Konig, 1988; Jones et al. 1987; Wolin and Miller, 1985; Stanier et al. 1986; Sprott, 1989; Vogels et al. 1988). The taxonomy

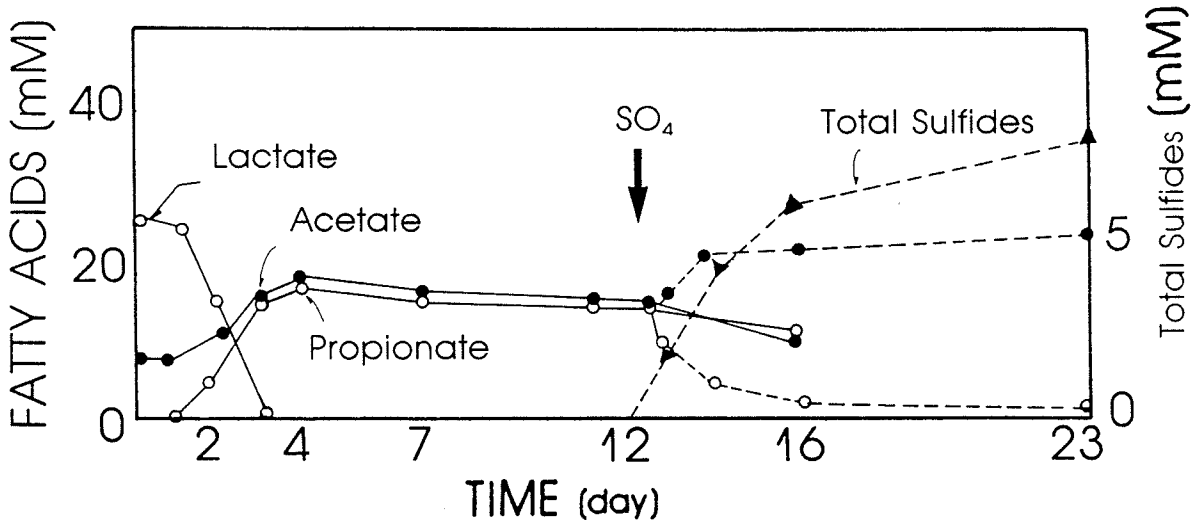


Figure 2-7 Effect of sulfate addition on propionate decomposition in cattle waste. After 12 days of incubation in the absence of sulfate, 20 mM Na_2SO_4 was added. Solid line, before sulfate-addition; dashed line, after sulfate-addition (after Ueki, et al., 1986).

of MPB based on 16s rRNA cataloguing was first presented in Balch et al. (1979) and later reproduced in Wolin and Miller, (1985). Likewise the determinative key to MPB species identification based on simple phenotypic characteristics was given in both publications.

The common property of methanogens is the production of methane from a limited number of one-carbon compounds and the two-carbon compound acetate (Vogels et al. 1988). The compounds of interest to this investigation are carbon dioxide, formate and acetate. The most detailed evaluation of the various MPB pathways is given by Daniels et al. (1985).

The best elucidated pathway of methane formation from H_2 and CO_2 is that for *Methanobacterium thermoautotrophicum* and since all MPB investigated contain similar compounds, the intermediary reactions presumably proceed along a common

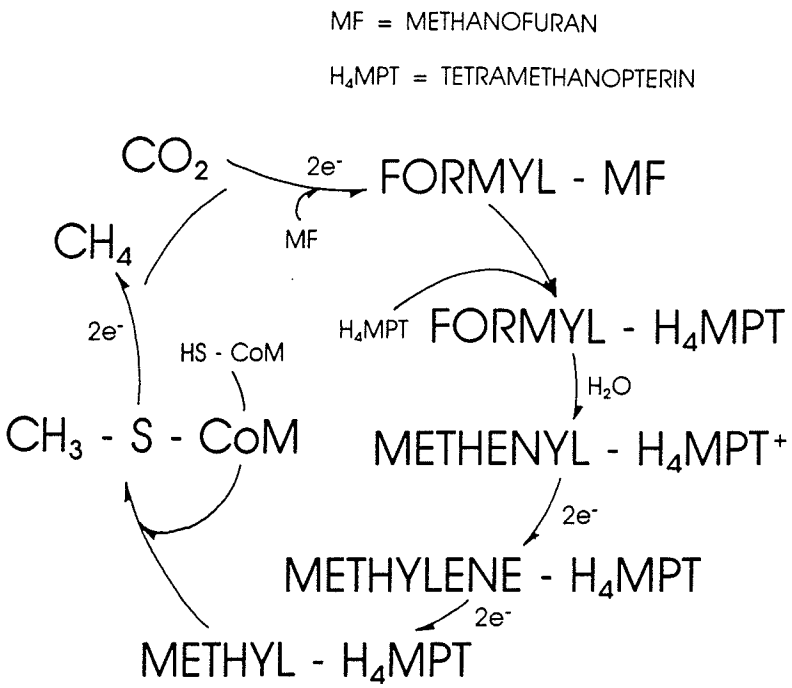


Figure 2-8 Conversion of carbon dioxide into methane in *M. thermoautotrophicum* (after Jones et al. 1987). Note: H_4 MPT = tetrahydromethanopterin.

line (Vogels et al. 1988; Konig, 1988). The simplified pathway is presented in Figure 2-8. The structures of and detailed discussions on the intermediates are given in various publications (Vogels et al. 1988; Jones et al. 1987; Daniels et al. 1985). The pathway was drawn as a cycle, due to the particularly intriguing initial CO_2 reduction step which lacks a requirement for stoichiometric amounts of ATP and also requires methylcoenzyme M reduction (Jones et al. 1987). The coenzymes methanofuran (MF), tetramethanopterin (H_4MPT), and coenzyme M are unique to methanogens and are the one-carbon carriers during the sequential reduction of CO_2 to CH_4 (Figure 2-8; Jones et al. 1987). About half the species of methanogenic

bacteria are able to grow on formate, but it is unclear whether formate is directly channelled into the reduction pathway (Figure 2-8) or indirectly via CO_2 (Vogels et al. 1988).

In all methanogenic bacteria tested, the terminal stage of methanogenesis involves the reductive demethylation of the simple compound methylcoenzyme M (CH_3-S-CH_2-CH_2-SO_3^-) to coenzyme M (HS-CH_2-CH_2-SO^-_3) and methane (Vogels et al. 1988).

$$CH_3\ S - CoM + H_2 \xrightarrow[reductase]{methylcoenzymeM} CH_4 + HS - CoM \qquad 2\text{-}6$$

Coenzyme M derivatives are unique to MPB and equation 2-6 is catalyzed by a complex system of proteins and coenzymes. Details of this system are given in Jones et al. (1987). The presence of the active sulfide group on coenzyme M indicates a likely potential for free sulfide competition, as would the same group on acetylcoenzyme A which is involved in MPB metabolism. An abundance of dissolved sulfide may interfere with the binding sites of the active sulfide group, therefore inhibiting the coenzymes.

Studies on the biochemistry of methanogenesis from acetate have focused on *Methanosarcina barkeri* and have demonstrated that the methyl group of acetate ends up almost completely in methane at which methylcoenzyme M could be identified as an intermediate, whereas the carboxylic group is oxidized to CO_2 (Vogels et al. 1988). However, a study by Krzycki et al. (1982) reported that the reverse was true for 15% of the acetate converted (Vogels et al. 1988). The standard free energy of the reaction for acetoclastic methanogenesis (Reaction 6, Table 2-2) is nearly equal to that

required for synthesis of a molecule of ATP from ADP and inorganic phosphate (+31.8 kJ $mole^{-1}$), therefore it is not as thermodynamically favorable when compared with methanogenesis from one-carbon substrates (Reactions 14 and 15, Table 2-2; Jones et al. 1987). If better growth substrates are available, the cell will utilize acetate for biosynthesis and form methane via the energetically more favorable pathways (Jones et al. 1987).

There are similarities between acetate cleavage and acetate synthesis in methanogens. The *Methanosarcinaceae* are capable of growth by acetate cleavage, and can also synthesize acetate autotrophically from H_2 and CO_2. The other methanogens are limited to growth on single-carbon substrates, i.e. they synthesize acetate autotrophically, but do not cleave it (Jones et al. 1987).

2.5 SULFATE REDUCING BACTERIA

Currently 10 genera comprising 33 species of SRB have been identified and described (Widdel, 1988; Stouthamer, 1988). Widdel (1988) presents a relationship tree of SRB with other eubacteria based on 16s rRNA catalogues and a table listing the properties of the classified SRB. Some SRB species can completely oxidize organic substrates (SRB_c) such as lactate and acetate to CO_2 while others are incomplete oxidizers (SRB_i) leaving acetate as a product. These are the two major metabolic groups of SRB (Widdel, 1988; Stouthamer, 1988). Apparently the SRB_i do not have an operating enzyme mechanism, like the tricarboxylic acid cycle, that allows the oxidation of the acetate unit (acetylcoenzyme A) originating from substrates, therefore it is excreted as acetate (Widdel, 1988). In general the SRB_c are

nutritionally more versatile than the SRB_i, however, the various species of the *Desulfovibrio* genera, which is the most versatile SRB_i, can use hydrogen, lactate, pyruvate, malate, fumarate, ethanol, glycerol, choline, and a number of amino acids (Widdel, 1988). It has been observed that the SRB_i may grow significantly faster than the complete oxidizers, therefore batch experiments with electron donors which can be used by both groups, such as lactate, usually select for SRB_i (Widdel, 1988). Besides SRB and NRB, only a few other types of metabolic anaerobes can grow with hydrogen or acetate as sole energy sources: hydrogen may be used by the MPB or the homoacetogenic bacteria (Reaction 13, Table 2-2) and acetate by some MPB (Widdel, 1988).

The advancement of the understanding of SRB bioenergetics has been hindered by the reaction product, H_2S. The inhibition of SRB by H_2S and other environmental factors have caused a great number of contradicting data in the literature (Stouthamer, 1988). General observations, however, have been corroborated such that some conclusions can be made.

At least seven SRB species have been shown to utilize the acetylcoenzyme A pathway for CO_2 fixation; therefore it has been concluded that autotrophy and CO_2 fixation by this pathway is wide spread in SRB (Stouthamer, 1988). The acetylcoenzyme A pathway is also used for the same purpose by the MPB and the acetogenic bacteria. According to Stouthamer (1988), it has recently been demonstrated that a number of SRB also utilize the acetylcoenzyme A pathway for energy generation during the degradation of acetate in the presence of sulfate. It has

also been reported that some SRB oxidize acetate by the normal tricarboxylic acid cycle, using the glyoxylate shunt (Stouthamer, 1988).

Postgate (1984) and Widdel (1988) have reported a well-established observation, which is the small quantity of methane which is formed in cultures of SRB (Widdel, 1988). This phenomenon occurs in all SRB that contain carbon monoxide dehydrogenase which is regarded as the key enzyme of the acetylcoenzyme A pathway (Stouthamer, 1988).

The SRB reduce their electron acceptor, sulfate, usually to sulfide as the entire and final product, with only one report observing that sulfite and thiosulfate were

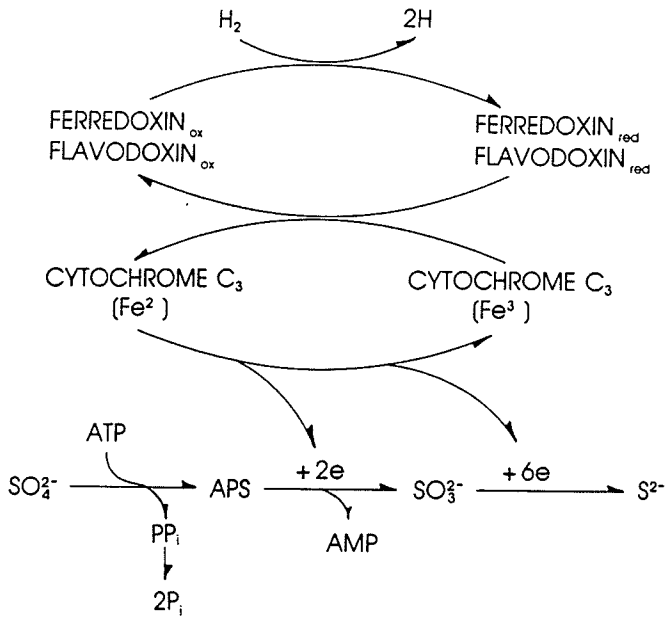


Figure 2-9 Dissimilatory sulfate reduction in *Desulfovibrio*.

excreted at low concentrations (Widdel, 1988). Most SRB can grow with sulfite or thiosulfate as electron acceptors instead of sulfate (Stouthamer, 1988; Widdel, 1988). Growth yield data has shown that sulfate reduction to sulfide is associated with a net synthesis of 1 mol of ATP and that approximately to 3 moles of ATP are formed during sulfite reduction to sulfide (Badziong and Thauer, 1978). The reduction of the sulfate ion SO_4^{2-} to the sulfide ion S^{2-} must be preceded by sulfate activation. Once activated, the reduction of sulfate can be performed for assimilatory or dissimilatory purposes. The amount of sulfate used by dissimilatory sulfate reduction far exceeds the amount that is used for cell synthesis (assimilatory). An example given by Widdel (1988) indicated that if 100 g of hydrogen sulfide were formed by sulfate reduction, approximately 14 g of dry cell mass would be synthesized, which results in only 0.2 g of hydrogen sulfide assimilated into the cells. The dissimilatory reduction process is shown schematically in Figure 2-9. The overall process can be described by the reaction equation,

$$8H^+ + SO_4^{2-} \rightarrow H_2S + 2H_2 + 2OH^- \qquad 2\text{-}7$$

where the hydrogen donors are alcohols, organic acids or molecular hydrogen.

The initial activation step of sulfate results in the formation of adenosine - 5′ - phosphosulfate (APS; Figure 2-9). The free energy of formation of this reaction is +46 kJ $mole^{-1}$, therefore the removal of pyrophosphate (PPi) is of great importance in driving the reaction in the direction of APS synthesis (Stouthamer, 1988; Legall and Fauque, 1988). APS is then reduced to sulfite SO_3^{2-}. Two different mechanisms have been proposed for the reduction of sulfite. The difference between the two mechanisms is whether trithionate $S_3O_6^{2-}$ and thiosulfate $S_2O_3^{2-}$ occur as free

intermediates during the reduction of sulfite to sulfide (Stouthamer, 1988). Legall and Fauque (1988) speculate that, both of these intermediates actually form in situ with an enzymatic complex allowing a fast transfer and reduction of the compounds. Many species of SRB can reduce sulfite or thiosulfate and a few elemental sulfur (Widdel, 1988). Incomplete oxidizing SRB using lactate are usually able to grow just as well with hydrogen as the electron donor, which has been demonstrated with different *Desulfovibrio* species, *Desulfotomaculum* species, *Desulfobulbus* species and *Thermodesulfobacterium commune* (Widdel, 1988). In fact, *Desulfovibrio* species may grow rather fast on hydrogen (Widdel, 1988). A key issue, which according to Legall and Fauque (1988) is not yet resolved, is whether molecular hydrogen is a residual product during growth on lactate and sulfate, or is it an essential metabolite? The species of the genera *Desulfobacter* are the only group that do not have a species that can grow on lactate. The only SRB_i species that have been shown to be capable of growth on propionate are *Desulfobulbus propionicus* and *D. elongatus*. However, many species of the SRB_c can oxidize propionate. Butyrate is used by many species of the SRB_c and by *Desulfovibrio thermophilus* of the SRB. The optimum temperature for the latter species is 65°C (Widdel, 1988), therefore it was not expected to play a significant role in this thesis investigation.

In the absence of sulfate, several types of SRB can grow by fermentation on various substrates. Facultative hydrogen-forming bacteria, in the presence of hydrogen-consumers, can shift their metabolism to the formation of more oxidized end products plus hydrogen (Dolfing, 1988). Under sulfate-limited conditions and in the presence of a hydrogen-consuming organism, SRB can act as facultative

hydrogen-formers when growing on lactate or ethanol (Bryant et al. 1977; McInerney and Bryant, 1981; Dolfing, 1988). Examples of some of these SRB are *Thermoanaerobium brockii, Desulfovibrio desulfuricans, Desulfovibrio vulgaris,* and the *S-organism* (Dolfing, 1988). Excess sulfate conditions were always present in this thesis investigation, therefore facultative SRB reactions were not expected to play a significant role.

The SRB may use low-molecular-weight compounds (fermentation end-products) as electron donors, therefore SRB are terminal degraders and their role is analogous to that of MPB (Widdel, 1988). According to Widdel (1988), recognizing dissimilatory sulfate reduction and methanogenesis as two alternative terminal processes has contributed a lot to our understanding of anaerobic mineralization.

2.6 COMPETITION BETWEEN SRB AND MPB

Hydrogen and acetate are the key precursors to methane formation during normal anaerobic wastewater treatment. In Section 2.5, the metabolism of the SRB was reviewed and it was noted that hydrogen and acetate may also serve as electron donors for sulfate reduction. Therefore, MPB and SRB must be considered as competing for these available substrates in anaerobic wastewater treatment systems where significant sulfate is present. When comparing degradation processes there is a general rule based on external electron acceptors. This rule states that the available electron acceptors are used in the following order of preference: $O_2 > NO_3^- > SO_4^{2-} > CO_2$. Therefore, NO_3^- will not be used until the O_2 concentration is low and so on, with CO_2 reduction being the least favorable. This rule is based on free energy

changes and it indicates that SRB will outcompete MPB in the presence of excess sulfate. However, thermodynamics alone is insufficient to address microbially catalyzed reactions. The effects of product inhibition and the reaction kinetics must also be considered. The product in this case is H_2S which inhibits both the SRB and the MPB.

2.6.1 Thermodynamic Considerations

Table 2-2 indicates that the free energy change for acetate (Reaction 10) and H_2 (Reaction 16) utilization by SRB were -59.9 and -151.9 kJ per reaction, respectively, while for the same respective MPB reactions (6 and 14) the free energy changes were -31.0 and -135.6 kJ per reaction. Therefore at these standard conditions the SRB would be expected to outcompete the MPB. Recalling the discussion of Section 2.2. reminds us that the actual free energy changes are dependent upon the activities of the reactants and the products of each reaction. The potential for MPB to outcompete SRB exists when the electron donor (acetate or H_2) to sulfate ratios become very high or when a buildup of sulfide occurs. Conversely, it would be expected that SRB would outcompete MPB when the electron donor to sulfate ratios were low and if sulfide concentrations were not allowed to buildup. The SRB reactions are also pH dependent and theoretically would be less favorable at higher pH values. Although species of hydrogen-utilizing SRB are routinely isolated from anaerobic digesters, according to Tursman and Cork (1989), acetoclastic SRB have not yet been isolated. Therefore, it would appear that in anaerobic digesters SRB and MPB may be

competing for H_2 only. The reasoning for this observation may be derived from the kinetic theory of substrate utilization.

2.6.2 Kinetic Considerations

The kinetics of substrate utilization is described using the Michaelis-Menten equation which is similar to the Monod equation for cell growth. Monod's classical model was based on enzyme theory (Monod, 1949). The Michaelis-Menten substrate utilization equation is as follows:

$$V = \frac{V_M S}{K_M + S} \qquad 2\text{-}8$$

where,

V = specific consumption rate (units of substrate per unit time and cell mass);

V_M = maximum specific consumption rate;

S = substrate concentration (units of substrate); and

K_M = a kinetic parameter, which is defined as the substrate concentration at which the consumption rates are half of V_M (units of substrate).

Using this equation Widdel (1988) developed an equation which relates the kinetic parameters of SRB and MPB. Letting V_1 and V_2 represent the specific substrate utilization rates of SRB and MPB, respectively, the ratio of the two rates would be:

$$\frac{V_1}{V_2} = \frac{V_{M1}(K_{M2} + S)}{V_{M2}(K_{M1} + S)} \qquad 2\text{-}9$$

At limiting, very low substrate concentration (S<< Km) the following equation is derived:

$$\frac{V_1}{V_2} = \frac{V_{M1}/K_{M1}}{V_{M2}/K_{M2}} \qquad 2\text{-}10$$

Therefore, the ratio of V_m/K_m governs the relative competition between species. At high substrate concentration ($S >> K_m$), the maximum specific consumption rate governs according to equation (2-11):

$$\frac{V_1}{V_2} = \frac{V_{M1}}{V_{M2}} \qquad 2\text{-}11$$

Therefore, in anaerobic reactors where $S >> K_m$, the substrate removal rate would be controlled by the organism with the maximum specific consumption rate, and here we should perhaps see what affects that rate.

Selected data comparing the kinetic parameters of SRB and MPB for growth on H_2 and acetate was taken from Widdel (1988) and is summarized in Table 2-4. To understand the significance of this table, the concentrations of H_2 and acetate that can be expected in anaerobic reactors are needed. From Figure 2-3, it is apparent that H_2 must be maintained at approximately 10^{-4} to 10^{-7} atm to prevent thermodynamic inhibition. The solubility of gaseous H_2 in water is calculated from Henry's law expressed as:

where,

$$x = P/H \qquad 2\text{-}12$$

x = mole fraction of solute in the liquid phase (moles solute per mole solution);

P = partial pressure of the solute in the gas phase (atm.); and

H = proportionality constant (atm of solute pressure in the gas phase per unit concentration of the solute in the liquid phase, this unit concentration is expressed as moles solute per mole solution).

Therefore, using a value of 7.42E4 atm $mole^{-1}$ H_2 $mole^{-1}$ H_2O for H_2 at 35∝C (Perry et al. 1984) the concentration of H_2 in the solution would range from 0.15 to 0.15 E-3 μmol L^{-1}. These concentrations are much lower than the values of K_m listed in Table 2-4, therefore it is expected that equation 2-10 will govern. The ratio of the mean V_m/K_m values for SRB to MPB values from Table 2-4 is 2.41. Robinson and Tiedje (1984) conducted kinetic parameter determinations for 5 species of SRB and 4 species of MPB growing on H_2. The ratio of the highest V_m/K_m values for the SRB and MPB group was 2.2, thus supporting the data contained in Table 2-4. These results indicate a definite kinetic advantage for SRB over MPB. Tursman and Cork (1989) suggest that SRB have a lower K_m for H_2 due to the location of the common hydrogenase enzyme system. In MPB the system is located in the cytoplasm, while in SRB it is located in the periplasmic space, therefore resulting in less of an osmotic barrier.

The acetate concentrations in a healthy digester and in this study would range from approximately 8,000 to 170,000 μmol L^{-1}. These concentrations are much higher

than the K_m values, therefore it is expected that equation 2-11 will govern. The ratio of the mean V_m values for SRB to MPB from Table 2-4 is 1.35. This also indicates an

Table 2-4 Kinetic Parameters for SRB and MPB growth on H_2 or acetate.

SPECIES	TEMP. (°C)	V_M (μmol g^{-1} h^{-1})	K_M (μM)	V_M/K_M (L g^{-1} h^{-1})
		FOR H_2 CONSUMPTION		
SRB:				
Desulfovibrio				
vulgaris	37	1770	1.9	930
desulfuricans	37	5280	1.8	2930
sp. (G11)	37	3300	1.1	3000
sp. (PS1)	37	3300	0.7	4710
MPB:				
Methanobrevibacter				
arboriphilus (AZ)	35	8510	6.6	1290
Methanospirillum				
hungatei (JF-1)	37	4200	5.0	840
sp. (PM1)	37	5400	2.5	2160
Methanosarcina				
barkeri (MS)	37	6600	13.0	510
		FOR ACETATE CONSUMPTION		
SRB:				
Desulfobacter				
postgatei	30	830	230	3.6
	30	3100	64	48
	30	3200	77	42
	30	7200 to 9600	nd	nd
MPB:				
Methanosarcina				
barkeri (Fusaro)	30	2240	3000	0.75
barkeri (227)	37	6800	nd	nd
mazei (S-6)	37	1620	nd	nd

After Widdell (1988); nd, not determined in the study.

advantage to the SRB, but only a slight advantage, which is probably insignificant if one considered the potential for variations based on experimental conditions. These variations are evident in the experiments utilizing *Desulfobacter postgatei*, in which results varied by an order of magnitude for the same species (Table 2-4).

Using bench-scale attached growth reactors researchers have shown that MPB outcompeted SRB for acetate (Yoda et al. 1987), and acetate and ethanol (Isa et al. 1986b) under excess SO_4^{2-} conditions. Both experiments used high influent acetate concentrations. Isa et al. (1986b) showed that, during excess sulfate conditions, a ten-fold decrease in acetate/ethanol substrate concentration from 5 g COD L^{-1} to 0.5 g COD L^{-1} resulted in MPB electron flow dropping from 89% to 66% of the total electron flow. This not only indicates the importance of high substrate concentrations, but also indicates that MPB were able to outcompete the SRB for both acetate and ethanol, the latter being a good source of H_2. Isa et al. (1986a) estimated that on the average only 50% of the available H_2 was being utilized by the SRB. Their experiment was conducted at 35°C. Another three separate studies were cited by Isa et al. (1986a) that indicated MPB outcompeted SRB at SO_4^{2-} concentrations from 960 to 18,000 mg L^{-1}. A table was presented by Isa et al. (1986b) to support their contention that the COD to SO_4^{2-} ratio was very important in determining whether SRB would outcompete MPB. Choi and Rim (1991) observed that at COD:SO_4^{2-} (g g^{-1}) ratios from 1.7 to 2.7, there was active competition between MPB and SRB. Below this range, SRB were dominant, while MPB dominated at higher ratios. Prasad et al. (1991) found at COD:SO_4^{2-} ratios above 1, MPB predominated. This ratio plays a role in determining the outcome of the competition, and it must be kept in mind that two

factors are involved in the interpretation of this ratio. The first and most important is the stoichiometric sulfate requirements for SRB metabolism and second the kinetics of substrate utilization. The first takes precedent when the SO_4^{2-} concentration is limiting SRB growth, and the second when SO_4^{2-} is in excess. The table presented by the Verstraete group (Isa et al. 1986b) could also be interpreted to show that at high substrate concentrations MPB will outcompete SRB.

In another study, MPB were also found to outcompete the SRB at a volumetric loading of 18 g HAc L^{-1} d^{-1}, but at a volumetric loading of 1.3g HAc L^{-1} d^{-1} the SRB became the dominant microflora (Yoda et al. 1987). This research group estimated

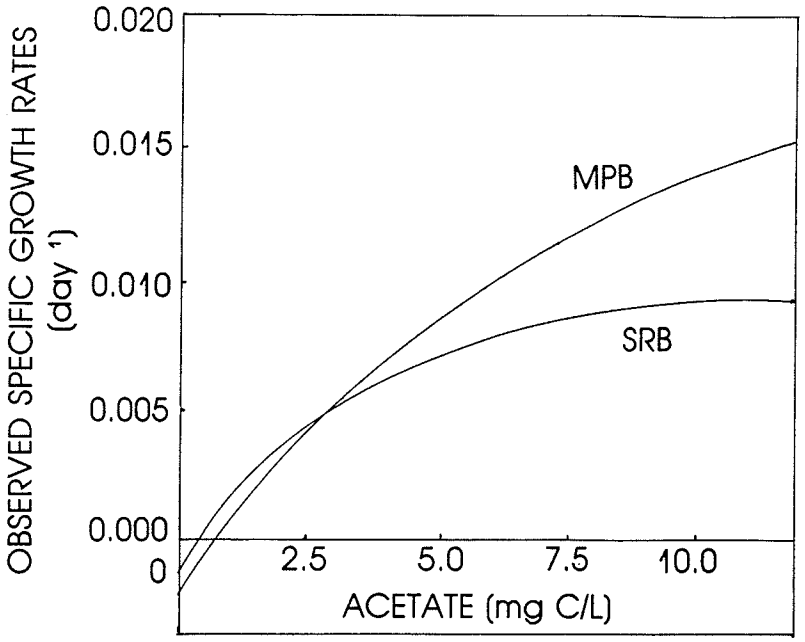


Figure 2-10 Observed growth curves for MPB and SRB in a biofilm (after Yoda et al., 1987).

the kinetic coefficients of acetate utilization in their study. They calculated V_m to be 507 and 257 μmol h^{-1} g^{-1} for MPB and SRB, respectively, with the respective K_m values determined to be 547 and 158 μmol L^{-1}. The growth curves for MPB and SRB from this study are presented in Figure 2-10. This illustrates the ability for MPB to outcompete SRB at an acetate concentration greater than 208 μmol L^{-1} (12.5 mg L^{-1}). Based on their observations (Figure 2-10) Yoda et al. (1987) recommended maintaining on acetate concentration of higher than 147 μmol L^{-1} (8.8 mg L^{-1}) in the bulk liquid of the reactor. Their reactors operated at 30°C. Isa et al. (1986b) reported that at an acetate concentration of 9 mmol L^{-1} (480 mg L^{-1}) the SRB began to increase significantly their share of the electron flow.

The investigations of Isa et al. (1986a) and Yoda et al. (1987) were carried out using attached growth reactors. Isa et al. (1986a) used a batch operation and Yoda et al. (1987) used a continuous feed system, with both systems utilizing recycle. Isa et al. (1986b) demonstrated the importance of differential bacterial attachment in maintaining a higher MPB population as compared to the SRB population. They reported that the MPB population was 200 times larger in the biofilm when compared to the effluent, while the population of the SRB was only 30 times higher in the biofilm. It was also found that by increasing the hydraulic retention time (HRT) of the reactor the percent of electron flow attributed to SRB increased. Both of these observations support their contention that SRB do not attach as readily to the support media, therefore are easily washed out. However, Thiele and Zeikus (1988) have suggested that SRB were performing syntrophic methanogenesis within the microniche of the aggregates (discussed in Section 2.2.2) because the bulk liquid SO_4^{2-}

could not penetrate into the aggregates. Whatever the mechanism, it indicates the advantage of biofilm reactors over suspended growth reactors when conditions exist which may allow SRB to compete with MPB for available substrate.

2.6.3 Rate Limiting Step

In theory the kinetic models of substrate utilization and bacterial growth should be developed for each specific substrate conversion. This is usually impossible unless simple soluble substrates are being treated. However, the cumulative effect of all the conversions will generally produce a smooth fit to a single equation (Stronach et al. 1986). The growth rate of bacteria can then be related to the substrate utilization rates using biokinetic constants. The population of each bacterial group will be in proportion to the amount of its particular substrate in the reactor system (Stronach et al. 1986). These series of bacterial or microbial groups form the catalysts which allow the activation energies of these biologically mediated reactions to be overcome.

The overall reaction rate will be determined by the reaction with the lowest rate. This is commonly referred to as the rate-limiting step. Hydrolysis is usually the rate-limiting step during complex wastewater treatment. However, if a simple soluble substrate is used, methanogenesis can be expected to be the rate-limiting step (Lin et al. 1989). There are important implications when one of the final reactions becomes the rate-limiting step. In the case of methanogenesis being the rate-limiting step, a buildup of acetate and/or H_2 can occur if the feed rate suddenly increases. These reaction products may then cause feed back inhibition or inhibit other

reactions, subsequently causing the process to fail. This problem can be avoided by basing the feed rate to the reactor on the rate-limiting step, and avoiding shock loadings.

A problem of more concern during operation is the potential for inhibition. An inhibitory substance can change the rate-limiting step of a process, resulting in the buildup of metabolic intermediates and eventually process failure. The final reactions of anaerobic digestion are thought to be the most sensitive to various inhibitory conditions. Studies into the effect of the end product of sulfate reduction, sulfide, on anaerobic processes have indicated that inhibition of propionate utilization occurs (Rinzema and Lettinga, 1988). Subsequently, propionate will build up in the reactor, which may lead to process failure if not rectified.

2.7 SULFIDE INHIBITION OF MPB AND SRB

According to Lawrence et al. (1966), the first documented report of methanogenesis inhibition due to SO_4^{2-} salts was reported in 1932. Then in 1952 it was reported that 200 mg L^{-1} sulfide had decreased gas production by 70%. As research progressed it became clear that there were many mechanisms which influenced the flow of electrons during the final reactions of anaerobic digestion. The competition between SRB and MPB for acetate and hydrogen has already been discussed in Section 2.5. The level of sulfide produced by SRB has usually been implicated in the inhibition of MPB and also SRB.

The most reduced form of sulfur, sulfide, can accumulate in anaerobic reactors from various sources. If SO_4^{2-} is present in a wastewater, it will be reduced to sulfide

by SRB. Organic sulfur is in the reduced form, therefore during anaerobic degradation of sulfur containing organics, sulfides will be released. This is analogous to ammonia release during degradation of proteinaceous wastes. However, organic sulfur concentrations are normally low in most wastewaters. Sulfide may also be in high concentrations in the influent.

The aqueous sulfide system is quite complicated due to the dissociation equilibria of soluble sulfide, the equilibrium between the gaseous and aqueous hydrogen sulfide and the solubilities of the various metal sulfides. The dissociation equilibria of soluble sulfide is pH dependent:

$$H_2S \overset{k1}{\leftrightarrow} H^+ + HS^- \overset{k2}{\leftrightarrow} H^+ + S^{2-} \qquad 2\text{-}13$$

The dissociation constant k_1 controls the species equilibrium at the normal pH of anaerobic reactors. The fraction of the total soluble sulfide (TS) which exists as hydrogen sulfide at 35°C is given by the equation (McCartney and Oleszkiewicz, 1990):

$$H_2S = (1 + 1.28 \cdot 10^{(pH-7)})^{-1} \cdot TS \qquad 2\text{-}14$$

This relationship is presented graphically in Figure 2-11. The equilibrium between the gaseous and the soluble TS is governed by the solubility of the un-ionized H_2S species, which is given by Henry's law similar to equation 2-12 for H_2. The equilibrium at 35°C can be expressed as:

$$P_{H_2S} = K_H[H_2S]_a \qquad 2\text{-}15$$

where

P_{H2S} = the partial pressure of H_2S above the liquid (atm);

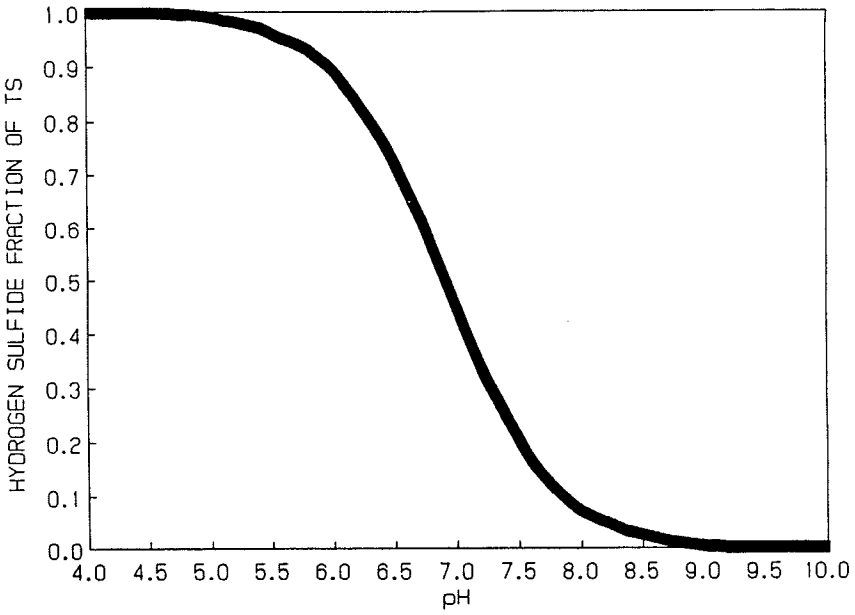


Figure 2-11 Effect of pH on hydrogen sulfide using equation 2-14, developed from the sulfide species equilibrium at 35°C.

K_H = absorption coefficient based on Henry's law (0.0121 atm mM^{-1}); and

$[H_2S]_a$ = the aqueous H_2S concentration (mM).

The main ions capable of precipitating metals in anaerobic reactors are S^{2-}, CO_3^{2-} and to a lesser extent PO_4^{3-} ions. The species concentrations of the sulfide and carbonate ions at a typical reactor pH of 7.3 are given in Table 2-5 (after Callander and Barford, 1983). The most important information from this table is the low fraction of S^{2-} and CO_3^{2-} at pH 7.3. In normal environments this allows the maintenance of microbially useful levels of the ions in Table 2-5 and also metal availability as nutrients. Sulfides can also play the role of heavy metal detoxification by precipitating out large concentrations of heavy metals. According to Tursman and Cork (1989), Lawrence and McCarty (1965) unequivocally demonstrated this effect.

Therefore, in all cases the presence of sulfide should effectively reduce the soluble metal concentration well below its toxic threshold (Tursman and Cork, 1989).

For cellular organisms, it is the neutral H_2S that effects the direct toxic actions because the ionic species are prevented from penetrating the cell membrane, unless there is a specific active transport system (Tursman and Cork, 1989). Therefore driven by osmotic gradients, H_2S readily permeates the membrane, and once inside the cytoplasm, H_2S may denature native proteins through the formation of sulfide and disulfide cross-links between the polypeptide chains (Conn et al. 1987). However, this theory does not support the observations of Parkin et al. (1980). These researchers stated that the recovery from sulfide inhibition was reversible. The H_2S may also interfere with the various coenzyme A and M sulfide linkages causing inhibition of these key metabolic proteins. The acetyl coenzyme A pathway for CO_2 fixation is common to SRB and MPB (Stouthamer, 1988). Hydrogen sulfide may also interfere with the assimilatory metabolism of S, but unfortunately the assimilation pathways are still obscure (Vogels et al. 1988).

It has been demonstrated that anaerobic reactors can recover from sulfide inhibition. At a shock concentration of 15.2 mM (500mg L^{-1}) TS, Parkin et al. (1980) showed that CH_4 production was inhibited 100%, but completely recovered 10 days after the TS concentration was lowered. The same researchers demonstrated that methanogenesis recovery could occur even after a 4 d exposure to 45.5 mM (1500 mg L^{-1}) of TS. The operating pH level was not reported in this investigation. They concluded that larger biological solid retention times and lower hydraulic retention times would help protect against toxic shock loadings. More recent work by Parkin

et al. (1990) has found that biological solids retention time and various organic loading rates did not significantly affect sulfide inhibition.

Table 2-5 Sulfide and carbonate ion distribution at pH 7.3 and 25°C (after Callander and Barford, 1983).

Species	Percent of total (%)
H_2S	33
HS^-	67
S^{2-}	0.00017
CO_2	11
HCO_3^-	89
CO_3^{2-}	0.089

Hydrogen sulfide concentrations higher than 1.75 mM (56 mg L^{-1}) were observed to inhibit cellulose degradation to methane (Khan and Trottier, 1978). These researchers rated the various sulfur compounds for inhibition potential and found that H_2S > TS > sulfite > thiosulfite > sulfate. Van den Berg et al. (1980) also reported that low concentrations of sulfate were stimulatory to the microbial conversion of acetic acid to CH_4.

Kroiss and Wabnegg (1983) reported a 50% inhibition of methanogenesis from acetate at a H_2S concentration of 1.7 mM (55 mg L^{-1}). They used inoculum from an upflow sludge blanket reactor in a completely mixed reactor. These same researchers developed a model for predicting H_2S toxicity concentrations from a given set of

operating data (Kroiss and Plahl-Wabnegg, 1984). The model was calibrated and verified using data from a full-scale plant treating sugar factory wastewater and also from lab-scale experiments using citric acid wastewater. The model predicts that below a COD:SO_4^{2-} (g g^{-1}) ratio of 33 in the influent, problems with H_2S toxicity will arise. Isa et al. (1986a) reported a 50% inhibition of methanogenesis at an extraordinarily high H_2S concentration of 35.3 mM (1200 mg L^{-1}) using both an acetate substrate and an acetate/ethanol substrate. These researchers also reported that H_2S stripping gave only slightly higher SO_4^{2-} removals that were not statistically significant. Another research team concluded that increased COD removal efficiency and sulfate reduction was achieved using an H_2S-stripped reactor (Oleszkiewicz and Hilton, 1986; Hilton and Oleszkiewicz, 1987). Anaerobic upflow sludge bed reactors fed whey and waste sulfite liquor were used in their investigation. The two latter observations of Isa et al. (1986b) seem to indicate that H_2S is not very toxic to MPB or SRB. They attributed this lack of sensitivity to H_2S as being due to the adaptation of the MPB to H_2S in the attached film reactor. Other researchers (Koster et al., 1986) have suggested that a pH or sulfide gradient may exist in the biofilm, which limits the H_2S concentration inside the biofilm.

Koster et al. (1986) reported a 50% MPB activity inhibition at an H_2S concentration of 7.4 mM (250 mg L^{-1}) or a TS concentration of 25.8 mM (825 mg L^{-1}) depending on the pH range. They used 1.16 L serum bottles and biomass inoculum from an upflow anaerobic sludge blanket reactor treating potato waste. Acetate was used as a substrate. The MPB activity was found to be dependent on H_2S concentrations at a low pH (Figure 2-12b) and on TS concentrations at a high pH

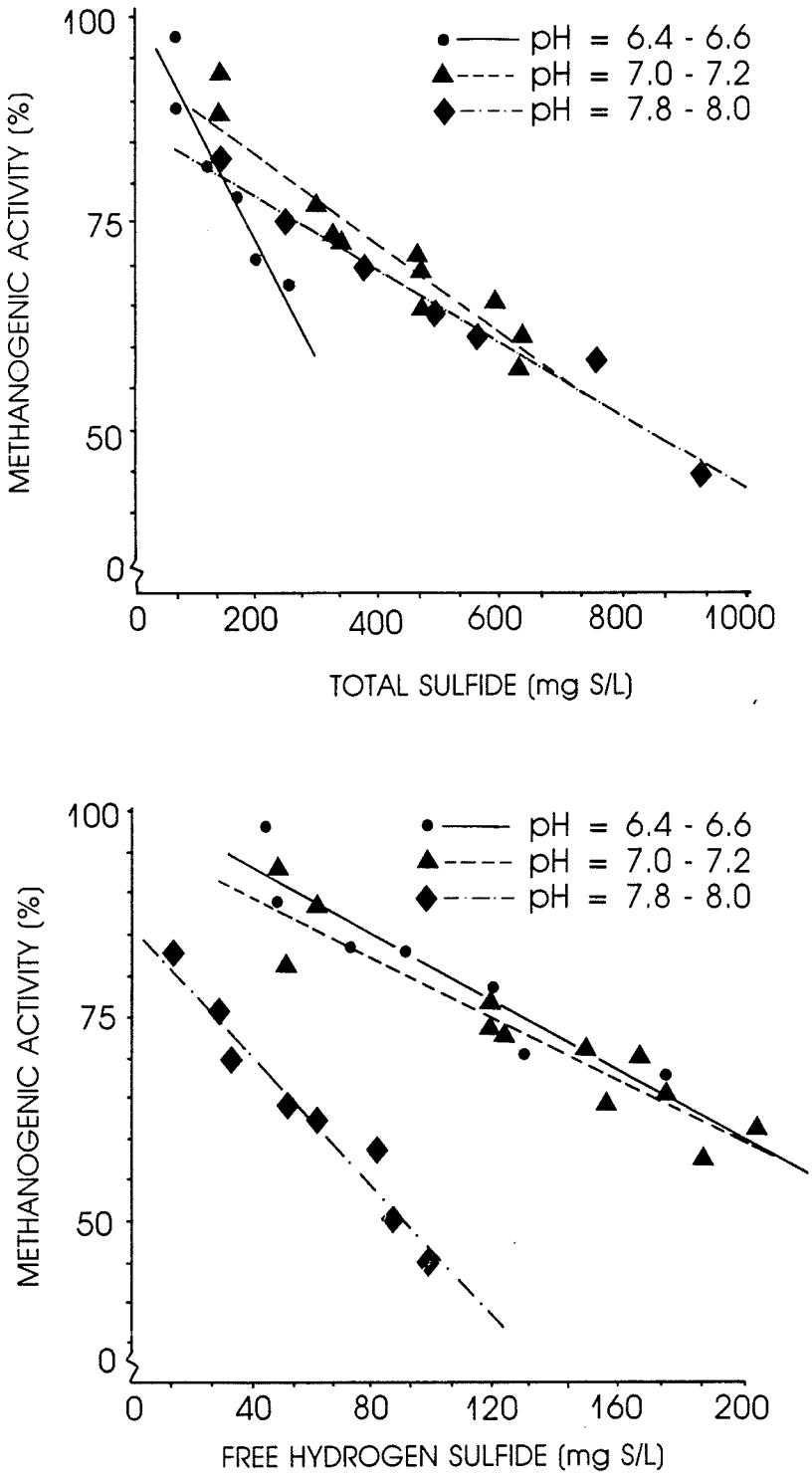


Figure 2-12 The maximum specific methanogenic activity (expressed as percentage of the uninhibited maximum specific methanogenic activity at the concomitant pH range) of acetate-fed granular sludge incubated at 30°C as a function of: A = TS; and B = free hydrogen sulfide (UIS) (after Koster et al., 1986).

(Figure 2-12a). They attributed the latter phenomenon to either an inhibitory effect of HS^-, which becomes apparent only at increased concentrations or by an increased sensitivity to H_2S of the acetoclastic MPB near the limit of their physiological pH range (Koster et al. 1986). A factor which may have influenced this observation is that the MPB were not acclimated to the high pH environment. Their methods indicate that NaOH was used for pH adjustment. This would indicate that the pH was adjusted up from a lower value, presumably around 7.1 (data not given). This may have effected the MPB activity. The linear regression lines for the pH 7.8 to 8.0 reactors intersects the vertical activity axis at 85%, while those for the lower pH sets intersect at close to 100% activity (Figure 2-12a, b). This would indicate a background inhibition exists in the high pH set at low sulfide concentrations. However, moving the high pH line in Figure 2-12b so that it passes through 100% activity still does not account for the difference in activity when compared to the lower pH lines. The high pH line is steeper, which supports the authors' contention that another factor besides H_2S is affecting this reactor set.

Using a synthetic distillery wastewater Karhadkar et al. (1987) observed a 50% CH_4 production inhibition at a TS concentration of 3.13 to 7.0 mM (100 to 224 mg L^{-1}) which corresponded to a headspace gaseous H_2S of 5%. According to Henry's law at 37°C this corresponds to an aqueous H_2S concentration of approximately 4.13 mM (140 mg L^{-1}); however, equation 2-14 estimates the H_2S concentration to be 1.13 to 2.52 mM (38.3 to 85.8 mg L^{-1}) based on a reactor pH of 7.1. Their work indicated how difficult it was to achieve a desired concentration of aqueous sulfide in batch systems. The measured sulfides were 20 to 50% less than the sulfides which had been added

to the reactors. They attributed the difference to precipitation with metals and the gaseous H_2S equilibrium. It was also noted that reactors with 1.25 and 2.5 mM (40 and 80 mg L^{-1}) TS performed better than the reactors without sulfide addition. This would indicate that sulfide was growth limiting as is the author's contention (Karhadkar et al. 1987), or that the added sulfide helped lower the oxidation-reduction potential of the media. Rinzema and Lettinga (1988) observed that H_2S concentrations of above approximately 2.94 mM (100 mg L^{-1}) caused a sharp drop in propionate removal efficiency. This contrasted their earlier work which used the same biomass and found a 50% inhibition of acetoclastic methanogenesis at approximately 7.35 mM (250 mg L^{-1}) H_2S (Koster et al. 1986). This points to the sensitivity of propionate degradation to elevated sulfide concentrations. They found that inhibition would not occur provided the H_2S concentration was kept below 2.94 mM (100 mg L^{-1}) no matter what the TS concentration was (Rinzema and Lettinga, 1988), therefore implicating H_2S as the inhibitory species of sulfide.

A study by Hilton and Oleszkiewicz (1988) concluded that SRB were inhibited in proportion to the TS concentration and not the H_2S concentration, while acetate utilization (MPB) was inhibited more by H_2S than TS. They observed that in the presence of high TS and at a high pH the electron flow was via the methanogenic pathways, while at lower pH values both the MPB and SRB were inhibited.

At COD:SO_4^{2-} ratios of 1.7 to 2.7, MPB and SRB were observed to be in competition for available substrate (Choi and Rim, 1991). These researchers reported that MPB were inhibited at total sulfide concentrations of 3.75 to 4.38 mM (120 to 140 mg L^{-1}), while SRB were inhibited at concentrations of 5.0 to 6.25 mM (160 to 200 mg

L^{-1}). The sulfide inhibition of both groups was reported to be dependent on organic loading rates and the type of substrate used; for example, MPB were not inhibited at sulfide concentrations of 7.5 mM (240 mg L^{-1}) when fed seafood waste (Choi and Rim, 1991). These researchers did not distinguish between ionized and un-ionized sulfide in their work. The seafood waste reactor operated at a pH of 7.3, while the control reactor operated at a pH of 7. The un-ionized sulfide (H_2S) concentrations were calculated (Equation 2.14) to be 52.6 to 61.4 mg L^{-1} and 67.5 mg L^{-1} for the control and seafood waste reactors, respectively. This indicates that un-ionized sulfide concentrations may have been responsible for these observed inhibition differences, rather than a dependence on substrate type.

Parkin et al.(1990) concluded that during sulfide inhibition, the most important relationship was COD:SO_4^{2-}. They observed that loading rates or SRT did not affect inhibition concentrations, if COD:SO_4^{2-} kept constant. Hydrogen sulfide concentrations, which resulted in process failure, were 1.94 mM (62 mg L^{-1}) and 1.88 mM (60 mg L^{-1}) for acetate and propionate fed chemostats, respectively. Parkin's research group (Parkin et al., 1990) also reported that SRB shut down before MPB. Prasad et al. (1991) reported that at COD:SO_4^{2-} ratios of < 1, sulfide inhibition of SRB and MPB was similar.

2.8 RELEVANT MODELS

Engineers and scientists have always strived to understand fully the mechanisms which affect the inhibition of microbial processes. Models have been developed for product inhibition kinetics. The majority of these models related to

inhibition caused by organic products. Mulchandani and Luong (1989) presented a critique of these various models. Parkin and Speece (1982) discussed various inhibition models developed for anaerobic digestion processes. These models dealt mainly with toxic compounds and the effects these compounds had on methanogenic reaction kinetics. Kroiss and Plahl-Wabnegg (1984) developed a model for sulfide toxicity to anaerobic digestion based on COD and sulfate loading, as follows:

$$P^2_{H_2S} \cdot \frac{C_s}{f} - P_{H_2S}[0.5 n \cdot S_o + \frac{C_s}{f}(1-P_{co_2})] + S_s + n_s(1-P_{CO_2}) = 0 \qquad 2\text{-}16$$

where P_{H_2S,CO_2} = partial pressures of H_2S and CO_2 (atm);

C_s = saturation concentration of H_2S at 1 atm of H_2S, which is a function of temperature (mole L^{-1});

f = free aqueous H_2S fraction of TS, which is a function of pH (fraction);

n = COD removal efficiency (fraction);

n_s = sulfate reduction efficiency (fraction);

S_o = molar COD influent concentration (mole L^{-1}); and

S_s = molar sulfate influent concentration (mole L^{-1}).

Various experimental (bench-scale) and full-scale results were found to fit the results predicted by the model. The model predicts that for COD to sulfate ratios below 30, the problem of H_2 S inhibition becomes important (Kroiss and Plahl-Wabnegg, 1984). The model also predicts that raising the pH (reducing f) within the pH range 7.5 to 8.0, will increase the COD removal efficiency.

2.9 SUMMARY

The degradation of complex wastes during anaerobic treatment was shown to be a complicated web of reactions mediated by several different species of bacteria. Even the details of the reactions involving the formation and consumption of the immediate precursors to methane formation were found to be complicated and variable depending on the environmental and operating conditions of the reactors. The key precursors to methane formation are hydrogen and acetate. The SRB have been shown to outcompete the MPB for both of these energy sources at the low substrate concentrations found in lake sediments, but MPB have been observed to outcompete SRB for acetate in most wastewater reactors. The SRB definitely outcompete MPB for H_2, but the degree of this competition seems to depend strongly on the operating conditions of the reactor. The competition mechanisms were qualified based on the reaction characteristics such as, thermodynamic free energies of formation, and the kinetic coefficients V_M and K_M. Other less qualified characteristics are the effects of sulfide inhibition on MPB and SRB, and the role of diffusion in mediating various processes. Differential diffusion rates create micro environments within the biofilms, which have different conditions than the bulk liquid. Some of the suggested effects not yet supported by direct experimental evidence are lower SO_4^{2-} and sulfide concentrations, and elevated pH. The former will allow MPB to outcompete the SRB, while the latter two allow both groups to avoid sulfide inhibition. Biofilm reactors have been reported to be able to tolerate an order of magnitude higher H_2S concentrations when compared to suspended growth reactors.

The important role of hydrogen was highlighted, not only in competition mechanisms, but also in mediating syntrophic degradation reactions. The hydrogen-forming reactions are dependent upon syntrophic interspecies hydrogen and/or electron transfer to pass the reduced intermediates to hydrogen-consumers. If the delicate balance between these two trophic groups is upset, hydrogen may build up, which inhibits the hydrogen-forming reactions at very low concentrations. The buildup of either propionate or butyrate in reactors was identified as an indication of this type of inhibition. Another key observation was that hydrogen-consuming SRB are able to keep lower concentrations of hydrogen in a reactor as compared to their MPB analogs. This allows the syntrophic hydrogen-formers to obtain more energy from their substrates. Direct evidence was presented which indicated that SRB play a key role in propionate reduction. It has yet to be identified whether the key role of SRB is to cleave propionate or to remove hydrogen from the system or both.

The majority of research has pointed to H_2S as being the inhibiting form of sulfide. It has been suggested that this un-ionized form can readily penetrate biofilms and cell membranes via osmotic forces. Propionate degradation was shown to be particularly sensitive to elevated hydrogen sulfide concentrations in one experiment. MPB and SRB were observed to be equally sensitive to un-ionized sulfide. It has also been observed that SRB were more sensitive to TS than MPB, while both were sensitive to H_2S. The TS sensitivity suggests thermodynamic inhibition of SRB reactions. The thermodynamic inhibition occurs due to product buildup in much the same way that hydrogen-formers are inhibited by hydrogen

buildup. The COD:SO_4^{2-} ratio and pH were identified as important parameters when studying competition between MPB and SRB, as well as sulfide toxicity.

Chapter 3

RESEARCH OBJECTIVES AND TECHNIQUES

The primary objective of the investigation was to determine the limiting substrate utilization rate in artificially elevated sulfide environments. The investigation consisted of four experimental phases. Phase 1 was a general screening experiment, in which the utilization rates of the immediate precursors to methane formation were investigated. Directions for Phases 2 to 4 were developed from the observations of each preceding phase, i.e., Phase 1 gave direction to phase 2, and so on. Phases 2 to 4 focused on the utilization of lactate and acetate. The presence of (or lack of) propionate as an intermediate during lactate degradation was also investigated. The specific objectives and techniques of each phase are discussed in this chapter.

In all phases, biomass was acclimated using breeder reactors. Inoculum was then taken from the breeders for serum bottle reactor sets. The serum bottles were used as single-run batch reactors. Phase 1 used a single breeder reactor and seventy serum bottle reactors. Phase 2 used a single breeder reactor and thirty serum bottle reactors. Phase 3 used four breeder reactors and twenty-eight serum bottle reactors. Phase 4 used four breeder reactors and twenty-four serum bottle reactors. In total, 160 reactors were operated, with 10 of these being operated semi-continuously and the rest batch.

3.1 PHASE 1--OBJECTIVES AND TECHNIQUES

Phase 1 was designed as a screening experiment to investigate the various results reported in the literature. Sulfides have been reported to have a significant effect on the degradation of simple organic compounds. Therefore, the immediate organic precursors to methane were selected for this phase. The precursors selected were two even-numbered carbon compounds, acetate and butyrate, and two odd-numbered carbon compounds, propionate and racemic lactate. The objective was to observe the difference in the effects of UIS and TS on the degradation of these organics in a matrix of batch serum bottle reactors.

3.1.1 Experimental Techniques

The serum bottle experimental technique was developed from that of Shelton and Tiedje (1984) and Owen et al. (1979). The technique is outlined in Table 3-1. The formulations of the stock solutions used in the technique are given in Table 3-2. Strict anaerobic conditions were maintained during all transfers. The pH of the serum bottle inoculum (500 mL anaerobic biomass mixed with 500 mL of nutrient media) was adjusted to 6.5 or 7.7 using hydrochloric acid or sodium hydroxide solutions. The final concentration of inorganic feed supplements in each serum bottle is given in Table 3-3. Each single serum bottle reactor consisted of two serum bottles which were connected via needles and tubing (Figure 3-1). The needles were left in the system throughout the experiment. One bottle served as the reactor, while the other bottle was a gas container. The gas container was filled with N_2 gas and sealed under water to avoid oxygen contamination. The construction avoided venting the

Table 3-1. Phase 1 serum bottle start-up technique

Step	Description
1	Add 400 mL deionized water to 1 L erlenmeyer flask
2	Add: - 5 mL of S1 or S2, depending on pH required - 13.5 mL of S3
3	Bring to boil while flushing with N_2 gas
4	At the same time, boil and gas (N_2) 1 L of deionized water
5	Cool solutions to 15°C and continue to flush with N_2 gas
6	Add: - 1 mL of S4 - 0.5 mL of S5 - 1 mL of S7 - 5 g of $NaHCO_3$ as powder - 10 mL of S11 - required volume of S6
7	Add 500 mL of breeder organisms and bring volume to 1 L with previously boiled and cooled deionized water. Continue to flush with N_2 gas.
8	Adjust pH to either 6.5 or 7.7.
9	Using a 100 mL syringe, transfer 100 mL into each serum bottle.
10	Add 1 mL of S8, S9, or S10 (organic substrates) to each serum bottle, using syringe.
11	Bring bottles to 35°C and equilibrate gas pressure to atmospheric.
12	Incubate bottles at 35°C.

Table 3-2. Phase 1 stock media solutions used.

Solution No.	Compound and Description	Concentration (gL^{-1} except where indicated)
S1	pH 6.5 buffer (3 mM)	
	K_2HPO_4	52.2
	KH_2PO_4	81.6
S2	pH 7.7 buffer (3 mM)	
	K_2HPO_4	65.2
	KH_2PO_4	8.2
S3	Dissolve 1.59 Nitrilotriacetic acid in 500 mL of pH 6.5 solution adjusted with 3 or 4 N KOH	
	$MgCl_2 \cdot 6H_2O$	120
	$CaCl_2 \cdot 2H_2O$	16.7
	H_3BO_3	0.74
	$MnSO_4 \cdot H_2O$	0.185
	$CoCl_2 \cdot 6H_2O$	0.740
	$CuSO_4 \cdot 5H_2O$	0.037
	$AlK(SO_4) \cdot 12H_2O$	0.006
	$Na_2MoO_4 \cdot 2H_2O$	0.170
	Na_2SeO_3 - anhydrous	0.004
	$NiCl_2 \cdot 6H_2O$	0.074
	$ZnCl_2$	0.14
	Na_2WO_4	0.4(mgL^{-1})
	NH_4Cl	26.6
S4	$FeCl_3 \cdot 6H_2O$	96.7
S5	Wolfe's Vitamin Solution (all in mg L^{-1})	
	Biotin	40
	Folic Acid	40
	Pyridoxine-HCl	200
	Riboflavin	10
	Thiamine-HCl	100
	Nicotinic Acid	100
	Pantothenic acid (Ca salt)	100
	V_{B12}-cyanocobalamine	2
	P-aminobenzoic acid	100
	Thioctic acid	100
S6	$Na_2S \cdot 9H_2O$	186.6

Table 3-2 (continued)

Solution No.	Compound and Description	Concentration
S7	Cysteine HCl	300
S8	NaOH	70.4 g
	Acetic acid (100% solution)	105.4 mL
S9	NaOH	38.0 g
	Propionic acid (100% solution)	70.2 mL
S10	NaOH	25.0 g
	Butyric acid (100% solution)	55.1 mL
S11	Na_2SO_4	221.5
S12	NH_4Cl	267.2

Table 3-3 Inorganic feed supplements in each serum bottle during Phases 1 and 2

Compound	mg added per 100 ml serum bottle volume
$Na_2S \cdot 9H_2$	76.5-721.5
Cysteine HCl	30.0
$NaHCO_3$	500.0
$K_2H\ PO_4$ - pH 7 medium	19.92
- pH 8 medium	44.92
$KH_2\ PO_4$ - pH 7 medium	25.23
- pH 8 medium	5.7
$FeCl_3 \cdot H_2O$	9.67
NH_4Cl	35.9
$MgCl_2 \cdot 2H_2O$	162.0
$CaCl_2 \cdot 2H_2O$	22.55
H_3BO_3	1.0
$MnSO_4 \cdot H_2O$	0.25
$CoCl_2 \cdot 6H_2O$	1.0
$CuSO_4 \cdot 5H_2O$	0.05
$AlK(SO_4)_2 \cdot 12H_2O$	0.008
$Na_2MoO_4 \cdot 2H_2O$	0.23
Na_2SeO_3	0.0054
$NiCl_2 \cdot 6H_2O$	0.1
$ZnCl_2$	0.189
Na_2WO_4	0.00054
Biotin	0.002
p-Aminobenzoie acid	0.005
Folic acid	0.002
Ca pantothenate	0.005
Nicotinic acid	0.005
Vitamin B12-cyanocobalamine	0.0001
Thiamine-HCl	0.005
Pyridoxine-HCl	0.01
Thioctic acid	0.005
Riboflavin	0.0005

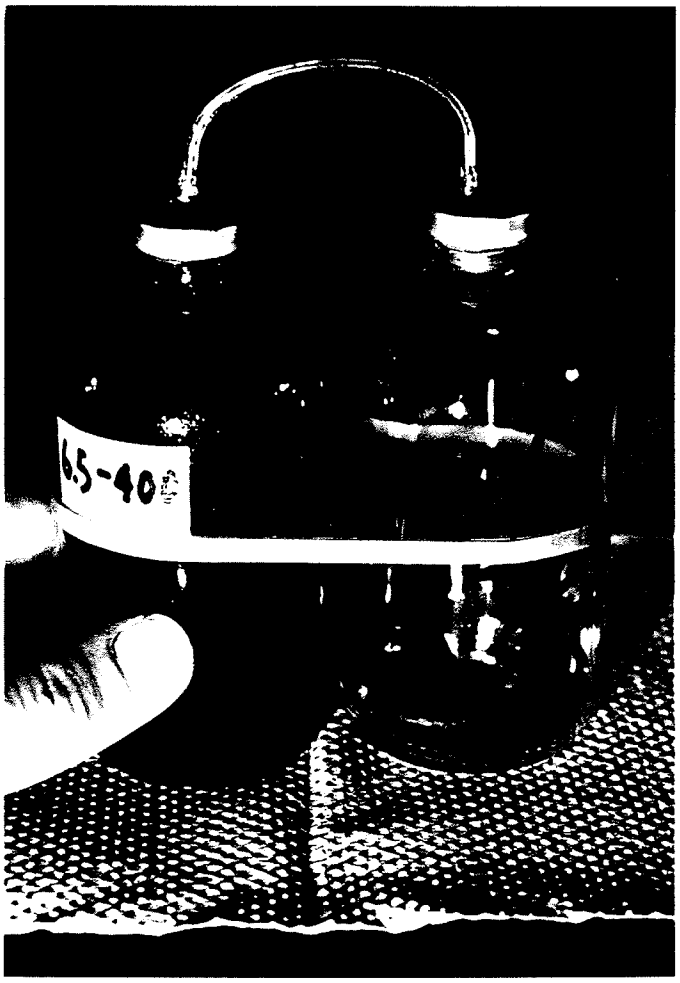


Figure 3-1 The two serum bottle reactor setups, where one bottle served as a reactor and the other bottle was used for gas storage. The reactors were connected via needles and tubing.

gaseous UIS, while minimizing the pressure buildup during subsequent gas production.

The anaerobic biomass was obtained from a 20L breeder reactor operating at 35°C. The breeder reactor was maintained on equal amounts (by mass) of butyrate, racemic lactate, propionate, and acetate. During each feeding, 4L of supernatant was withdrawn and replaced with 4 L of feed. The feed consisted of 20 g of COD using a mixture of the aforementioned organics. The total sulfate added each feeding was 0.0563 moles (5.4 g). Supplemental inorganic nutrients were added according to the pH 7.7 medium components and the lowest sodium sulfide concentration shown in Table 3-3.

In total, there were 5 sets of 14 serum bottles each. Each distinct serum bottle set was injected with 1 g COD per L at startup, using different starting COD sources, as follows:

1. Set B - butyrate
2. Set A - acetate
3. Set P - propionate
4. Set L - racemic lactate, and
5. Set AP - 50:50 mix of acetate and propionate

Each reactor set was divided into a low pH and a high pH subset, resulting in 7 reactors starting at pH 6.5 and 7 reactors starting at pH 7.7. The target starting TS concentrations in each subset (high and low pH) were 1.56, 1.56, 6.24, 12.48, 21.88, 31.25, and 56.25 mM (50, 50, 200, 400, 700, 1000, and 1800 mg L^{-1}).

Table 3-4 Serum bottle monitoring programs used in the four experimental phases

	Phase Number (Frequency*)			
Analysis	1	2	3	4
pH	S;E	S;E	S;M;E	S;M;E
lactate	S;D;E	S;D;E	S;D;E	S;D;E
acetate	S;D;E	S;D;E	S;D;E	S;D;E
propionate	S;D;E	S;D;E	S;D;E	S;D;E
butyrate	S;D;E	NA	NA	S;D;E
total sulfides (TS)	S;E	S;E	S;M;E	S;E
sulfate	S;E	S;E	S;D;E	S;D;E
hydrogen	NA	NA	D;E	D;E
gas production	E	E	E	E
gas composition	E	E	E	E

* S = Startup; E = End; D = Daily; NA = Not Analyzed;
M = Midway through experimental run

The serum bottles were monitored as described in Table 3-4. Samples were collected using a syringe and needle apparatus to pierce the system and extract a sample.

3.1.2 Analytical Techniques

Lactate was analyzed on a Waters HPLC equipped with a UV variable wavelength detector set at 210 nm and a Biorad HPX-85H column. The volatile acids (butyrate, propionate, and acetate) were analyzed on a Gow-Mac gas chromatograph equipped with a flame ionization detector. Sulfate and sulfide were analyzed in

accordance with the respective APHA et al. (1989) methods 4500-SO_4^2 F, and 4500-S^{2-} E. All the above samples were centrifuged in 1.5 mL microcentrifuge cups at high RPM for 5 minutes before analysis. A 1 mL sample of the supernatant was then removed for analysis. Only lactate samples were filtered; all other samples were not filtered before analysis.

The pH was measured according to APHA et al. (1989). Measurements were taken in the centrifuge cup as samples were discharged from the syringe used for sampling. Gas composition was analyzed in each serum bottle (reactor and gas container) using a Gow-Mac gas chromatograph with a series 550 thermal conductivity detector. Gas volume was determined in each serum bottle as well, using a pressure transducer apparatus similar to that described in Shelton and Tiedje (1984).

3.1.3 Experimental Apparatus

The following equipment was used during the serum bottle startup procedure:

- 160 mL Wheaton serum bottles complete with grey butyl rubber stops and aluminum seals
- serum bottle top crimper
- gas line bottle connections, each consisting of two cutoff syringe needles and tygon tubing
- 70 large rubber bands to hold serum bottles together
- 11 - 1 L erlenmeyer flasks
- 100 mL glass syringe
- 10 large magnetic stir bars
- magnetic stirrer
- hot plate
- peristaltic pump used for biomass transfer
- various sizes of graduated cylinders
- numerous 1.5 mL microcentrifuge sample cups (total depends on number of samples required)
- pH meter

- water bath
- ice
- various sizes of syringes

The breeder reactor consisted of a 20 L nalgene carboy. The biomass was mixed via a connection at the spigot to a line tapped in at the 10 L water level. Biomass was removed using this mixing line. Mixing was accomplished using a Cole-Parmer Masterflex peristaltic pump and a series 17 head set at 500 RPM (1.1 L per minute). The pump was turned on 5 minutes every hour. Gas flowed through a wet gas meter attached to the top of the carboy.

3.2 PHASE 2--OBJECTIVES AND TECHNIQUES

Phase 2 was designed to investigate further the apparent high sensitivity of propionate degradation. Since lactate is a known substrate for SRB and its degradation produces both propionate and acetate, it was used as a substrate. Acetate was also used as a substrate. The objective was to increase the understanding of the inhibition of the acetate utilization rate and the propionate utilization rate at various concentrations of TS and UIS. A secondary objective was to study the TS and UIS inhibition of SRB and MPB.

3.2.1 Experimental Techniques

The serum bottle experimental technique was modified from that used in Phase 2. The technique is outlined in Tables 3-5 and 3-6. The stock solutions used were identical to those reported in Table 3-2, except for S1 and S2. The solutions were changed to pH 7.0 and 8.0 buffer solutions, respectively. The resulting K_2HPO_4

Table 3-5. Nutrient preparation and serum bottle startup techniques for Phase 2. Stock solutions were as reported in Section 3.2.1 Recipe makes 2 L of both pH 7 and pH 8 nutrient solutions. Final serum bottle volume is 100 mL

Step	Description		
1.	Label two 2-L erlenmeyer flasks, pH 7 and pH 8. Flush flasks with N_2 gas, and add approximately 900 mL of deionized water to each. If possible, set up flasks on magnetic stirring hot plates. Continue to flush headspace with N_2 gas.		
2.	Cover flask tops with aluminum for and boil vigorously for 5 minutes. Purge with N_2 gas for 5 minutes, i.e., a total of 10 minutes boiling.		
3.	Add:	pH 7 - 10 mL S1 - 2 mL S4	pH 8 - 10 mL S2 - 2 mL S4
4.	Prepare pH 7 and pH 8 top-up water solutions (500 mL of each). Add 10 mL of S1, and S2 to pH 7 and pH 8 flasks, respectively. Follow step 2. Let flasks cool while continually gassing headspace.		
5.	Cool flasks in ice bath until temperature less than 50°C. Ensure headspace gassed with N_2 during this time.		
6.	Add:	pH 7 - 27 ml S3 - 1.0 mL S5 - 2 mL S7 - 40 mL S11 - 10 g $NaHCO_3$	pH 8 - 27 mL S3 - 1.0 mL S5 - 2.0 mL S7 - 40 mL S11 - 10 g $NaHCO_3$
7.	Cover 2 L flasks with aluminum foil and continue to gas headspaces. mix with magnetic stir-bar for 5 minutes.		
8.	Begin flushing ten 500 mL erlenmeyer flasks with N_2 gas, minimum of 10 minutes flushing. Ensure each flask has a 400 mL mark calibrated on it. Label each flask consecutively from 1 to 10.		
9.	Add one L of breeder inoculum into each 2-L flask. Bring volume of 2-L flasks to two L with top-up water prepared in step 4.		
10.	Transfer 400 mL from 2-L flasks into 500 mL flasks. Each of the ten flasks will be used for the standard sulfide concentration across the three reactor sets.		
11.	Add required sulfides into each 500 mL flask as outlined in Table 3-6.		

Table 3-5. (continued)

Step	Description
12.	Adjust pH to 7.0 for flasks labelled 1 to 5 and to pH 8.0 for flasks labelled 6 to 10.
13.	Begin flushing serum bottles with N_2 gas, minimum of ten minutes.
14.	Using a 100 mL syringe and tubing, transfer 100 mL of nutrient preparation into each respective serum bottle, while flushing with N_2 gas, and seal.
15.	Shake well and let stand for one hour at room temperature, then vent to atmospheric pressure.
16.	Sample for pH, TS, sulfate, and organics.
17.	Add organics using a 1 mL syringe, based on S8 and S9 solutions in Table 3-2.

Table 3-6. Sulfide solution requirements for Phase 2 serum bottles

Serum Bottle Number in Set	pH	Sulfide Concentration	Stock Solution (mL)* required	multiplier	added
1	7	100		2.0	
2	7	200		2.0	
3	7	300		2.2	
4	7	500		2.4	
5	7	800		2.6	
6	8	100		1	
7	8	200		1	
8	8	300		1.2	
9	8	500		1.5	
10	8	800		1.6	

* Stock sulfide solution: 25 mg S mL^{-1}

and KH_2PO_4 concentrations were 39.84 and 50.46 gL^{-1} for the pH 7 solution (S1) and 89.84 and 11.4 gL^{-1} for the pH 8 solution (S2). The final concentration of inorganic feed supplements to each serum bottle is given in Table 3-3. The serum bottle reactors were operated as described in Section 3.1.1 and pictured in Figure 3-1.

The anaerobic biomass was obtained from a 20 L breeder reactor, as described in Section 3.1.1. The reactor was fed 1 g COD per L reactor volume once per week. During each feeding, 4 L of supernatant was withdrawn and replaced with 4 L of feed medium. The feed medium contained 20 g of COD using a 1:1 COD mixture of acetate and racemic lactate, or 0.156 and 0.112 moles (2.35 and 10.1 g), respectively. The total sulfate added each feeding was 0.0563 moles (5.4 g). Supplemental inorganic nutrients were added according to the pH 8 medium components and lowest sodium sulfide concentration shown in Table 3-5. When the biomass was taken from the breeder, the pH was 8.1 (7.8 to 8.3), the gas production was 630 mL gas per g COD added, and the TS concentration was 14.4 mM (475 mg L^{-1}). Biogas production was determined from the cumulative totals of both gas and COD addition during the 62 days preceding Phase 2.

Three sets of serum bottles were used. Set 1 was started with racemic lactate, set 2 was started with acetate, and set 3 was started with a 1:1 COD mixture of lactate and acetate. Each set contained ten serum bottles and consisted of two subsets of five serum bottles each. One subset was started at pH 7, the low pH subset, and the other subset was started at pH 8, the high pH subset. The target startup TS concentrations were 3.1, 6.2, 9.3, 15.5, and 31 mM (100, 200, 300, 500, and 1000 mg L^{-1}) for each subset.

The serum bottles were monitored as described in Table 3-4. Samples were collected using syringe and needle apparatus to pierce the system and extract the sample.

3.2.2 Analytical Techniques

All analyses were conducted as described in Section 3.1.2.

3.2.3 Experimental Apparatus

The equipment and apparatus described in Section 3.1.3 was used for this experimental phase, except that 2 L and 500 mL erlenmeyer flasks were substituted for the 1 L flasks.

3.3 PHASE 3--OBJECTIVES AND TECHNIQUES

Phase 3 was designed to eliminate the effects of pH adjustment on the lactate degradation processes observed in Phases 1 and 2. The role of H_2 as a reaction mediator was also to be investigated. Breeder reactors were used to acclimate biomass to high pH and low pH conditions.

The objective was to repeat the experiment conducted in Phase 2 using two sources of inoculum, which were acclimated to high and low pH conditions. Also, the addition of daily H_2 and sulfate monitoring would allow the role of H_2 and SRB to be quantified. It was suspected that hydrogenotrophic SRB were inhibited by TS in Phase 2, resulting in the buildup of H_2 which consequently inhibited the obligate hydrogen forming acetogens. This would account for the propionate buildup

observed in Phase 2 (Figure 3-2). Another possibility was the direct inhibition of the acetogens (Figure 3-2B).

3.3.1 Serum Bottle Experimental Techniques

The serum bottle experimental technique was modified from that used in Phase 2. The method is outlined in Figure 3-3 and Table 3-7 and 3-8. The stock solutions used were as described in Section 3.2.1, except for S3. The new recipe for S3 is presented in Table 3-9. As with the two previous Phases, each serum bottle reactor consisted of two serum bottles. The serum bottle reactors used in Phase 3 are shown in Figure 3-4. In total, there were four sets of seven serum bottles each--one set for each breeder reactor. All serum bottles were injected with 1 g COD of L(+) lactate per litre at startup. Two sets were started at pH 7 and two at pH 8. The target starting UIS concentrations were 1.56, 2.19, 2.81, 3.75, 5.0, 6.25, and 7.50 mM (50, 70, 90, 120, 160, 200, and 240 mg L^{-1}) in each set. This resulted in target TS concentrations of 3.59, 5.0, 6.41, 8.59, 11.31, 14.38, and 16.88 mM (115, 160, 205, 275, 365, 460, and 540 mg L^{-1}) in the pH 7 sets and 21.41, 30, 38.44, 51.56, 68.75, 86.25, and 103.44 mM (685, 960, 1230, 1650, 2200, 2760, and 3310 mg L^{-1}) in the pH 8 sets. The anaerobic biomass was taken from the breeder reactors.

The serum bottles were monitored as described in Table 3-4. Samples were collected using syringe and needle apparatus.

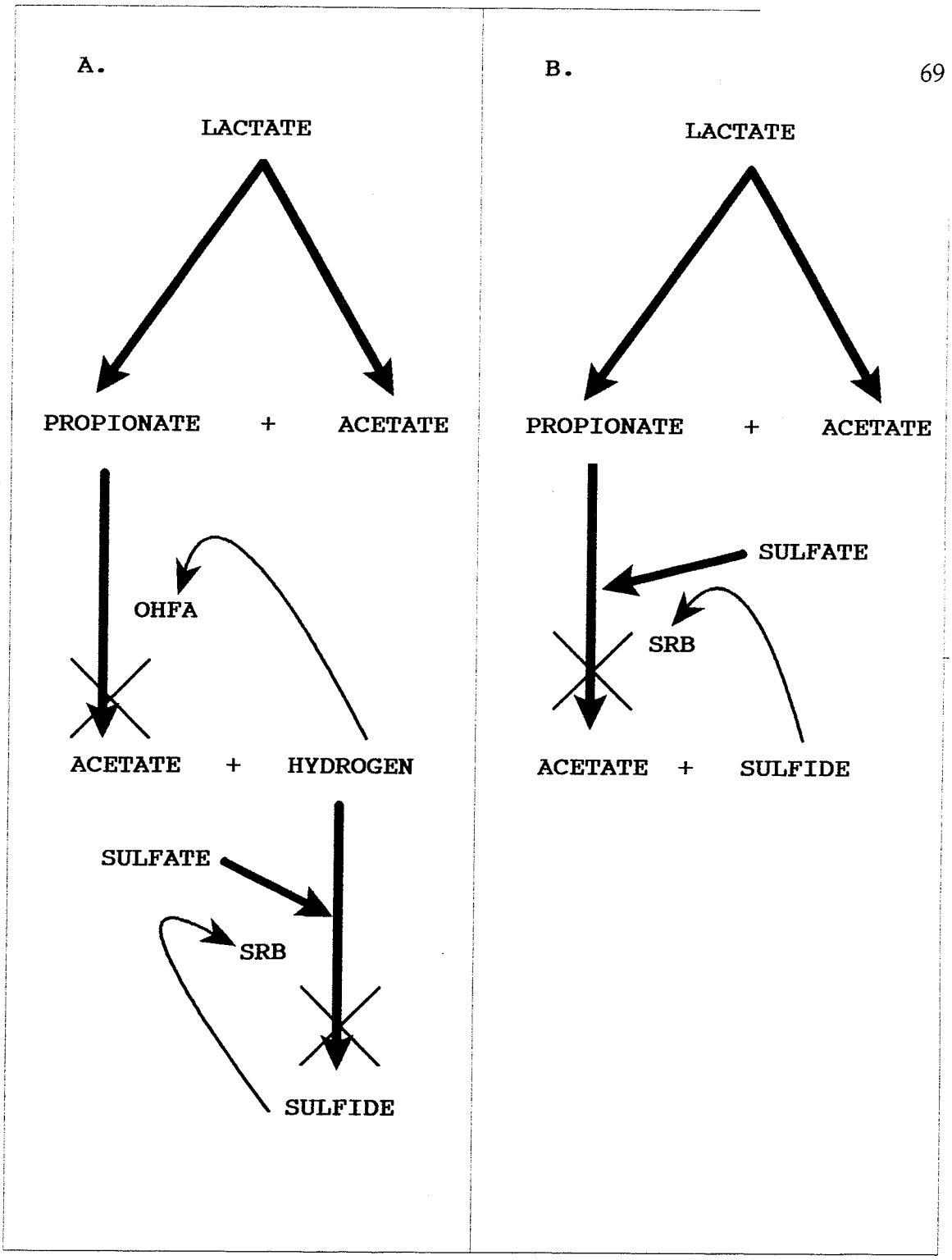


Figure 3-2. Possible inhibition mechanisms which fit the observations of Phase 2.
A. UIS inhibits the hydrogenotrophic SRB. The buildup of H_2 then inhibits the OHFA, which results in propionate to buildup.
B. UIS inhibits the incomplete oxidizing SRB responsible for propionate degradation.

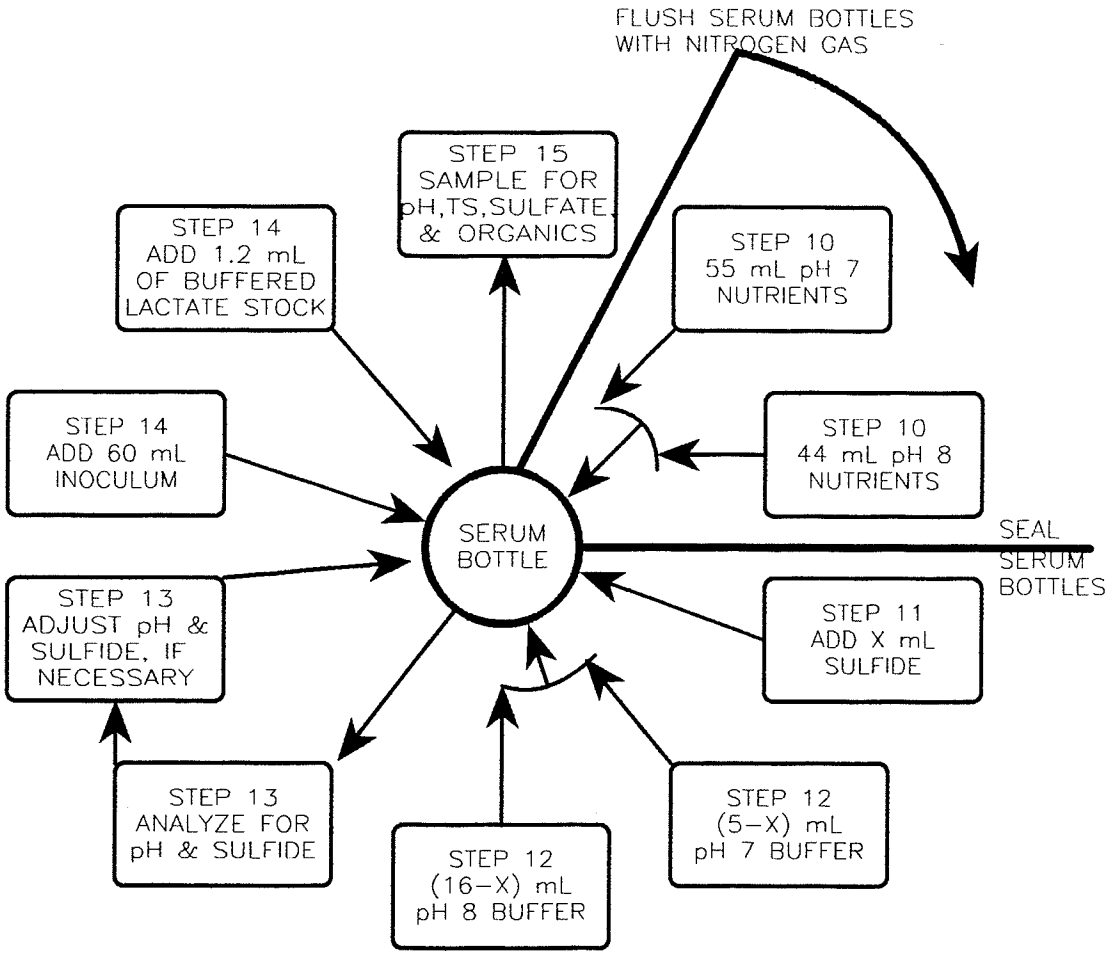


Figure 3-3. Outline of serum bottle startup technique used in Phase 3.

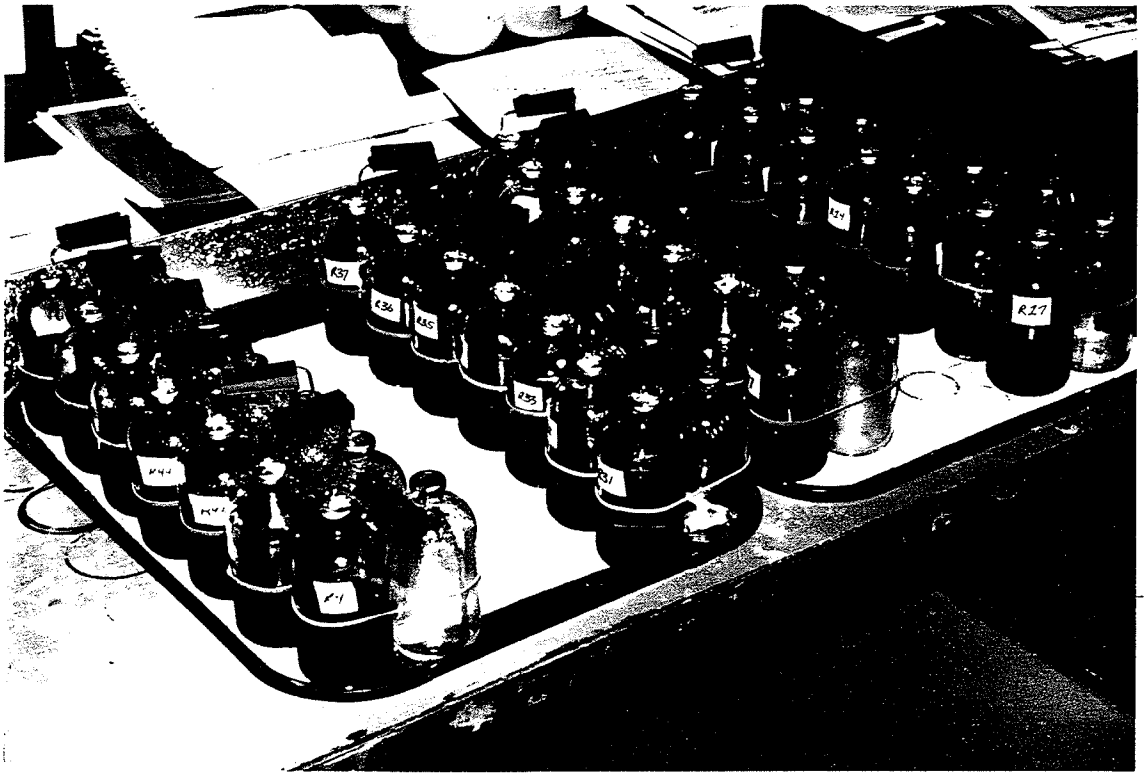


Figure 3-4. Serum bottle reactors used in Phase 3. Red clamps were used during gas volume and composition analysis.

Table 3-7. Nutrient preparation and serum bottle startup techniques for Phase 3. Stock solutions were as reported in Section 3.2.1 Recipe makes 1 L of both pH 7 and pH 8 nutrient solutions. Final serum bottle volume is 120 mL

Step	Description
1.	Label two 2-L erlenmeyer flasks, pH 7 and pH 8. Flush flasks with N_2 gas, and add approximately 700 mL of deionized water to each. If possible, set up flasks on magnetic stirring hot plates. Continue to flush headspace with N_2 gas.
2.	Cover flask tops with aluminum for and boil vigorously for 5 minutes. Purge with N_2 gas for 5 minutes, i.e., a total of 10 minutes boiling.
3.	Add: pH 7: - 22 mL S1; - 3.3 mL S4; - 363 mg K_2SO_4; - 291 mg Na_2SO_4. pH 8: - 27.3 mL S2; - 4.1 mL S4; - 455mg $K_2 SO_4$; - 365 mg Na_2SO_4
4.	Prepare pH 7 and pH 8 top-up water solutions (250 mL of each). Add 5 mL of S1, and S2 to pH 7 and pH 8 flasks, respectively. Follow step 2. Let flasks cool while continually gassing headspace.
5.	Cool flasks in ice bath until temperature less than 50°C. Ensure headspace gassed with N_2 during this time.
6.	Add: pH 7: - 65.4 ml S3; - 2.7 mL S5; - 10.9 mL S7. pH 8: - 81.9 mL S3; - 3.4 mL S5; - 13.7 mL $NaHCO_3$
7.	Cover 2 L flasks with aluminum foil and continue to gas headspaces. mix with magnetic stir-bar for 5 minutes.
8.	Begin flushing ten 500 mL erlenmeyer flasks with N_2 gas, minimum of 10 minutes flushing.
9.	Adjust pH of 1-L flasks to pH 7 and pH 8, if necessary. Bring 1-L flasks to volume (1 L) with respective buffered top-up solutions prepared in step 4.
10.	Add 55 mL of pH 7 solution to each Reactor 1 and 3 serum bottle, and 44 mL of pH 8 solution to each Reaction 2 and 4 serum bottle. Seal the serum bottles.
11.	Referring to Table 3-7, add required sulfide solution to each serum bottle.

Table 3-7 (continued)

Step	Description
12.	Top up each serum bottle with respective top-up water solutions, based on equations in Figure 3-3 and values in Table 3-7.
13.	Analyze each serum bottle for pH and TS, and adjust, if necessary.
14.	Add 60 mL inoculum from respective breeder reactors into each serum bottle. Add 1.2 mL of pH 7 and pH 8 lactate solutions to reactor sets 1 and 2, and 3 and 4, respectively. Final serum bottle volume equals 120 mL.
15.	Mix well and sample for pH, TS, sulfate, volatile acids, and lactate.
16.	Incubate at 35°C.

Table 3-8 Sulfide solution requirements for Phase 3 serum bottles

Serum Bottle Number	Sulfide Concentration (mgL^{-1})		Stock Solution Needed* (top-up water needed) (mL)	
	pH 7 R1/R3	pH 8 R2/R4	pH 7	pH 8
1	115	685	1.0(4.0)	3.3(12.7)
2	160	960	1.4(3.6)	4.6(11.4)
3	205	1230	1.8(3.2)	5.9(10.1)
4	275	1650	2.4(2.6)	7.9(8.1)
5	365	2200	3.1(1.9)	10.6(5.4)
6	460	2760	3.9(1.1)	13.3(2.7)
7	540	3310	4.6(0.4)	15.9(0.1)

* Stock Sulfide Solutions: - pH 7 buffered: 14 mg S mL^{-1}
- pH 8 buffered: 25 mg S mL^{-1}

Table 3-9 Description of recipe for stock solution S3 used in Phases 3 and 4

Compound and Description	Concentration ($g\ L^{-1}$ except where noted)
$MgSO_4 \cdot 7H_2O$	140.2
$CaCl_2 \cdot 2H_2O$	16.04
H_3BO_3	0.71
$MnSO_4 \cdot H_2O$	0.18
$CoCl_2 \cdot 6H_2O$	0.71
$CaSO_4 \cdot 5H_2O$	0.036
$AlK(SO_4)_2 \cdot 12H_2O$	5.778 mgL^{-1}
$Na_2MoO_4 \cdot 2H_2O$	0.163
Na_2SeO_3	3.822 mgL^{-1}
$NiCl_2 \cdot 6H_2O$	0.71
$ZnCl_2$	0.134
Na_2WO_4	0.384 mgL^{-1}
$(NH_4)_2SO_4$	31.22

3.3.2 Breeder Reactor Experimental Techniques

The breeder reactors consisted of four 2 litre batch operated reactors (Figure 3-5a and 3-5b). The reactors were maintained at 35±0.1°C using a temperature controlled water bath. The biogas was collected and measured using acidified salt solution displacement techniques, as described in Metcalf and Eddy (1979) (Figure 3-6). The reactors were mixed using Masterflex peristaltic pumps complete with variable speed drives and series 18 heads. The pumps operated for five minutes every half hour at 600 RPM (2.3 L per minute).

The UIS was scrubbed from the headspace using peristaltic pumps complete with variable speed Masterflex drives and series 17 heads were used. The headspace gas was bubbled through a zinc acetate solution and then returned to the headspace. The pumps operated at 100 RPM (0.3 L per minute) for five minutes every half hour.

This allowed MPB to outcompete SRB for available acetate at COD:sulfate ratios of 0.8 and 1.6 g g^{-1}

Breeder reactors B1 and B3 were maintained at a pH of 7.0 ± 0.2, while B2 and B4 were maintained at a pH of 8.0 ± 0.2. Chemcadet pH controllers were used. The breeders were fed 1 g COD per L of reactor volume approximately three times per week. The substrate contained all micro- and macronutrients needed, including excess sulfate. The batch feeding procedure consisted of allowing the biomass to settle during 2.5 h of no-mix and then manually decanting and feeding 200 mL. One hundred mL glass syringes were used to decant and feed the reactors. The feed was prepared as described in Table 3-10. A photograph of the feed storage container is presented in Figure 3-7.

3.3.3 Analytical Techniques

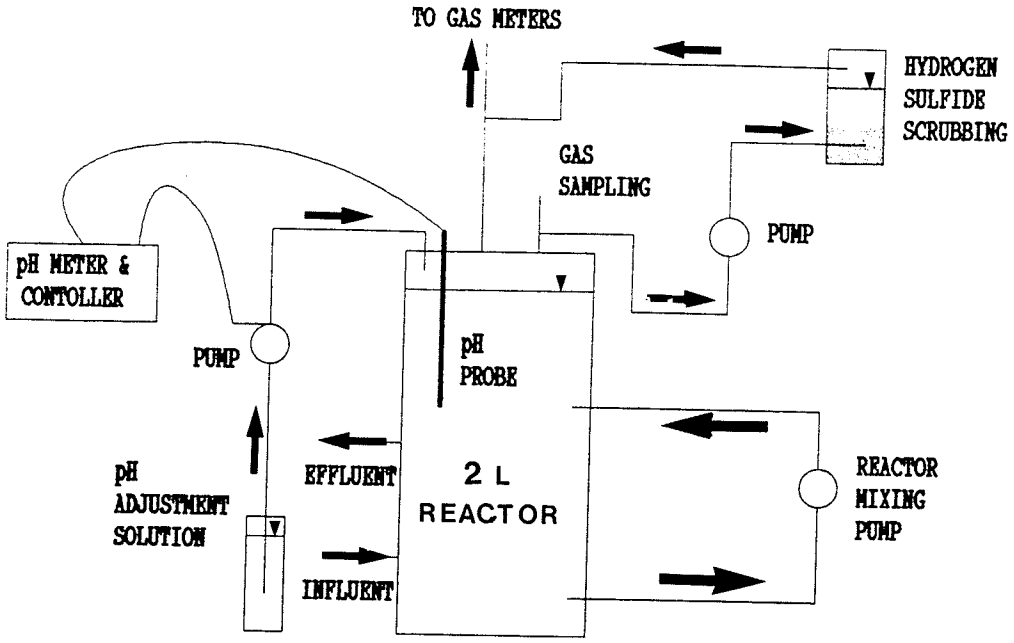
All analyses were conducted as described in Section 3.1.2.

The hydrogen gas analysis method was developed from that of Andrawes and Ramsey (1986), and Andrawes and Deng (1985). The headspace gas of the serum bottles was analyzed using an Antek 3000 gas chromatograph equipped with a helium ionization detector.

3.3.4 Experimental Apparatus

The apparatus listed in Section 3.1.3 was used for the serum bottle startup procedure.

A



NOTE: pH adjustment solution was caustic for pH 8 breeders and acidic for pH 7 breeders.

B

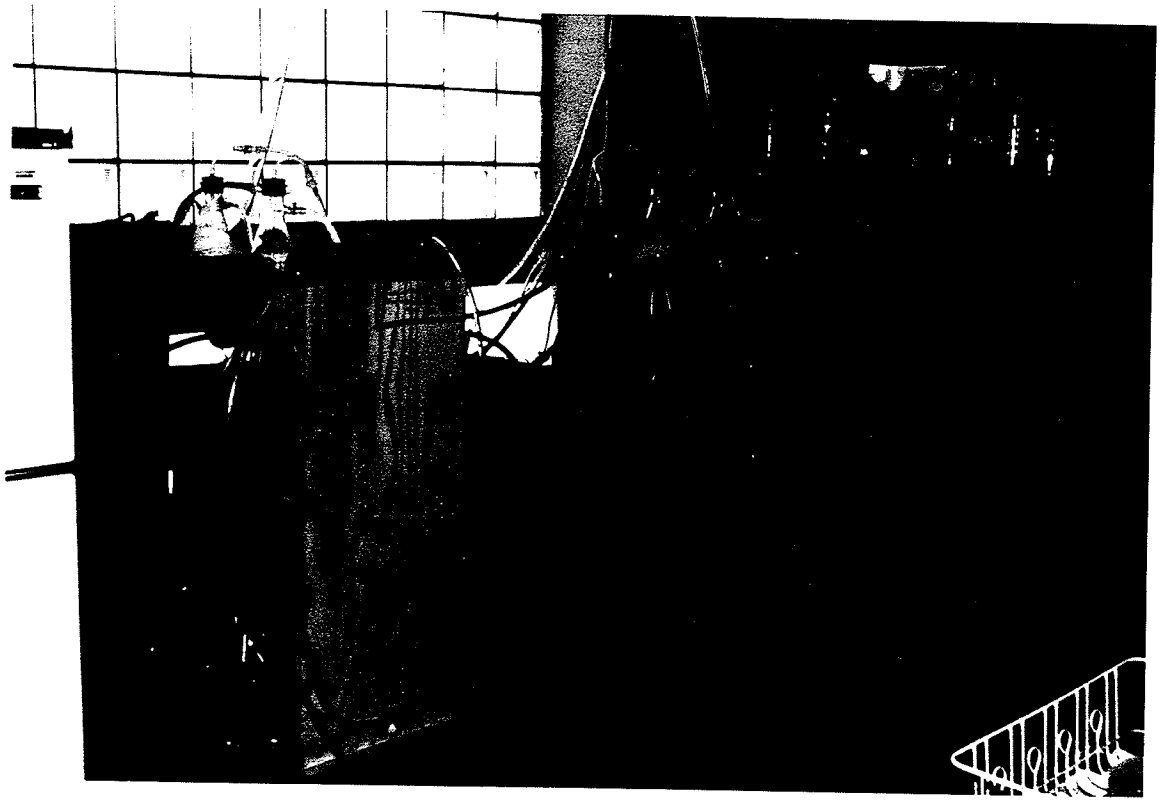


Figure 3-5. A. Schematic of a breeder reactor; B. View of breeder reactor system used to acclimate biomass in Phases 3 and 4. Breeders located in an insulated water bath with pumps for gas scrubbing and mixing located on shelves either side of bath. Gas collection and measurement system in background.

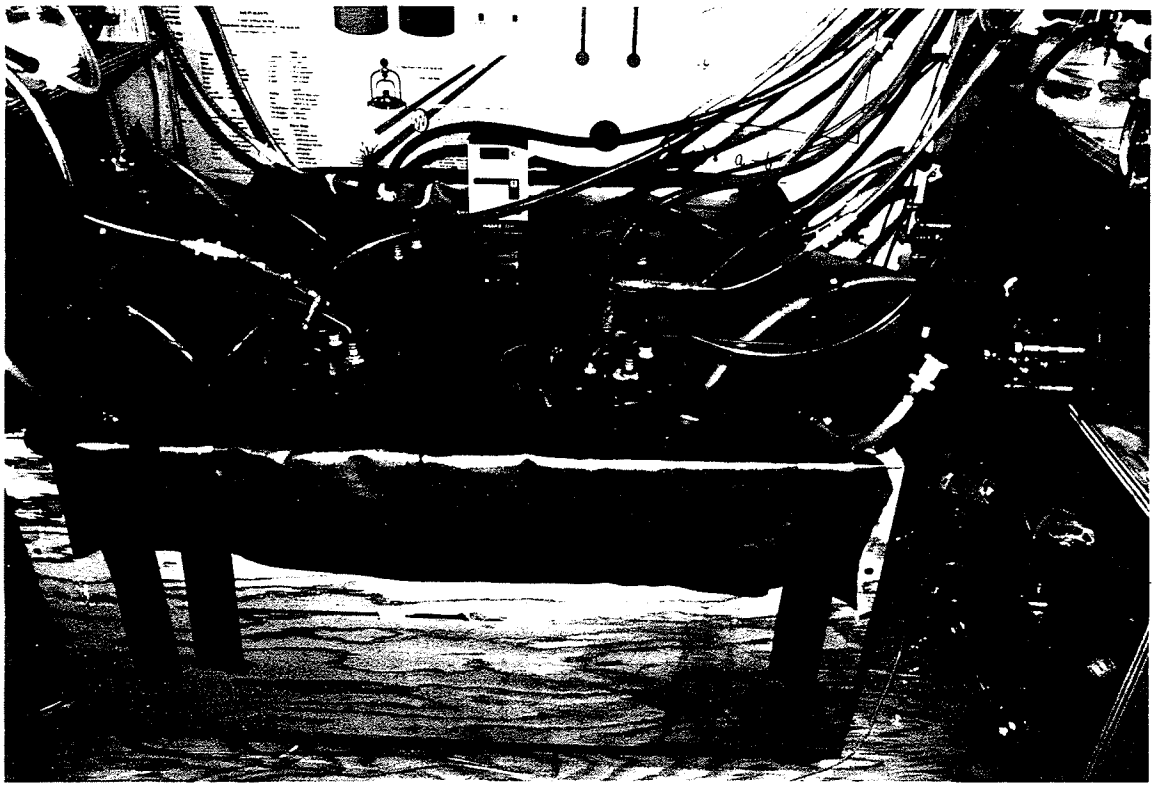


Figure 3-6. View of breeder reactor water bath. The pH control pumps and solutions for all four reactors located on tabletop to right of bath. Mixing pumps located on first shelf.

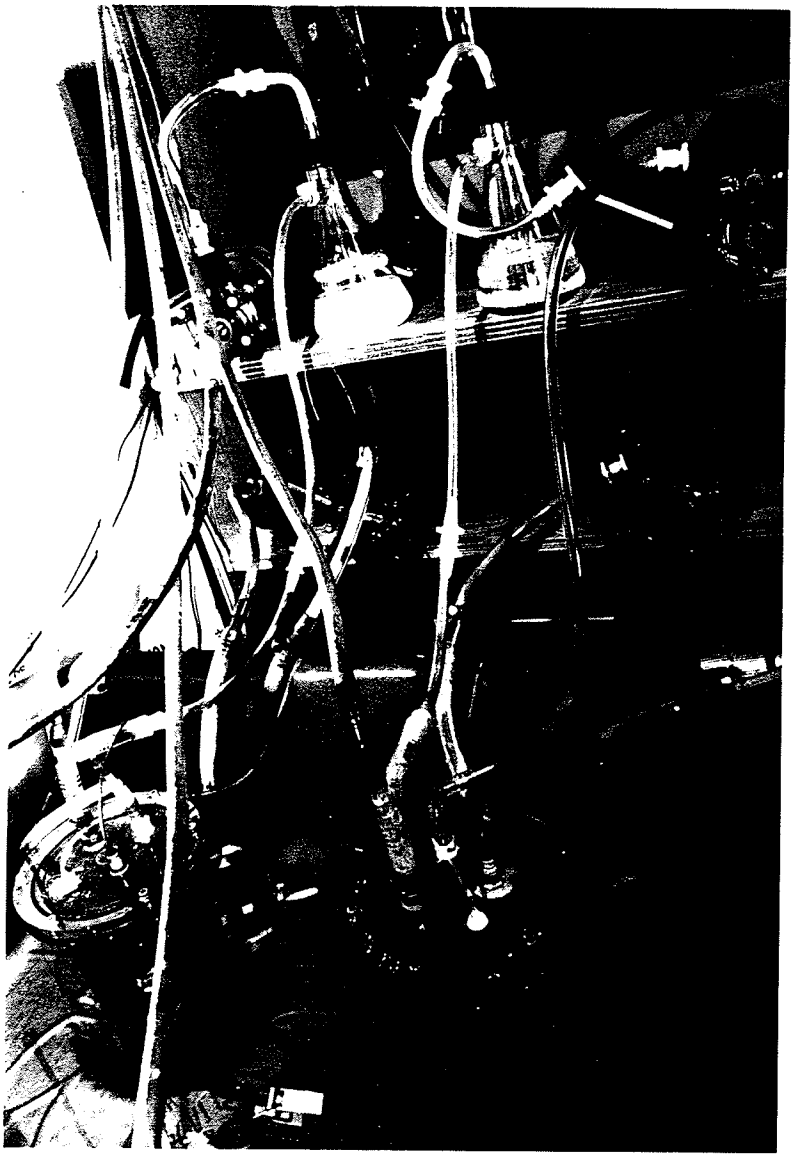


Figure 3-7. View of breeders 2 and 4. Gas scrubbing pumps and solutions located on top shelf. Gaslines, pH probe and pH solution lines attached to top of reactor. Mixing pump lines, and feed/sample lines located on sides of reactors.

Table 3-10. Breeder reactor feed preparation method used in Phases 3 and 4. Recipe makes 18 L of sterile feed media. Note: All stock solutions given in Tables 3-2 and 3-9, except where noted.

Step	Description
1	Place 18 L feed container on magnetic stirrer located on the floor, complete with three-inch magnetic stir bar
2	Loosely attach container stopper apparatus and cover with large piece of aluminum foil
3	Begin gassing feed container with N_2 gas
4	Bring 3.5 L of deionized water in a 4 L erlenmeyer flask to a boil and boil for five minutes
5	Cover top of 4 L erlenmeyer with aluminum foil and purge with N_2 gas for five minutes while continuing to boil
6	Add boiled and purged deionized water to 18 L feed container
7	Repeat steps 4 to 6 until 17 L of water in the feed container. Continue gassing headspace and purging liquid of the feed container during this time
8	Add: - 180 mL of S1* - 23 mL of S4 - 3 g K_2SO_4 - 2.4 g Na_2SO_4 to the feed container
9	Cool feed container, continue gassing until temperature less than 50°C
10	Remove N_2 gas purging and gas only the headspace
11	Add: - 540 mL of S3 (Table 3-9) - 22.5 mL of S5 - 280.3 mL L(+) lactic acid (30% solution) - 84.1 mL acetic acid (100% solution) - 240 mL 10 N NaOH solution - 90 g $NaHCO_3$ to feed container and mix well
12	Stop gassing feed container

Table 3-10 (continued)

Step	Description
13	Add: - 18 mL of S7 - 100 mL of S6 to feed container
14	Using pH probe and 10 N NaOH solution, adjust pH to 7.5
15	Adjust liquid volume to 18 L with boiled and gassed deionized water, seal and pressurize feed container with N_2 gas

* pH 7.5 buffer (3 mM solution) prepared by adding 42.8 and 14.2 g of K_2HPO_4 and KH_2PO_4, respectively, into 1 L of deionized water.

3.4 PHASE 4--OBJECTIVES AND TECHNIQUES

Phase 4 was designed to investigate the experimental variables which may have led to the observations of Phase 3. The observations of Phase 3 appeared to contradict those of Phase 2. Phase 4 serum bottle reactor sets were set up to isolate the operating conditions which were different between Phases 2 and 3.

Four different variables were investigated, as follows: adjustment of inoculum pH from 8 to 7 at startup; unacclimated inoculum substituted for acclimated inoculum; racemic lactate used as substrate instead of L(+) lactate; and high sulfate concentration at startup.

Phase 4 also investigated the effect of elevated H_2 concentrations on propionate degradation. Two different experimental sets were runs, as follows: the headspace of the serum bottles were flushed with H_2 gas, and one set was fed lactate, while the other set was fed a mixture of lactate and propionate.

The objective of Phase 4 was to reproduce the observations made during Phase 2, particularly the presence of propionate as an intermediate of lactate degradation. A secondary objective was to observe the effect of the H_2 on propionate degradation.

3.4.1 Serum Bottle Experimental Technique

A new serum bottle technique was used which increased the amount of biomass in the reactors. This was done to achieve faster organic removals, and therefore shorter experiment time as compared to the three previous phases. The method is outlined in Figure 3-8 and Tables 3-11 and 3-12. The serum bottle reactors did not consist of dual reactors as in the previous three phases, but rather a single serum bottle without the gas serum bottle. The serum bottles were then incubated upside down to prevent H_2 gas from escaping through the septum. The serum bottles were placed on a shaker tray which operated for five minutes every hour (Figure 3-9).

In total, there were eight sets of three serum bottles each. The operating conditions of the serum bottles are described in Table 3-13. The target TS concentrations for the serum bottles operating at pH 7 were 1.56, 4.68, and 12.5 mM (50, 150, and 400 mg L^{-1}). The serum bottles were monitored as described in Table 3.4.

3.4.2 Breeder Reactor Experimental Techniques

The breeder reactor apparatus was operated as described in Section 3.3.2.

3.4.3 Analytical Techniques

All analyses were conducted as described in Sections 3.1.2 and 3.3.3.

3.4.4 Experimental Apparatus

The apparatus listed in Section 3.1.3 was used for the serum bottle startup procedure.

Table 3-11. Nutrient preparation and serum bottle startup techniques for Phase 4. Stock solutions were as reported in Section 3.2.1 Recipe makes 150 mL of both pH 7 and pH 8 nutrient solutions. Final serum bottle volume was 94 mL in pH 7 bottles and 97 mL in pH 8 bottles.

Step	Description
1.	Label 150 mL volumetric mark on 3 serum bottles. Label one pH 8 and two pH 7. Flush serum bottles with N_2 gas, and add approximately 100 mL of deionized water to each. If possible, set up bottles on magnetic stirring hot plates. Continue to flush headspace with N_2 gas.
2.	Cover bottles with aluminum foil and boil vigorously for 5 minutes. Purge with N_2 gas for 5 minutes, i.e., a total of 10 minutes boiling.
3.	To each serum bottle, add: pH 7: - 11.5 mL S1, - 1.7 mL S4; pH 8: - 11.5 mL S2, - 1.7 mL S4
4.	Prepare pH 7 and pH 8 top-up water solutions (100 mL of each). Add 2 mL of S1 and S2 to pH 7 and pH 8 bottles, respectively. Follow step 2. Let serum bottles cool while continually gassing headspace, then seal.
5.	Cool serum bottles in ice bath until temperature less than 50°C. Ensure headspace gassed with N_2 during this time.
6.	Add: pH 7: - 10.0 mL S3, - 1.4 mL S5, - 3.0 g $NaHCO_3$; pH 8: - 10.0 mL S3, - 1.4 mL S5, - 3.0 g $NaHCO_3$
7.	Cover serum bottles with aluminum foil and continue to gas headspaces. Mix with magnetic stir-bar for 5 minutes.
8.	Fill serum bottles to be used as reactors with N_2 gas while under water,i.e., immerse serum bottle in water until all air displaced, invert, bubble N_2 gas into bottle, and seal. Label bottles as required. Add: - 285 mg K_2SO_4, - 232 mg Na_2SO_4 to serum bottles H1, H2, and H3 before sealing.
9.	Adjust pH of nutrient serum bottles to pH 7 and pH 8, if necessary. Bring volume to 150 mL with respective buffered top-up solutions prepared in step 4, and seal.
10.	Add 9 mL of pH 7 and pH 8 nutrient solutions to respective serum bottle reactors using a needle and syringe.

Table 3-11 (continued)

Step	Description
11.	Referring to Table 3-12, add required sulfide solution to each serum bottle.
12.	Top up each serum bottle with respective top-up water solutions, based on values in Table 3-12.
13.	Analyze each serum bottle for pH and TS, and adjust, if necessary.
14.	Inject 81 mL inoculum from respective breeder reactors and NEWPCC into each serum bottle. Add required organic substrate (1 mL). Final serum bottle reactor volumes were 94 and 97 mL in pH 7 and pH 8 bottles, respectively.
15.	Mix well and sample for pH, TS, sulfate, volatile acids, and lactate.
16.	Incubate at 35°C.

Table 3-12 Sulfide solution requirements for Phase 4 serum bottles

Serum Bottle Number	Sulfide Concentration (mgL^{-1})		Stock Solution Needed* (top-up water needed) (mL)	
	pH 7 R1/R3	pH 8 R2/R4	pH 7	pH 8
1	50	200	0.4(12.6)	0.8(5.2)
2	150	600	1.1(1.9)	2.3(3.7)
3	400	1600	3.0(0.0)	2.3(3.7)

* Stock Sulfide Solutions: - pH 7 buffered: 14 mg S mL^{-1}
- pH 8 buffered: 25 mg S mL^{-1}

Table 3-13. Phase 4 serum bottle operating condition description.

Set Number	Operating pH	Biomass Source	Substrate (1 g COD L^{-1})	Startup Differences
A	7	Breeder 1 (pH 7)	L(+) lactate	-
B	7	Breeder 4 (pH 8)	L(+) lactate	-
C	7	NEWPCC* (pH 7)	L(+) lactate	-
D	7	Breeder 1 (pH 7)	L(+) lactate	100% H_2 atm.
E	7	Breeder 1 (pH 7)	propionate/L(+) lactate (1:1)	100% H_2 atm.
F	7	Breeder 1 (pH 7)	racemic lactate	-
G	8	Breeder 4 (pH 8)	L(+) lactate	-
H	8	Breeder 4 (pH 8)	L(+) lactate	high[SO_4^{2-}]

* North End Water Pollution Control Centre municipal digester sludge, Winnipeg, MB, Canada.

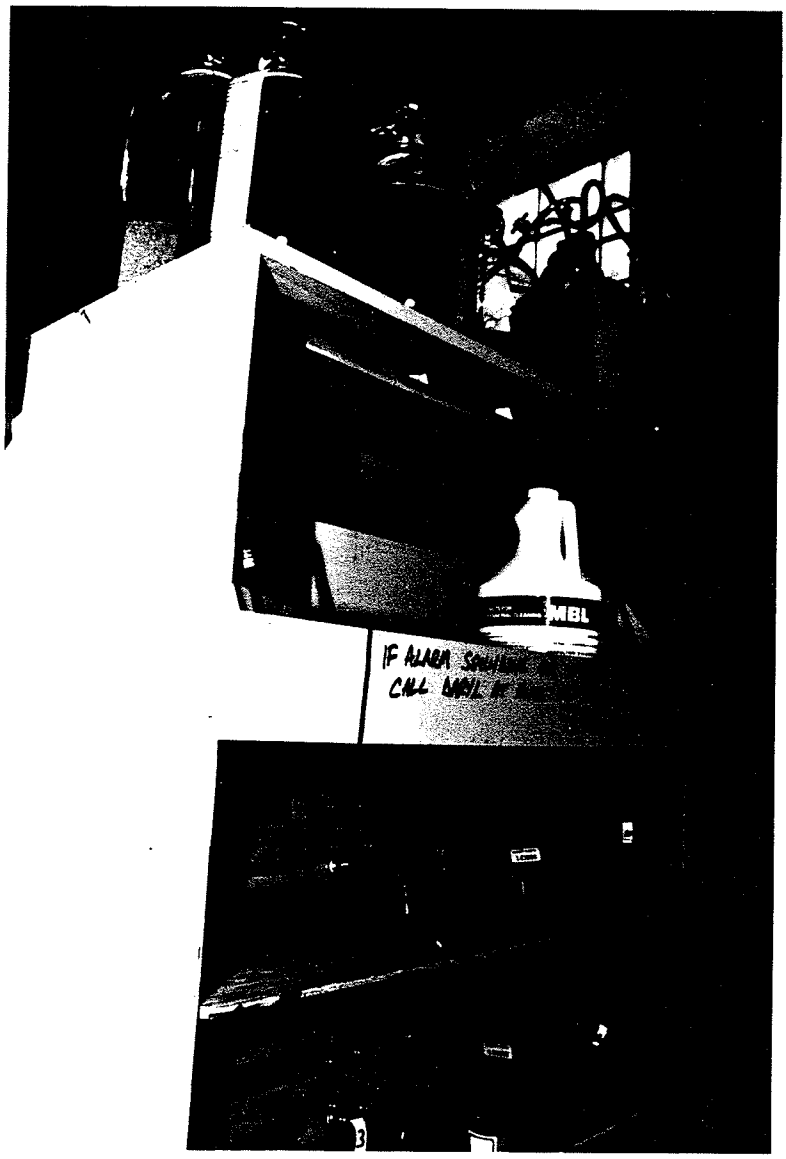


Figure 3-8. Biogas displacement meters and pH control apparatus for the breeder reactors.

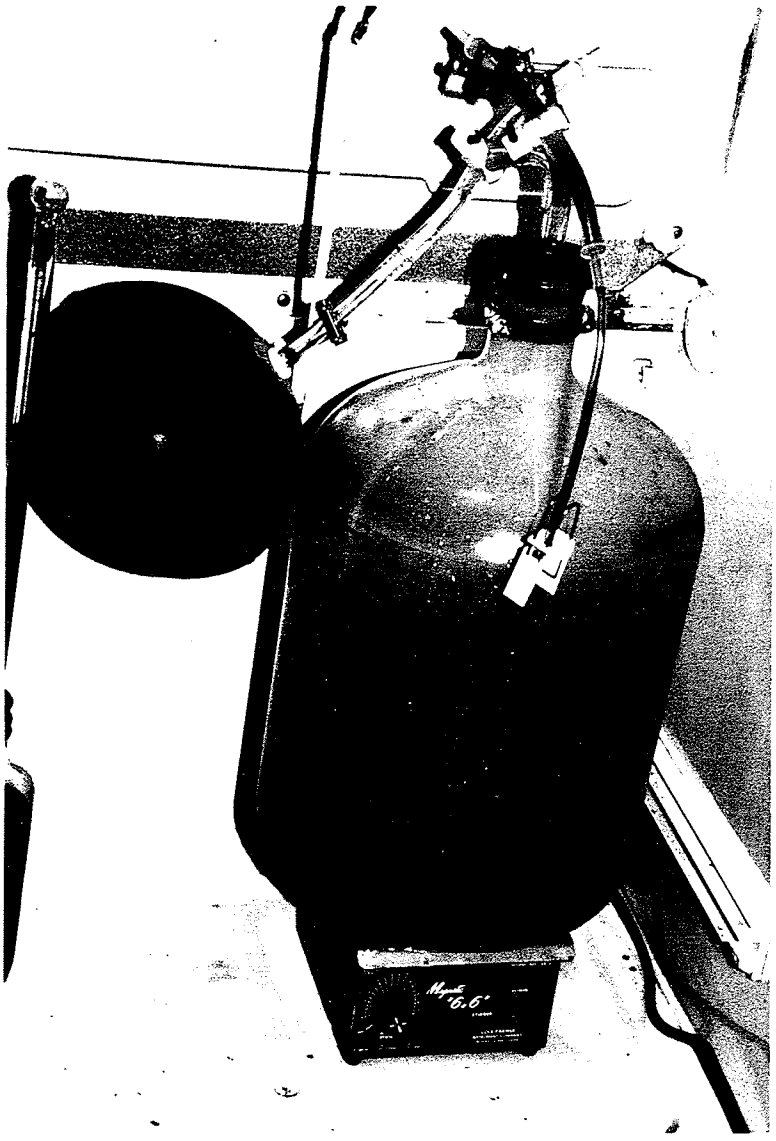


Figure 3-9. Breeder feed storage container inside cooler. System was kept pressurized with H_2 gas balloon to minimize oxygen contamination. Feed removed with syringe.

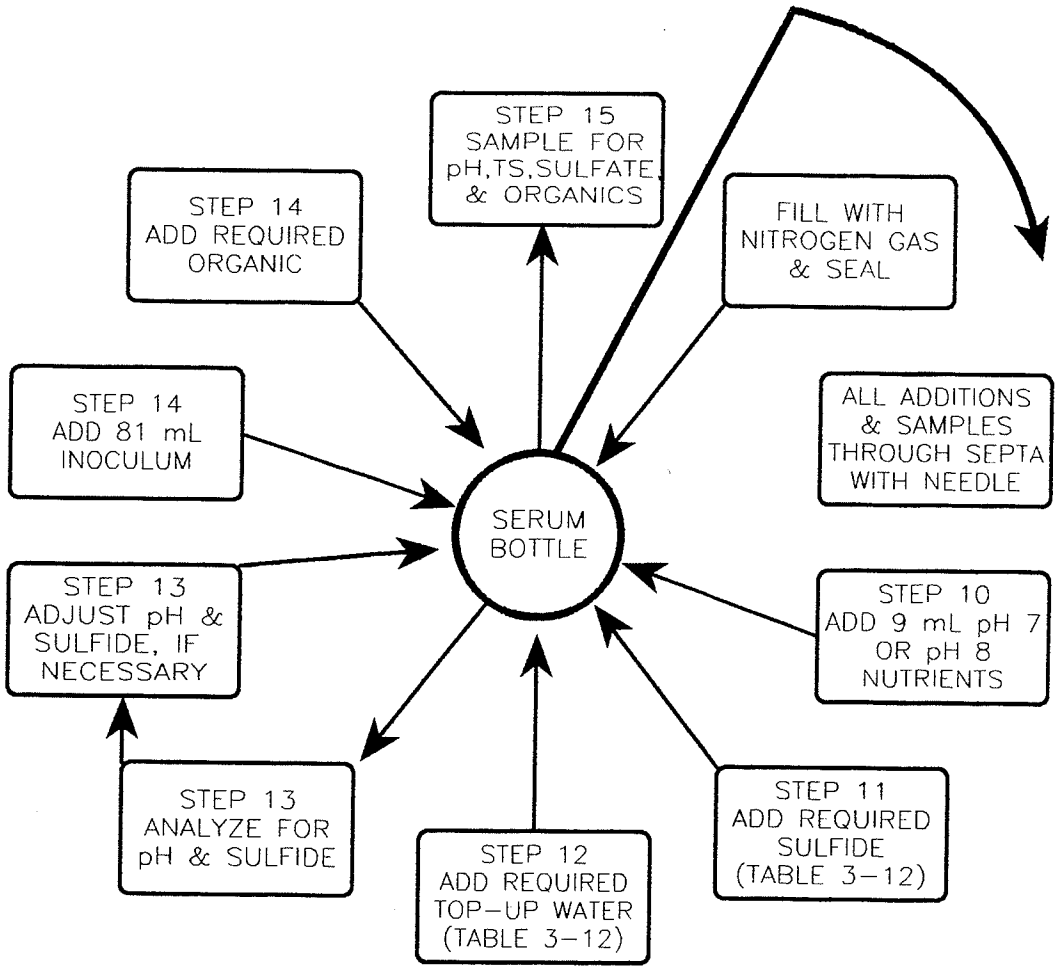
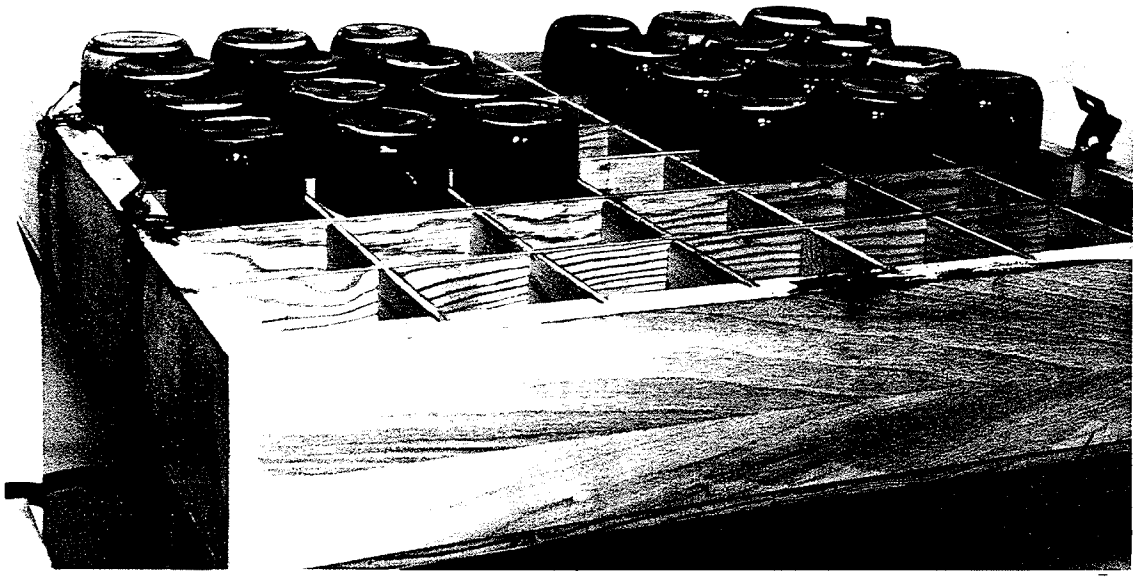


Figure 3-10. Outline of serum bottle startup technique used in Phase 4.

Figure 3-11. The Phase 4 inverted serum bottles shown on shaker apparatus. Pictures inside the incubator. Power to shaker controlled with 1-hour mechanical timer.

A.



B.

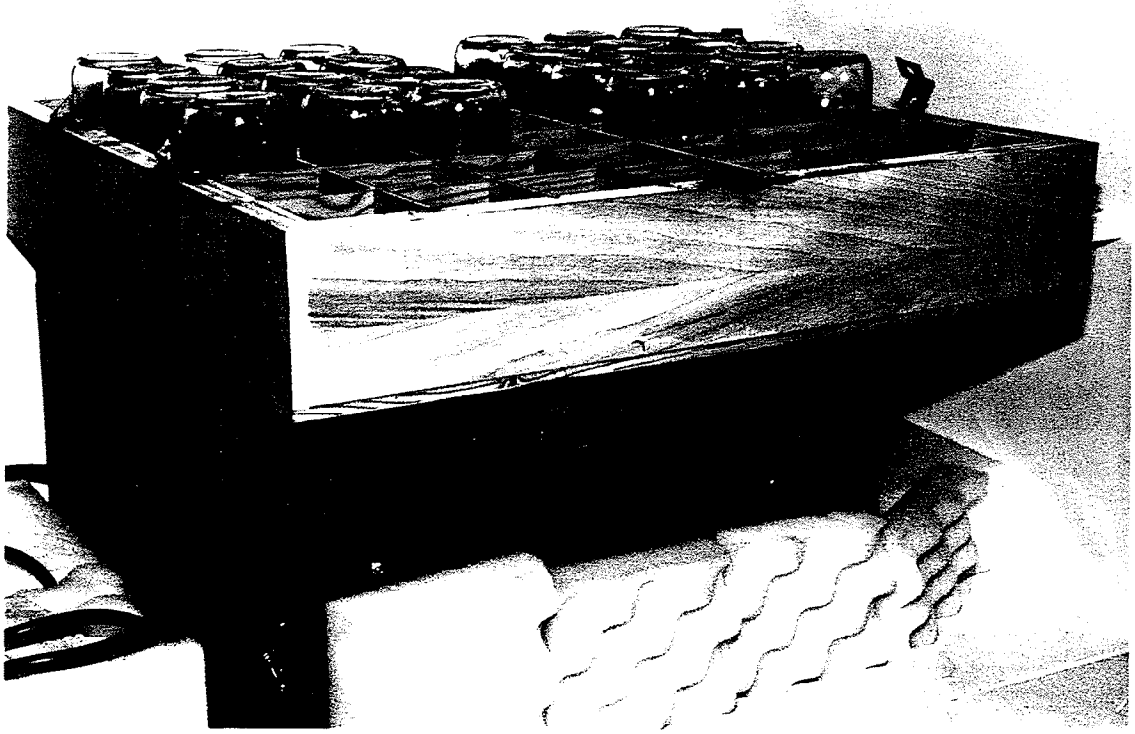


Figure 3-12. Phase 4 serum bottles. A. During a shaking period. B. After shaking period. Samples were shaken 5 minutes of every half-hour.

Chapter 4.

EXPERIMENTAL RESULTS AND DISCUSSION

The experimental programs consisted of four phases, as described in Chapter 3. All tables and figures in this chapter were constructed from the results of these programs. The phases proceeded chronologically, with the results of the previous phase giving direction to the next phase. All phases were conducted using high sulfate conditions (COD:sulfate ratios of 0.8 to 3.7) and various sulfide concentrations. Phase 1 was a general screening experiment looking at the utilization of the simple substrates which immediately precede methanogenesis. Phases 2 and 3 focused on the utilization of lactate and acetate as well as the role of propionate as an intermediate. Phase 4 investigated the utilization of lactate under various environmental conditions and the roles of propionate and hydrogen as intermediates. Each phase was discussed separately, with phase summaries immediately following the detailed discussions and an overall summary contained in Chapter 5.

4.1 PHASE 1--RESULTS AND DISCUSSION

Five series of batch reactors were incubated at various levels of TS and at a high and low pH level. The five series contained 1 gL^{-1} as COD of either acetate, propionate, butyrate, lactate, or a 1:1 mixture of acetate and propionate.

The raw data and substrate depletion profiles for all Phase 1 serum bottle runs are contained in Marstaller (1989). Marstaller's research was directed and interpreted by the author of this thesis. The effect of the initial TS on the degradation rates of

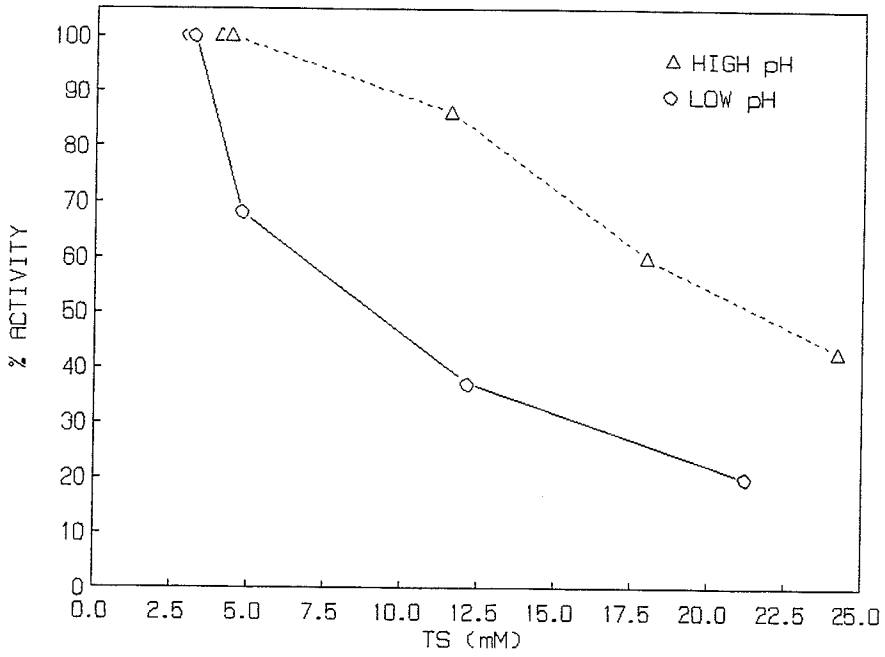


Figure 4-1. Phase 1--Effect of TS on acetate depletion activity at high and low pH

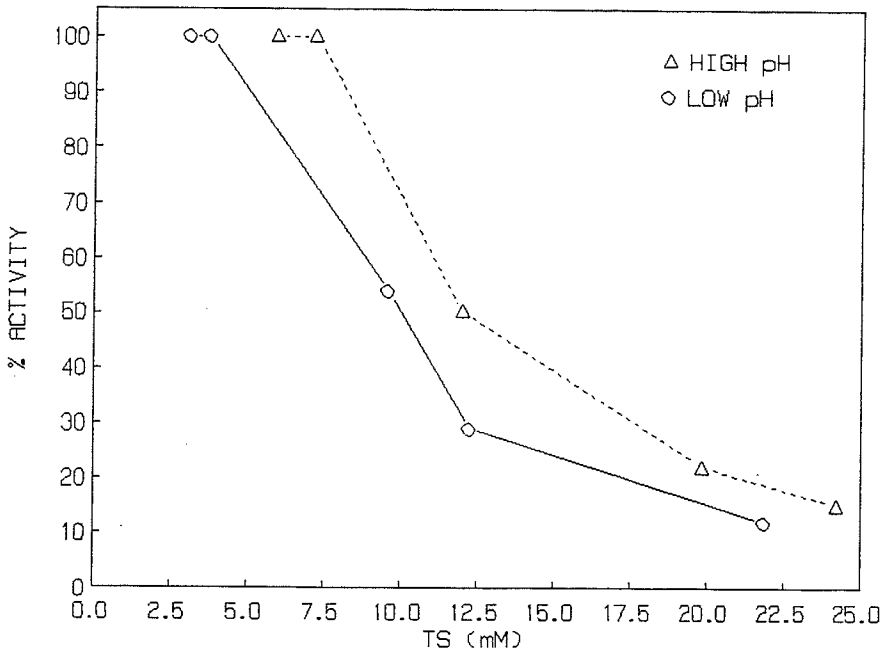


Figure 4-2. Phase 1--Effect of TS on propionate depletion activity at high and low pH

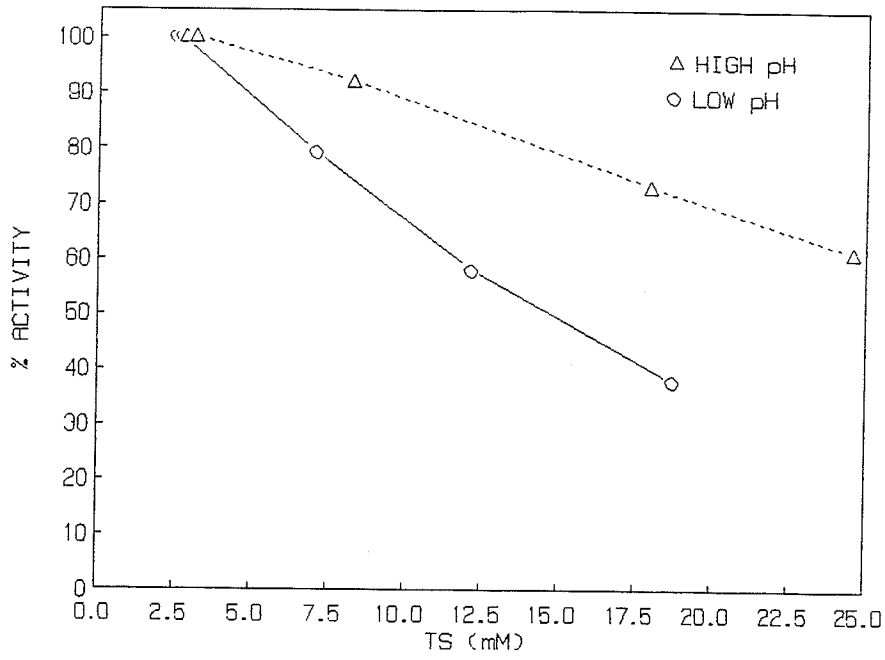


Figure 4-3. Phase 1--Effect of TS on butyrate depletion activity at high and low pH

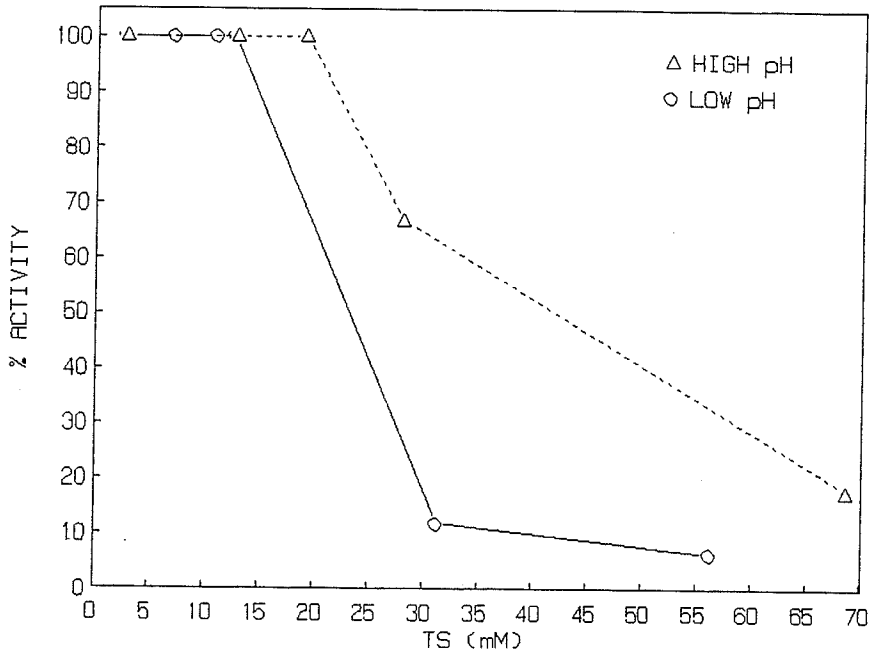


Figure 4-4. Phase 1--Effect of TS on lactate depletion activity at high and low pH

the pure organic substrates investigated were summarized in Figures 4-1 to 4-4. The 1:1 acetate:propionate series was not discussed, as it does not add any significant information to that already found in the pure substrate series. The pure substrate removal rates were expressed as a percentage of the control reactor substrate removal rates and are referred to as percent activities throughout the thesis. The percent activities were then plotted against the TS concentration in the serum bottle. The substrate removal activities for pure substrate sets were dependent upon TS concentrations and pH values (Figures 4-1 to 4-4). In general, a higher TS and/or a lower pH resulted in a lower activity.

These observations may be due to lower activities at the lower pH levels or to the higher UIS concentrations at the lower pH levels. The UIS concentrations for each serum bottle were calculated using equation 2-14. The effect of initial UIS on activities for all pure substrate sets is presented in Figure 4-5. The decrease in activities correlate well to the increasing UIS concentrations.

The concentrations of TS and UIS which represented 50% inhibition of the substrate depletion activities are presented in Table 4-1.

Table 4-1. Phase 1 TS and UIS concentrations resulting in 50 percent inhibition of pure substrate utilization activities

	Propionate	Acetate	Butyrate	Lactate
TS; mM(mg L^{-1})				
low pH	9.38 (300)	10.0 (320)	12.50 (400)	17.19 (550)
high pH	14.69 (470)	18.75 (600)	>25.0 (800)	32.81 (1050)
UIS;mM (mg L^{-1})				
low pH	3.13 (100)	3.91 (125)	7.34 (235)	10.0 (320)
high pH	1.89 (60)	3.13 (100)	> 6.25 (200)	12.19 (390)

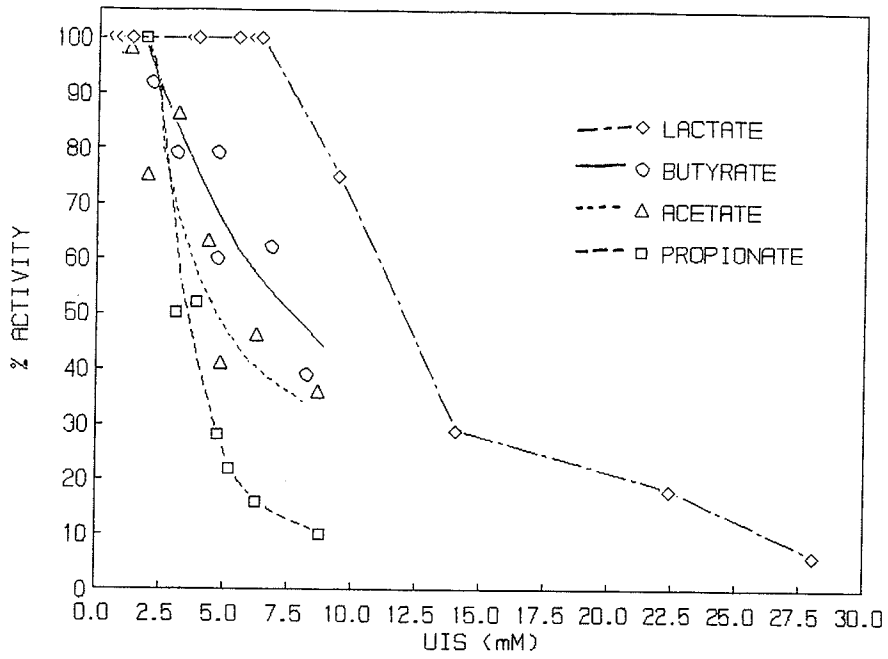


Figure 4-5. Phase 1--Effect of UIS on substrate depletion activity at high and low pH. Curves for butyrate, acetate, and propionate were drawn freehand as "best-fit" lines. All reactors achieved 100% activity at UIS of ≤ 2 mM.

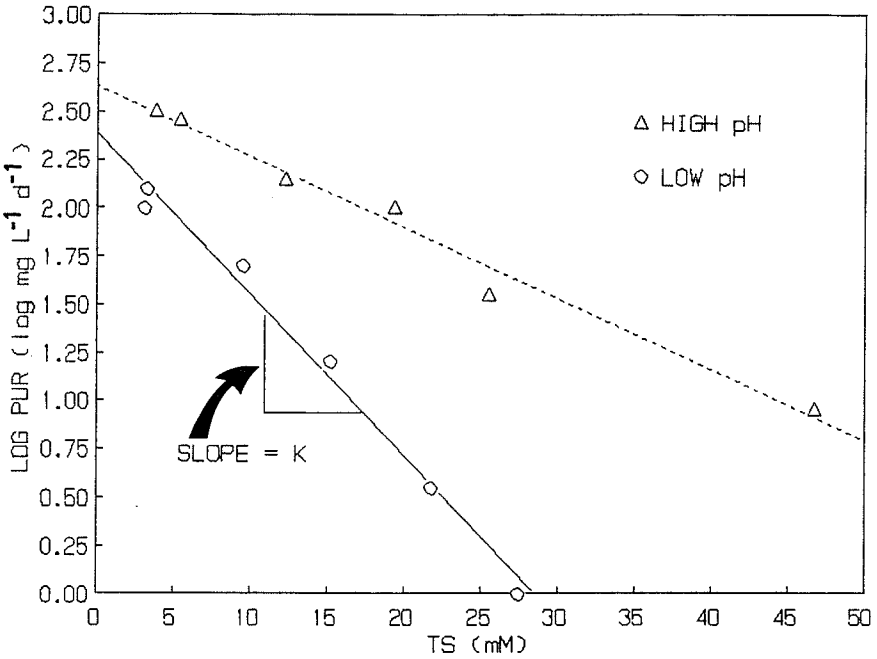


Figure 4-6. Phase 1--Effect of TS on PUR and representation of exponential inhibition constant (K). Linear regression fit for both lines.

These observations point to the significant sensitivity of propionate degradation mechanisms relative to acetate degradation. The substantially lower sensitivity of both butyrate and lactate degradation indicate that these species would not be subject to inhibition by sulfide before propionate and/or acetate inhibition.

Table 4-2. Phase 1 exponential inhibition constants (K; L g^{-1}) for acetate and propionate series

	Acetate	Propionate
K (L g^{-1}):		
low pH	1.4	2.7
high pH	0.6	1.2

Exponential inhibition constants (K) were developed for acetate and propionate utilization rates (AUR and PUR). The values for K were taken as the slopes of the best-fit lines through the semi-log plots of the data sets (e.g., Figure 4-6). This figure represents the log of the PUR plotted versus initial TS. The PUR values were determined from the slope of the best-fit line through the propionate concentration versus time curve for each of the serum bottle reactors. An example of the PUR calculation method can be seen in Appendix A. The exponential inhibition constants were calculated using the formula:

$$\frac{PUR_i}{PUR_o} = 10^{-K(TS)} \qquad 4\text{-}1$$

where $PUR_{i,o}$ are the respective inhibited and noninhibited rates (intercept) for given TS concentrations. The values for K at high and low pH are presented in Table 4-2. The values of K point to the higher sensitivity of the propionate removal step and to

the detrimental effects of the higher UIS of the lower pH values. The PUR was approximately twice as sensitive to TS when compared to AUR (Table 4-2). Rinzema and Lettinga (1988) concluded that propionate breakdown was the rate-limiting step under UIS inhibition of MPB. Koster et al. (1986) reported that at high pH, TS may become the dominant inhibition factor. Although a higher UIS tolerance at lower pH was generally observed here (Table 4-1), no trend could be quantified.

4.1.1 Phase 1--Observation Summary

The following observations were made: The degradation rates of all organic substrates tested were affected by high TS concentrations; similar TS concentrations at the high pH levels were not as inhibitory; activity inhibition of all substrates correlated to the UIS concentration, which indicates that UIS was the main inhibitory species of sulfide; propionate degradation was the most sensitive to UIS and TS, followed by acetate, butyrate, and lactate was the least sensitive; and the inhibition of acetate and propionate degradation was expressed by a relationship between the log of the utilization rates and the TS concentration, resulting in a numerical measure of the inhibition.

4.2 PHASE 2--RESULTS AND DISCUSSION

Phase 2 used three sets of serum bottles, all started with 1 gL^{-1} COD. Set 1 (S_L) was started with lactate, Set 2 (S_A) was started with acetate, and Set 3 (S_M) was started with a 1:1 COD mixture of lactate and acetate. Propionate was not used since it was observed as a free intermediate during lactate degradation in Phase 1,

therefore propionate observations were conducted in serum bottles started with lactate. Lactate degradation to propionate and acetate was also observed by Sorbes et al. (1989) in the absence of excess sulfate. Similar to Phase 1, various TS levels were used as well as high and low pH subsets. All raw data for Phase 2 is contained in Appendix A. The actual analytical pH, TS and UIS values obtained in the serum bottles deviated from the objective concentrations. The TS and pH also changed in the serum bottles during incubation. The variations can be reviewed in Table 4-3. For data interpretation, arithmetic means of the initial and final pH, TS, and UIS concentrations. Due to the significant sulfate reduction in S_L and S_M, the arithmetic mean UIS (UIS_a) and TS (TS_a) concentrations were significantly lower than the final recorded sulfide concentrations in these sets.

Substrate concentration versus time profiles were reviewed for each serum-bottle; such as that from S_L3, which is shown in Figure 4-7. Several general observations were made based on the analysis of the graphs for all 30 serum bottles, which are in Appendix A. Acetate utilization followed zero-order kinetics, i.e., the removal rate was independent of the acetate concentration above 2 mM (120 mg L^{-1}) acetate. AUR correlated with the UIS_a concentration of the serum bottles. Changes in lactate utilization rates could not be detected in any of the 30 serum bottles. This was due to all of the lactate being removed in the first day in all reactors. Significant residual propionate concentrations occurred in the serum bottles with a UIS_a concentration of ≥2.9 mM (100 mg L^{-1}), as indicated in Table 4-3. Serum bottle S_M10 was an exception to this observation and the lack of significant residual propionate in this serum bottle was attributed to the lower UIS concentration at the start of the

Table 4-3. **The initial and final pH, total sulfide (TS) and un-ionized sulfide (UIS) data for all serum bottles used in Phase 2. The values were determined from measurements taken at startup and when the serum bottles were removed from incubation.**

Serum		pH		TS mM (mg L^{-1} as S^{2-})		UIS mM (mgL^{-1} as S^{2-})	
		Initial	Final	Initial	Final	Initial	Final
S_L	1	7.3	7.1	5.1(162)	10.3(330)	1.5(49)	4.2(135)
	2	7.7	7.5	10.1(322)	13.8(440)	1.5(48)	2.7(87)
	3*	7.4	7.3	10.1(322)	13.1(420)	2.6(83)	3.7(118)
	4*	7.3	7.3	15.7(502)	19.4(620)	4.8(153)	5.4(174)
	5*	7.4	7.3	23.8(762)	29.4(940)	6.2(197)	8.3(264)
	6	8.1	7.5	8.8(282)	12.5(400)	0.6(18)	2.5(79)
	7	8.1	7.6	13.8(442)	18.1(580)	0.8(26)	3.0(95)
	8	8.1	7.7	11.1(362)	16.3(520)	0.7(21)	2.2(70)
	9	8.3	7.8	17.5(561)	20.9(670)	0.8(24)	2.3(74)
	10	8.1	7.7	30.1(962)	32.5(1040)	1.8(56)	4.4(140)
S_A	1	7.2	7.2	3.2(101)	6.3(200)	1.0(33)	2.1(66)
	2	7.6	7.5	10.1(321)	9.4(300)	1.7(53)	1.8(59)
	3*	7.5	7.3	11.2(357)	10.6(340)	2.2(71)	3.0(96)
	4*	7.4	7.3	13.8(441)	18.8(600)	3.3(105)	5.3(169)
	5*	7.4	7.3	23.8(762)	30.6(980)	5.7(181)	9.3(299)
	6	8.1	7.7	6.3(201)	5.0(160)	0.4(12)	0.7(22)
	7	8.1	7.8	13.8(442)	11.9(380)	0.8(26)	1.3(42)
	8	8.2	7.8	11.3(362)	9.4(300)	0.5(17)	1.0(33)
	9	8.4	7.9	16.3(522)	16.9(540)	0.5(18)	1.5(48)
	10	8.2	7.8	28.2(902)	28.1(900)	1.3(42)	3.1(99)
S_M	1	7.2	7.2	3.2(102)	7.5(240)	1.1(34)	2.5(79)
	2	7.6	7.5	8.8(282)	11.9(380)	1.4(46)	2.3(75)
	3*	7.3	7.3	11.3(362)	14.4(460)	3.2(102)	4.0(129)
	4*	7.4	7.2	11.3(362)	18.1(580)	2.7(86)	6.0(192)
	5*	7.4	7.2	23.8(762)	30.0(960)	6.2(197)	9.9(317)
	6	8.0	7.8	10.1(322)	9.4(300)	0.7(23)	1.2(37)
	7	8.1	7.7	12.6(402)	15.0(480)	0.7(23)	2.0(65)
	8	8.1	7.7	15.1(482)	16.6(530)	0.9(28)	2.2(71)
	9	8.2	7.7	16.3(522)	18.4(590)	0.8(25)	2.5(80)
	10	8.1	7.5	27.6(882)	33.1(1060)	1.6(52)	6.6(210)

* Significant residual propionate concentration observed in serum bottle.

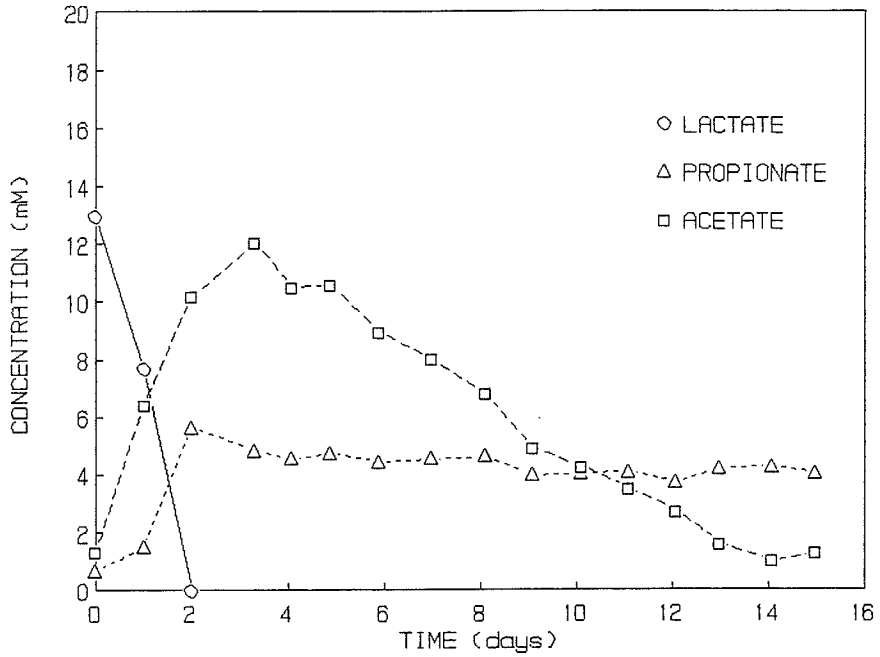


Figure 4-7. The substrate depletion profile of S_L3. The propionate residual was equal to approximately 4.2 mM (311 mgL^{-1}).

experimental run. The residual propionate did not break down and remained at a constant concentration for the duration of the experiment, while the acetoclastic MPB were able to remove acetate (Figure 4-7).

The percentage of organic COD, removed from the bulk liquid, attributed to SO_4^{2-} removed from the bulk liquid and CH_4 production are presented in Table 4-4. The total percentage of COD recoveries ranged between 122.7 to 31.7%; therefore, the discussion of this data must proceed with caution. However, some significant trends were observed and warrant discussion.

Table 4-4. The amounts of organic COD removals attributed to sulfate reduction and methane production.

Serum Bottle		% COD Removal attributed to:		
		SO_4^{2-}	CH_4	Total
S_L	1	55.5	67.2	122.7
	2	40.9	56.8	97.7
	3	24.5	65.4	89.9
	4	3.7	39.8	43.5
	5	0.0	56.6	56.6
	6	41.9	34.6	76.5
	7	44.8	37.4	82.3
	8	44.4	32.6	77.0
	9	36.6	27.9	64.5
	10	9.9	68.5	78.4
S_A	1	0.0	64.6	64.6
	2	0.0	47.9	47.9
	3	0.0	66.7	66.7
	4	0.0	53.8	53.8
	5	0.0	48.5	48.5
	6	6.3	47.0	53.4
	7	4.9	46.7	51.6
	8	4.6	44.7	49.3
	9	0.8	39.4	40.2
	10	0.8	52.7	53.5
S_M	1	34.4	67.1	101.6
	2	18.6	63.9	82.5
	3	4.1	65.4	69.4
	4	0.0	60.2	60.2
	5	0.0	31.7	31.7
	6	30.5	39.3	69.8
	7	26.1	39.5	65.6
	8	26.7	31.9	58.5
	9	20.8	39.2	60.0
	10	12.5	38.0	50.5

4.2.1 Acetate Utilization Rates

The AUR's were calculated during the period of zero-order removal rates for all 30 serum bottles. The effect of the pH and the UIS_a concentration on the AUR in each serum bottle was then investigated (graphs in Appendix A-2). It was concluded that the pH effect on AUR was significant at UIS_a concentrations of $\leq$ 2 mM (68 mg L^{-1}). Above this value, the pH effect was negligible. However, to eliminate pH as a variable, the effect of UIS_a concentration on AUR was investigated using serum bottles in which the recorded pH values were within an interval of 0.25 pH units. Only serum bottles 3, 4, and 5 of each set fit this criteria (Table 4-3). These reactors are indicated by the filled symbols of Figure 4-8.

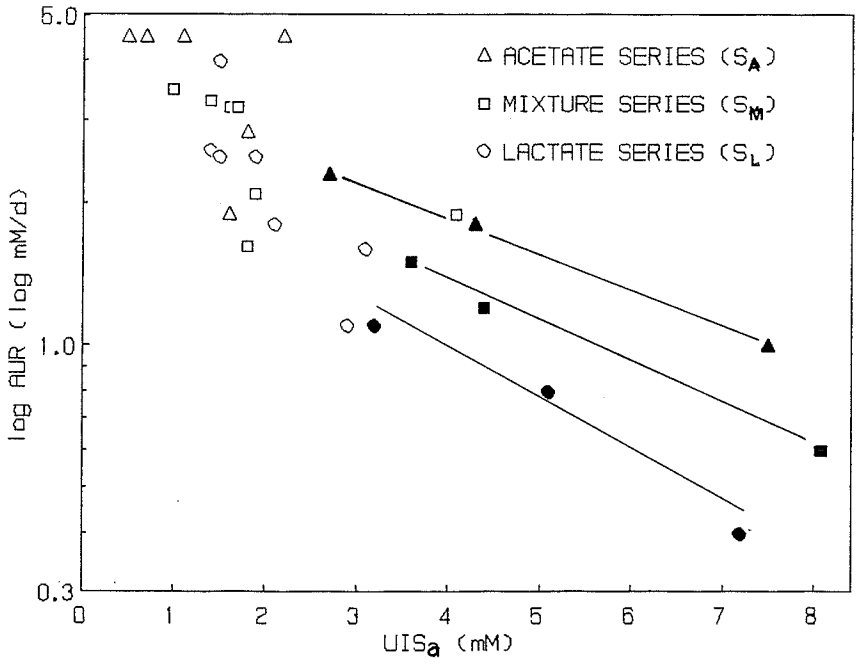


Figure 4-8. Effect of UIS_a on AUR for the serum bottles in all three series. Filled-in data points represent serum bottles at comparable pH levels.

The methodology for developing the K values in Section 4-1 was used. The value for UIS_a was substituted for TS in Equation 4-1. The best-fit lines through the semi-log plots of the data subsets (Figure 4-8) resulted in linear relationships over the UIS_a range of 2 to 8 mM (68 to 272 mg L^{-1}). Below 2 mM, there was no inhibition and 8 mM was the highest concentration evaluated in this investigation. The retardation of the AUR by UIS_a was then calculated as the slope of these lines. The exponential inhibition constants (K) were 0.071, 0.087, and 0.100 mM^{-1} for S_A, S_M, and S_L, respectively. The model (modified equation 4-1) was valid above a UIS_a concentration of 2 mM (64 mg L^{-1}). The increase in inhibition constants coincided with an increase in the initial lactate concentration (0, 7.6, and 14.0 mM for S_A, S_M, and S_L, respectively) and a decrease in the initial acetate concentrations (22.1, 17.1, and 11.2 mM for S_A, S_M, and S_L, respectively). The lower AUR's could not be attributed to the lower starting acetate concentrations, since the data was gathered in the region where the rates were zero-order. It was speculated that hydrogen buildup, as a result of lactate and propionate degradation, may be responsible for this observations, but since hydrogen was not measured, a correlation cannot be developed. However, the observation does indicate that the AUR may be dependent upon the reactions which lead to acetate formation.

It has been shown that UIS is the controlling species of TS, with respect to AUR retardation (Rinzema and Lettinga, 1988; Khan and Trottier, 1978). Therefore, pH control can be used to change to UIS:TS ratio according to equation 2-14. In some treatment processes, i.e., where available SO_4^{2-} or degradable organic sulfur limit the TS production, simply raising the operating pH may significantly increase

the removal rates. Control of pH could perhaps be used in conjunction with other operational strategies to control UIS inhibition; however, Koster et al. (1986) have shown that MPB may be more sensitive to UIS at higher pH levels.

4.2.2 Energy Flow Via SRB and MPB

The COD removal attributed to MPB was generally higher than that attributed to SRB (Table 4-4). Even with lactate as a substrate (S_L) MPB were able to utilize no less than 73% of the COD attributed to SRB. The fraction of COD removal attributed to sulfate reduction in the S_A serum bottles was very low. The low pH S_A subset had no detectable sulfate reduction, while in the high pH S_A subset, the organic COD removal attributed to sulfate removal ranged from 0.8 to 6.3%. This indicates that the SRB were not able to outcompete the MPB for acetate in this investigation. In general, the high pH reactors of all sets yielded lower CH_4 than the uninhibited low pH serum bottles. It was thought that two processes may have lead to these observations. An observed drop in pH associated with the high pH serum bottles indicated that some of the oxidized carbon remained as HCO_3^-. The incubation time of the serum bottles may also have had an effect on CH_4 yield. The serum bottles were removed when their acetate concentration dropped below 1 mM (60 mg L^{-1}). This resulted in incubation times of 18d and 9d for the low and high pH serum bottles, respectively, except for S_L10, which was incubated for 18d. The longer incubation times may have resulted in higher CH_4 yields. A comparison of the relatively uninhibited serum bottles of the low pH subsets of S_L and S_M show that methane production accounted for an average of 63.8% of the organic COD removed.

Similarly, in an uninhibited defined co-culture of SRB and MPB, Phelps et al. (1985) reported a methane production of 67% of the theoretical methane yield. Acetate and methanol were used in their study.

A significant trend, indicated in Table 4-4, is that of decreasing SRB activity as the TS concentration increased in all the subsets. In contrast to this, the MPB activity remained relatively steady in the high pH subsets, regardless of the TS concentrations, but decreased in the low pH subsets as the TS concentration increased. This indicates that the SRB appear to be more sensitive than the MPB to the elevated TS concentrations. A previous study found that the SRB were inhibited at lower TS concentrations than the MPB, while both groups were equally inhibited by UIS during the degradation of lactose and acetate (Hilton and Oleszkiewicz, 1988). To define this trend further, the effect of the TS concentration on the fraction of organic COD attributed to sulfate reduction and methane generation were analyzed graphically for S_L and S_M. The graphs resulting from the two reactor sets were similar, therefore only the data for S_M are presented. Figures 4-9 and 4-10 are for the low pH and high pH subsets, respectively. Sulfate reduction essentially stopped in the low pH subset before there was even a 50% inhibition of the methane generation (Figure 4-9). The 50% inhibition thresholds were 2.6 and 7.5 mM (83.2 and 240 mg L^{-1}) TS for SRB and MPB, respectively. The fraction of organic COD removal attributed to the MPB was relatively constant, while that attributed to the SRB decreased in the higher pH subset (Figure 4-10). These observations would indicate that in this study the SRB were more sensitive to both TS and UIS as compared to the MPB, which verified the work of Hilton and Oleszkiewicz (1988). Parkin et al.

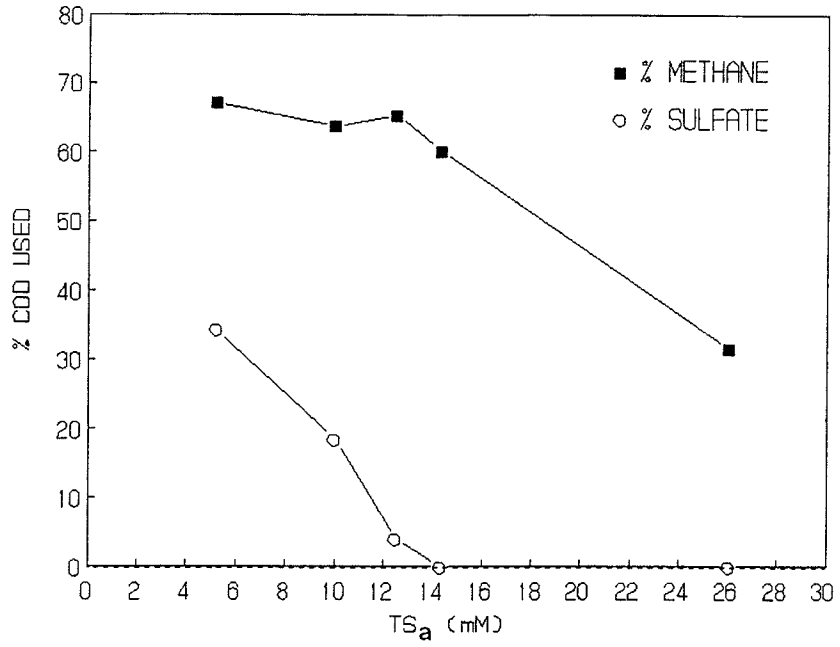


Figure 4-9. Effect of TS_a on the fraction of COD removal attributed to sulfate reduction and methane production in the low pH subset of S_M.

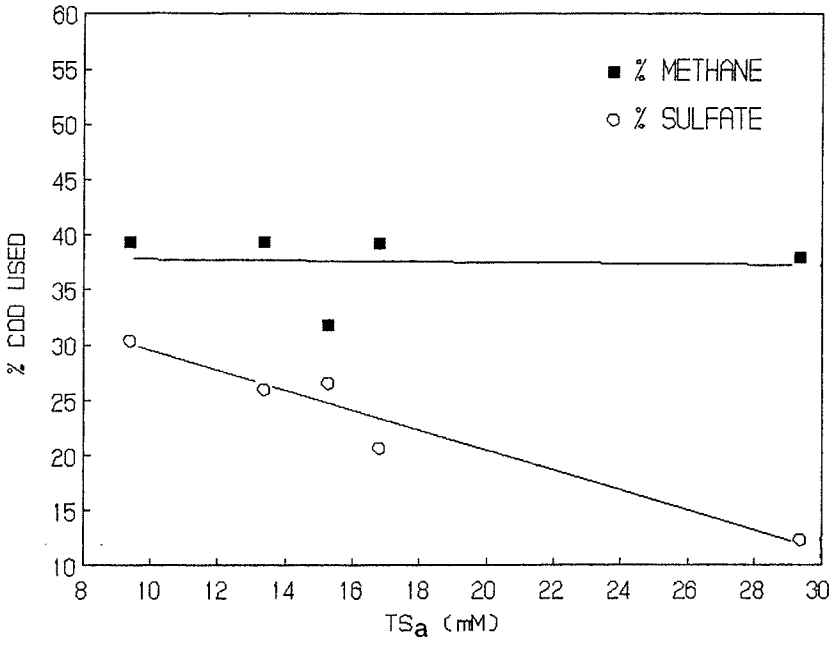


Figure 4-10. Effect of TS_a on the fraction of COD removal attributed to sulfate reduction and methane production in the high pH subset of S_M. Linear regression lines used to fit data points.

(1990) also found SRB to be more UIS sensitive than MPB when growing on propionate. Choi and Rim (1991) reported MPB to be more TS sensitive than SRB using more complex substrates. These contrasting observations may indicate that SRB responsible for degradation of simple substrates, such as propionate, acetate, and hydrogen, are more sensitive to sulfides than the SRB growing on higher substrates.

4.2.3 Degradation Stoichiometry

The stoichiometry of lactate degradation was estimated from serum bottle S_L5. This reactor was chosen because both propionate and acetate utilization were completely inhibited, while lactate degradation proceeded until completion. The molar ratios of lactate utilized to propionate formed to acetate formed were 1:0.45:0.64. This corresponds closely to that reported by Widdel and Pfennig (1982) for growth of *Desulfobulbus propionicus*. The pure culture was isolated from freshwater mud and sewage sludge. The stoichiometry for growth on lactate without sulfate reduction was 1:0.67:0.75 (lactate removed:propionate formed:acetate formed). The same culture also grew on propionate, but required sulfate for growth. The molar ratio was 1:0.76:1.0 for propionate utilized to sulfate reduced to acetate formed (Widdel and Pfennig, 1982). There was no sulfate reduction in S_L5, indicating that sulfate reduction was not related to lactate utilization. It was hard to develop a direct day-to-day correlation, however, since sulfate was only measured at the beginning and end of the experimental runs. The monitoring program was changed for Phases 3 and 4 to include daily sulfate analysis (Table 3-4). It was already stated that acetate utilization did not correspond to significant sulfate reduction (Section

4.2.2), therefore, by elimination, the majority of sulfate reduction was assumed to correspond to propionate utilization.

To calculate the propionate removed to sulfate reduced ratio, serum bottle S_L1 was used. The following assumptions were made: the ratios of lactate utilized to propionate and acetate formed calculated for S_L5 held for S_L1; and all sulfate reduction was attributed to propionate utilization. The resulting molar ratio was 1:1.53 (propionate removed to sulfate reduced).

The balanced stoichiometric equations for lactate degradation to propionate and acetate without sulfate reduction are as follows:

$$3CH_3CHOHCOO^- \rightarrow 2CH_3CH_2COO^- + CH_3COO^- + H^+ + HCO_3^- \quad 4\text{-}2$$

$$8CH_3CHOHCOO^- \rightarrow 4CH_3CH_2COO^- + 5CH_3COO^- + 3H^+ + 2HCO_3^- \quad 4\text{-}3$$

Equation 4-2 assumes no hydrogenotrophic acetogenesis, while equation 4-3 accounts for this. The respective molar ratios for these equations are 1:0.67:0.33 and 1:0.50:0.63 (lactate removed:propionate formed:acetate formed). The molar ratios of equation 4-3 correlated well to that of S_L5, which was 1:0.46:0.64, therefore was chosen as representative of lactate degradation stoichiometry for Phase 2.

The balanced stoichiometric equation for propionate degradation to acetate with sulfate reduction is as follows:

$$4CH_3CH_2COO^- + 3SO_4^{2-} \rightarrow 4CH_3COO^- + 4HCO_3^- + 3HS^- + H^+ \quad 4\text{-}4$$

The molar ratio of propionate utilized to sulfate reduced for this equation is 1:0.75. The ratio from S_L1 was 1:1.53. No explanation was developed for this difference. This relationship was investigated further in Phase 4.

4.2.4 Propionate Residuals

A propionate residual in the serum bottles inhibited by sulfides pointed to the possible inhibition of the syntrophic bacteria responsible for propionate metabolism. The effect of the UIS concentration on the residual propionate concentration for the S_L and S_M is presented in Figure 4-11. The S_M showed less residual propionate since it started with half the lactate concentration of the S_L. The propionate residuals could be due to the inhibition of the hydrogen-consuming metabolic pathways of the MPB or the SRB.

Another possible explanation may be the inhibition of the acetogenic or the incomplete-oxidizing SRB pathways (Table 2-2). The residual propionate concentrations of these two series were then plotted versus the quantity of sulfate reduced for each reactor. A strong correlation for the low pH subsets was observed, as shown in Figure 4-12. The combined information of Figures 4-11 and 4-12 strongly suggests that the SRB are responsible for the mediation of the propionate utilization step. The inhibition of propionate utilization is a frequently-noted phenomenon in anaerobic reactors. Hilton and Archer (1988) reported a rapid increase in propionate concentration when an SRB inhibitor was added to an anaerobic reactor treating molasses wastewater. Weigant et al. (1986) have shown that syntrophic propionate utilizers have extremely low growth rates and are very sensitive to an increase in the concentration of the reaction product H_2. Therefore, these organisms are dependent on other groups of bacteria to remove the H_2 produced during propionate degradation (Reaction 3 - Table 2-2). If the H_2 concentration increases the breakdown of propionate will essentially stop. However, Wilkie and Smith (1989) concluded that

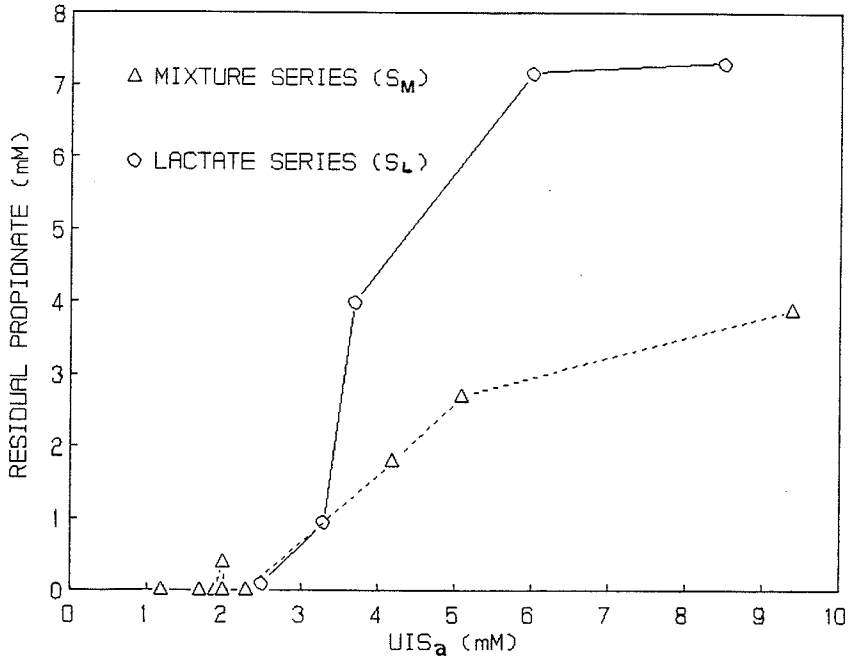


Figure 4-11. Effect of UIS_a on propionate residual in S_M and S_L. All residuals were observed in the low pH subsets.

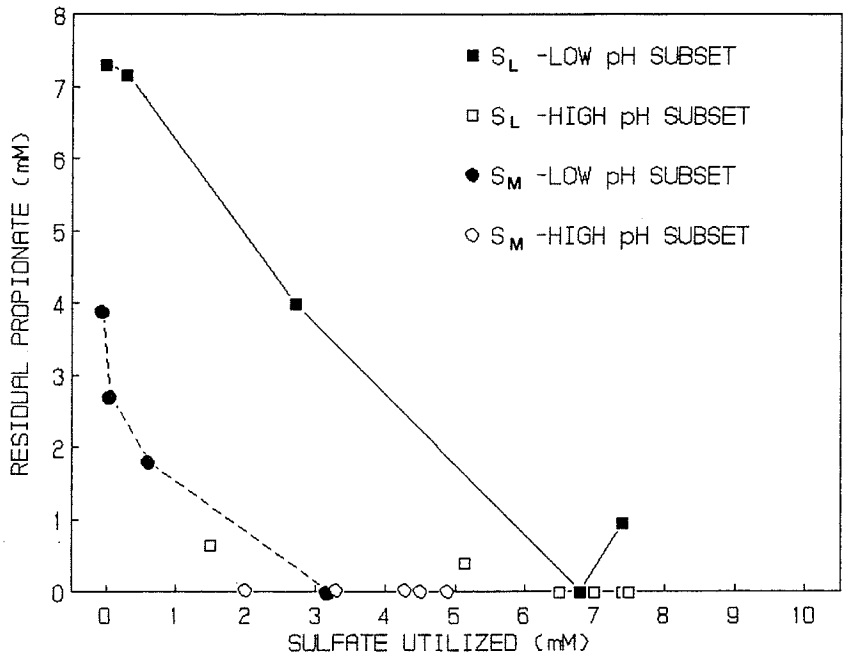


Figure 4-12. Propionate residual as a function of sulfate reduction from the low and the high pH subsets of S_M and S_L.

H_2 uptake was not a sign of process failure. Their work pointed to propionate utilization as being the most sensitive step during organic or hydraulic overloading. These observations were made in the absence of significant sulfide. From this discussion, it becomes apparent that as a result of the inhibition of SRB, the intermediate step of propionate degradation has stopped, therefore eliminating the potential for acetoclastic (Reaction 6) and hydrogenotrophic (Reaction 14) methanogenesis from the reaction products of propionate degradation (Reaction 3). Since H_2 was not measured in this investigation, it is not known whether SRB participate in H_2 removal. Unfortunately, according to Wilkie and Smith (1989), the understanding of propionate utilization is among the most primitive areas in microbiology, e.g., bacteria capable of utilizing propionate as an energy source have yet to be isolated in pure culture. The work of Widdel and Pfennig (1982) was not discussed by Wilkie and Smith (1989). Their work indicated that propionate utilization by a pure culture of *Desulfobulbus propionicus* was possible.

4.2.5 Phase 2--Observation Summary

The following observations were made: No inhibition of lactate degradation was detected; the AUR proceeded as a zero-order reaction above an acetate concentration of 2 mM (120 mg L^{-1}); the SRB did not out-compete the MPB for the available acetate in S_A; the SRB were more sensitive to increasing TS and UIS concentrations when compared to the MPB; and a direct relationship between the amount of sulfate reduced and residual propionate pointed to the critical role of SRB in mediating propionate degradation.

4.3 PHASE 3--RESULTS AND DISCUSSION

Phase 3 used inoculum that had been acclimated to either pH 7 or pH 8 conditions, depending on whether a low or high pH subset run was being conducted. The acclimation was accomplished in four breeder reactors. The average operating conditions for the breeders are presented in Table 4-5. Four sets of serum bottles were started with 1 gL^{-1} lactate as COD. Two sets were operated at pH 7, while two other sets were operated at pH 8. The set names R1, R2, R3 and R4 correspond to breeder reactors 1, 2, 3, and 4, respectively. All raw data and degradation profiles for Phase 3 are contained in Appendix B.

The pH, TS, and UIS data for all serum bottles at the start and end of the Phase 3 experimental run are presented in Table 4-6. The pH values changed significantly in the low pH reactors (R1 and R3 sets). This may be attributed to the generation of alkalinity during the conversion of sulfate to sulfide. The high pH reactors (R2 and R4 sets) showed a slight pH increase during the experimental run. The UIS concentrations were calculated using Equation 2-14, and the pH and TS values reported in Table 4-6.

Concentration versus time profiles were reviewed for each serum bottle, such as that from R37, which is shown in Figure 4-13. The following observations were based on the graphs for all 28 serum bottles, which are in Appendix B.

4.3.1 Propionate Intermediate

There was no propionate buildup and MPB were more sensitive to sulfides than the SRB, regardless of TS concentration or pH. These observations contradicted those of Phase 2, which reported a propionate buildup and that the SRB were more

Table 4-5 **Average operating conditions of the breeder reactors during 34 day period prior to the Phase 3 serum bottle startup. The breeders had been operating for 214 days when biomass removed for serum bottle inoculum**

	Breeder Number			
Parameter	1	2	3	4
Temperature, °C	35±0.2	35±0.2	35±0.2	35±0.2
pH	7.0±0.1	7.9±0.1	7.0±0.1	7.9±0.1
TS, mM	4.4±2.0	11.8±3.2	7.8±3.7	6.6±3.1
(mg L^{-1})	(142±64)	(376±103)	(249±118)	(210±100)
Average HRT†, d	29.0	25.7	26.1	25.2
Feed molar ratios				
Lactate:Acetate :Sulfate	1:1.67:2.83	1:1.67:2.83	1:1.67:2.83	1:1.67:2.83
COD:SO_4(gg^{-1})	0.79	0.79	0.79	0.79
OLR‡, gCOD $L^{-1}d^{-1}$				
- each batch feed	0.92	0.92	0.92	0.92
- average	0.31	0.36	0.35	0.37
Biogas production§ mL d^{-1}	282	205	271	234
Specific gas production✣ mL biogas/g COD added	455	285	386	321

† Average HRT determined using the cumulative feed volume during the 34 day period

‡ OLR given as per day rates, while in reality feeding took place 3 times per week

§ Biogas production determined using the cumulative production during the 34 day period

✣ Specific gas production determined using both cumulative gas production and cumulative COD fed during the 34 day period

Table 4-6. The pH, total sulfide (TS) and un-ionized sulfide (UIS) data for all serum bottles used in Phase 3.

Serum Bottle		pH		TS mM (mgL^{-1})		UIS mM (mg L^{-1})	
		Start	End	Start	End	Start	End
R1-	1	7.0	7.4	5.6(180)	21.3(680)	2.5(79)	5.0(161)
	2	7.0	7.4	7.0(225)	23.1(740)	3.1(99)	5.5(176)
	3	7.0	7.3	8.6(275)	23.8(760)	3.8(121)	6.7(214)
	4	7.0	7.4	10.3(330)	24.1(770)	4.5(145)	5.7(183)
	5	7.0	7.3	13.3(425)	27.5(880)	5.8(186)	7.8(248)
	6	7.0	7.4	16.3(520)	31.3(1000)	7.1(228)	7.4(237)
	7	7.0	7.4	18.8(600)	34.4(1100)	8.2(263)	8.2(261)
R2-	1	8.0	8.0	24.7(790)	42.2(1350)	1.8(57)	3.1(88)
	2	8.0	8.1	34.4(1100)	50.9(1630)	2.5(80)	3.0(95)
	3	8.0	8.0	42.8(1370)	61.6(1970)	3.1(99)	4.5(143)
	4	8.0	8.0	55.9(1790)	75.6(2420)	4.1(130)	5.5(175)
	5	8.0	8.0	73.1(2340)	90(2880)	5.3(170)	6.5(209)
	6	8.0	8.0	90.6(2900)	93.1(2980)	6.6(210)	6.8(216)
	7	8.0	8.1	107.8(3450)	111.3(3560)	7.8(250)	6.5(208)
R3-	1	7.1	7.3	5.9(190)	19.4(620)	2.3(73)	5.4(174)
	2	7.0	7.3	6.7(215)	22.5(720)	2.9(94)	6.3(203)
	3	7.0	7.3	8.3(265)	23.4(750)	3.6(116)	6.6(211)
	4	7.1	7.3	9.7(310)	25.6(820)	3.7(119)	7.2(231)
	5	7.1	7.3	13.0(415)	27.5(880)	5.0(159)	7.8(248)
	6	7.1	7.3	15.9(510)	29.1(930)	6.1(195)	8.2(262)
	7	7.1	7.4	18.4(590)	33.8(1080)	7.1(226)	8.7(279)
R4-	1	8.0	8.1	25.0(800)	41.9(1340)	1.8(58)	2.4(78)
	2	7.9	8.2	34.7(1110)	37.5(1200)	3.1(99)	2.5(80)
	3	7.9	8.1	43.1(1380)	59.4(1900)	4.3(137)	3.5(111)
	4	7.9	8.1	56.3(1800)	75.3(2410)	5.0(161)	4.4(141)
	5	7.9	8.1	73.4(2350)	86.9(2780)	6.6(210)	5.7(181)
	6	7.9	8.1	90.9(2910)	98.8(3166)	8.2(261)	5.8(185)
	7	8.0	8.1	108.1(3460)	111.3(3560)	8.7(279)	6.5(208)

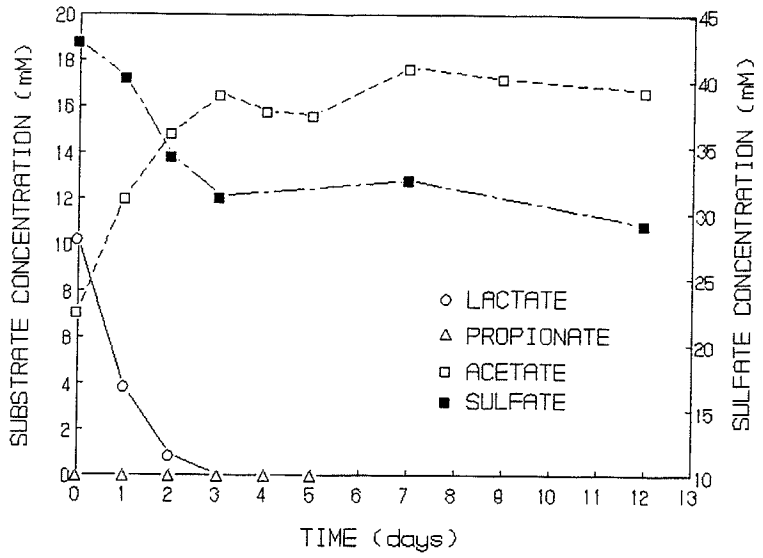


Figure 4-13. The concentration versus time profile of R37 in Phase 3. The average UIS concentration was 714 mM (229 mg L^{-1}).

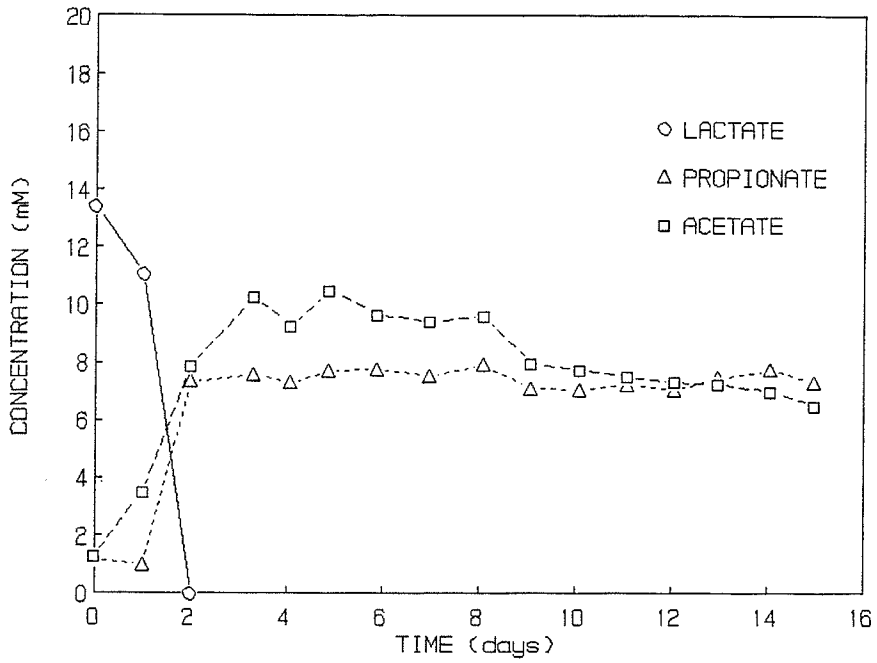


Figure 4-14. The concentration versus time profile of S_L5 in Phase 2.

sulfide sensitive than the MPB. The concentration versus time profile of serum bottle S_L5 from Phase 2 is presented in Figure 4-14. The UIS concentrations in serum bottles R37 and S_L5 (Figure 4-13 and 4-14, respectively) were almost identical (Table 4-7). Sulfate was not measured daily during Phase 2, but was in Phase 3.

Table 4-7. The comparison of UIS concentrations in serum bottles R37 and S_L5 from Phases 3 and 2, respectively

Description	UIS, mM (mgL^{-1})		
	Start	End	Average
Phase 3 - R37	6.09(195)	8.19(262)	7.14(229)
Phase 2 - S_L5	6.16(197)	8.25(264)	7.20(231)

When comparing the latter two figures, the response to similar starting substrate conditions was remarkably different. Similar to all serum bottles in Phase 3 there was no propionate buildup in R37, but considerable buildup in S_L5 from Phase 2. The SRB activity was measured from sulfate depletion observations in Phase 2. Sulfate concentrations were identical in S_L5 at the start and end of the run, indicating no SRB activity. However, as shown in Figure 4-13, there was significant sulfate reduction in R37, which indicates active SRB. It was also interesting to note that lactate was removed during Phase 2 without SRB activity (Section 4.2.3 and Figure 4-14), but lactate removal was tied to SRB activity in Phase 3 (R37; Figure 4-13).

4.3.2 Lactate Stoichiometry

The acetate removal observed in the serum bottles was not a function of SRB activity. A typical concentration versus time profile which had significant acetate depletion, i.e., no inhibition by sulfide was observed in R41 (Figure 4.15). The removal of acetate proceeded independently of sulfate reduction. Lactate removal, however, proceeded concurrently with sulfate reduction.

In general the lactate degradation to sulfate reduction molar ratios were consistent. In the reactors with significant lactate removal (R11 to R17 and R41 to R45), the ratios were 1:0.85±0.13 and 1:0.75±0.11, respectively. This suggests that at low pH the lactate degradation used more sulfate. The ratios differed dramatically for the reactors which did not completely remove lactate (R46 and R47). The molar ratios for these two reactors were 1:1.4 and 1:1.7, respectively. These values were approximately double the aforementioned means. This suggests that sulfate was removed from the bulk liquid before lactate during metabolism, or that lactate uptake was more sulfide sensitive than sulfate uptake. The lactate utilized to acetate formed molar ratios were investigated in serum bottles which had no acetate removal, but complete lactate degradation (R16, R17, R24, R25, R35, R36, R37, R43, R44, R45). The acetate formed was calculated as the difference between the start-up acetate concentration and that measured after all lactate was removed. The mean molar ratio for all the reactors was 1:0.99±0.12 (lactate:acetate).

The stoichiometric equation which represents lactate conversion to acetate by SRB is as follows:

$$12\ CH_3CHOHCOO^- + 6SO_4^{2-} \rightarrow 12CH_3COO^- + 12HCO_3^- + 6HS^- + 6H^+ \qquad 4\text{-}5$$

Equation 4-5 implies that the molar ratio should have been closer to 1:0.5. No plausible explanation was rationalized for the discrepancy between the observations and equation 4-5. This relationship was investigated further in Phase 4.

The lactate:acetate ratio had a strong relationship to the stoichiometric equation (4-5) presented. These observations suggest that a completely different metabolic pathway was utilized in the biomass when compared to Phase 2.

4.3.3 SRB and MPB Activities

The correlation of lactate removal and sulfate reduction led to the assumption that SRB were responsible for lactate removal. This relationship was confirmed in Phase 4. The variability of the sulfate results led to the questionability of their accuracy. For this reason, the use of lactate removal rates and overall sulfide production were investigated for suitable representation of SRB activity. Overall sulfide production was estimated from the aqueous TS concentrations at the start and end of the serum bottle runs. The initial removal rates of lactate and overall sulfide production were plotted against the initial UIS concentration in each serum bottle (Figure 4-16). The pH 7 and pH 8 results were taken from the R1 and R4 serum bottle sets, respectively. The lactate removal rates appeared to be more sensitive than sulfide production, but it must be remembered that sulfide production was measured over the entire length of the serum bottle run, while lactate removal was measured daily. The lactate removal rates were expected to give the best representation of SRB activity.

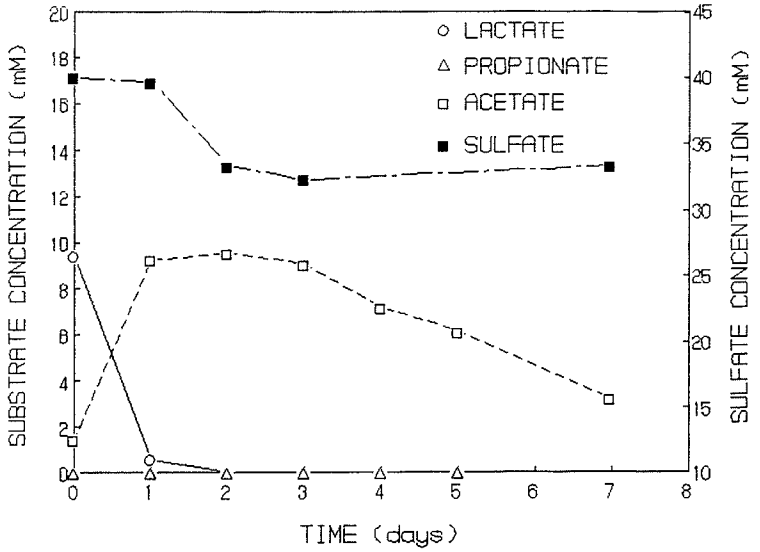


Figure 4-15. Concentration versus time profile of R41 during Phase 3.

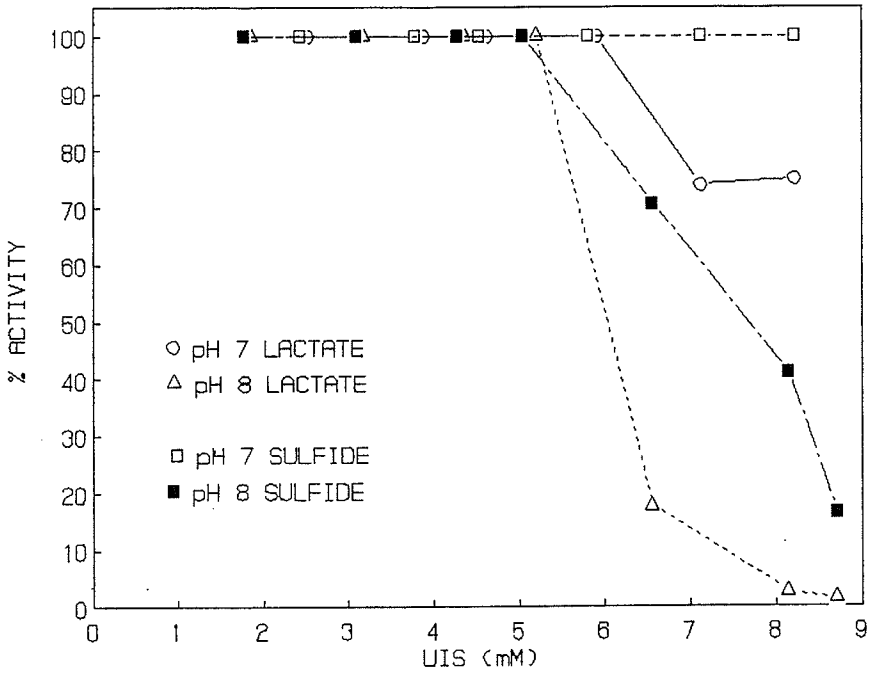


Figure 4-16. Effect of UIS on lactate removal rates and sulfide production during Phase 3. Each point represents a serum bottle. The pH 7 and pH 8 data were represented by serum bottle sets R1 and R4, respectively.

Similarly for MPB, the acetate removal rates and overall methane productions were plotted versus the initial UIS concentration for each serum bottle (Figure 4-17). Again, pH 7 and pH 8 results were taken from the R1 and R4 serum bottle sets, respectively. The acetate removal rates were selected to represent MPB activity, because the acetate removal rates were based on daily measurements, while the methane measurements were over the entire experimental run. The acetate removals would best represent the sensitivity of MPB to elevated sulfide.

The effect of initial UIS on the MPB and SRB activities during Phase 3 are presented in Figure 4-18. The MPB were equally sensitive to the UIS at a pH of 7 or 8, while the SRB had a different response to UIS at the two pH levels.

The UIS concentration corresponding to 50% inhibition of the MPB was 3.1 mM (100 mg L^{-1}). This value approximates those found for acetate removal in Phase 1, as reported in Table 4-1. The 50% inhibition concentrations of UIS for lactate removal, however, do not relate well between Phases 1 and 3. The UIS concentrations corresponding to 50% inhibition of lactate removal in Phase 1 averaged 11.1 mM (355 mg L^{-1}), while the UIS concentrations in the Phase 3 pH 8 set (R4) was only 5.8 mM (185 mg L^{-1}). This observation was due to the higher TS concentration in Phase 3. The Phase 3 pH 7 set (R1) did not reach 50% inhibition at the UIS concentrations investigated (< 8.4 mM; 300 mg L^{-1}). The higher sensitivity in the pH 8 set of Phase 3 (Figure 4-18) may relate to the high TS concentrations needed to achieve UIS concentrations equal to those of the pH 7 sets (Table 4-6). The same activity values from Figure 4-18 were plotted versus initial TS concentrations in Figure 4-19. The SRB were more sensitive to TS than UIS concentrations in the pH

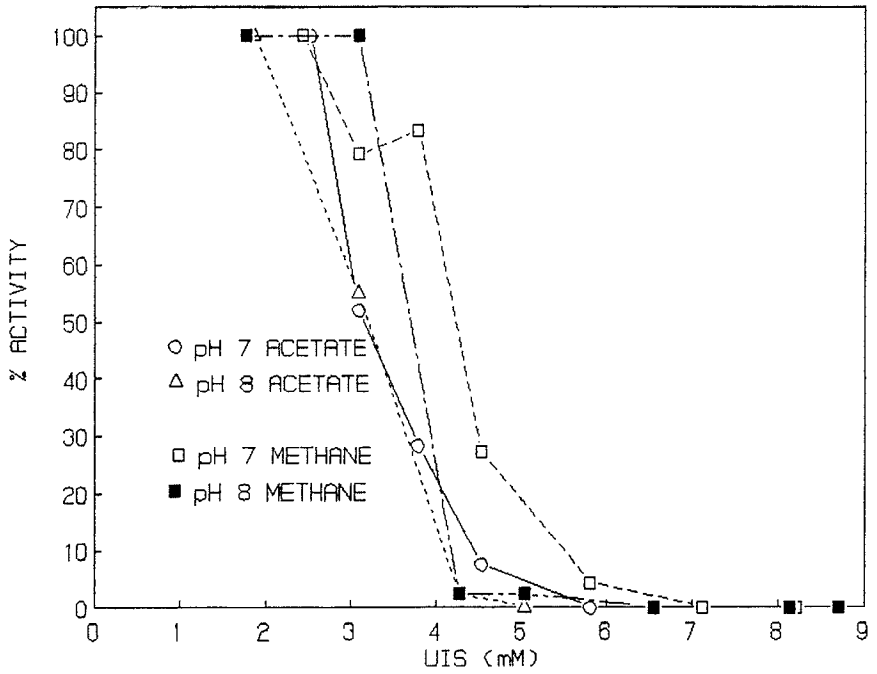


Figure 4-17. Effect of UIS on acetate removal rates and methane production during Phase 3.

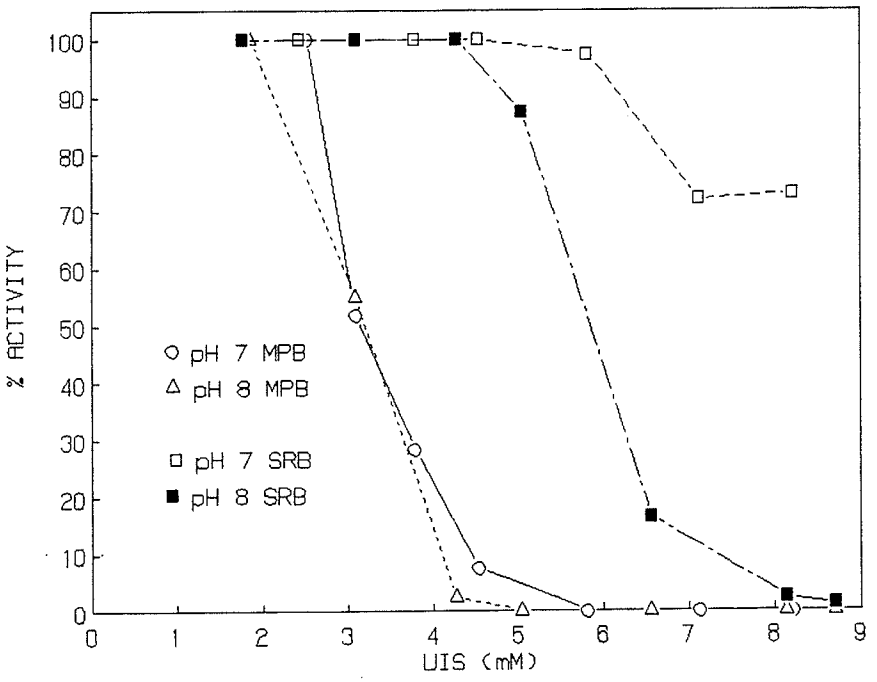


Figure 4-18. Effect of UIS on MPB and SRB activity during Phase 3. Serum bottle sets R1 and R4 represented the pH 7 and pH 8 sets, respectively.

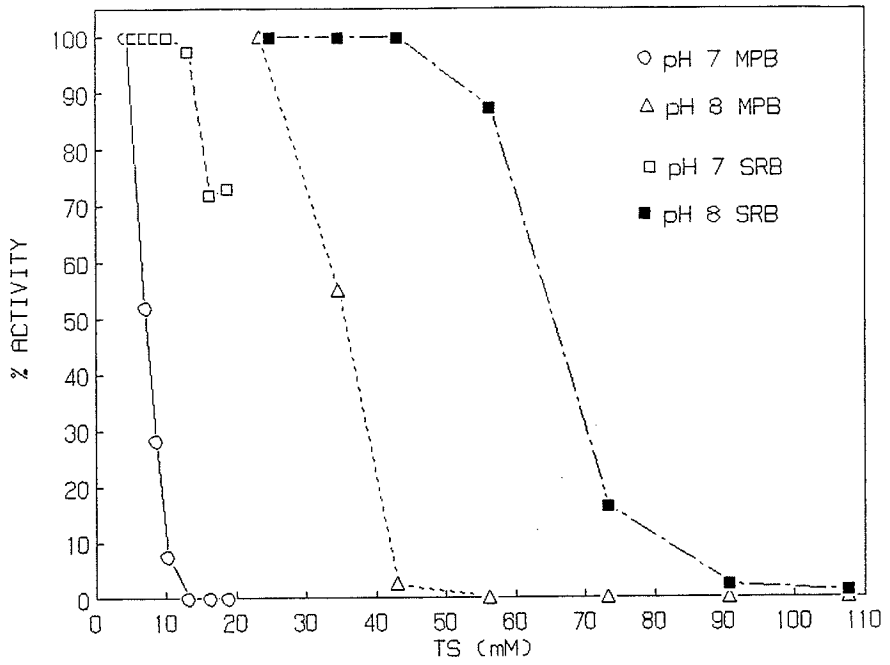


Figure 4-19. Effect of TS on MPB and SRB activity during Phase 3. Serum bottle sets R1 and R4 represented pH 7 and pH 8 sets, respectively.

8 set. The slight sensitivity of the pH 7 SRB (Figure 4-19) was probably related to the relatively high fraction of UIS in these serum bottles (Figure 4-18).

4.3.4 Phase 3--Observation Summary

The following observations were made: no propionate buildup was detected; the MPB were more sensitive to sulfide compared to the SRB; the inhibition of MPB correlated well with UIS concentrations, not TS concentrations; the SRB were slightly inhibited by the UIS concentrations used in this Phase (< 9.4 mM, or < 300 mg L^{-1}); the SRB were completely inhibited by high TS concentrations; acetate removal was a function of MPB; lactate was converted to acetate on a 1:1 molar ratio basis; and

sulfate and lactate removal proceeded concurrently at a molar ratio of 0.8 ± 0.2 (sulfate removed:lactate removed). The latter ratio was somewhat higher than the theoretical ratio of 0.5.

4.4 PHASE 4--RESULTS AND DISCUSSION

The observations made during Phase 3 seemed to be in conflict with those made during Phase 2. The most relevant observation was the different lactate degradation pathways observed in the two phases. Propionate was observed as an intermediate during the degradation of lactate in Phase 2, but not in Phase 3. As described in Section 3.4, the operating conditions of the serum bottles in Phase 4 were chosen to isolate the experimental differences between the two previous phases, as well as to investigate the effect of H_2 on propionate degradation. Hydrogen gas was measured in the headspace of the serum bottles, and all discussions will be based on gaseous headspace levels. Aqueous concentrations may be determined using the appropriate tables and Henry's law (Perry et al., 1984).

The biomass inoculum for Phase 4 was taken from either breeder reactor 1 or 4 (pH 7 and pH 8, respectively), as well as the City of Winnipeg's North End Water Pollution Control Centre (NEWPCC). The latter served as inoculum that had not been acclimated to the high lactate and high sulfate conditions of the breeder reactor feed. The average operating conditions for breeders 1 and 4 are presented in Table 4-8. Eight sets of serum bottles were started with 1 g L^{-1} COD. The description of the operating conditions for each set was given in Table 3-13. All raw data and degradation profiles are contained in Appendix C. The pH, TS, and UIS data for all

Table 4-8. Average operating conditions of breeder reactors 1 and 4 during the 32 day period prior to the Phase 4 serum bottle startup. The breeders had been operating for 306 days when biomass removed for serum bottle inoculum.

	Breeder Number	
Parameter	1	4
Temperature, °C	35±0.2	35±0.2
pH	7.1±0.2	7.8±0.2
TS, mM	8.2±2.9	6.4±3.5
(mg L^{-1})	264±92	206±110
Average HRT† (d)	58.5	95
Feed molar ratios:		
Lactate:acetate:sulfate	1:1.67:1.42	1:1.67:1.42
COD:SO_4(gg^{-1})	1.59	1.59
OLR‡, g COD L^{-1} d^{-1}		
- each batch feed	0.92	0.92
- average	0.31	0.19
Biogas production§, mL d^{-1}	275	114
Specific gas production✣	444	300
mL biogas/g COD added		

† Average HRT determined using the cumulative feed volume during the 32 day period

‡ OLR given as per day rates, while in reality feeding took place 3 times per week

§ Biogas production determined using the cumulative production during the 32 day period

✣ Specific gas production determined using both cumulative gas production and cumulative COD fed during the 32 day period

Table 4-9. The pH, TS, and UIS data at the start and end of Phase 4 for all serum bottles.

Serum Bottle		pH		TS mM (mgL^{-1})		UIS mM (mg L^{-1})	
		Start	End	Start	End	Start	End
A-	1	6.9	7.1	12.5(400)	13.8(440)	6.2(198)	5.3(168)
	2	7.0	7.1	15.6(500)	17.5(560)	6.8(219)	6.7(214)
	3	7.1	7.2	20.0(640)	21.9(700)	7.7(245)	7.2(231)
B-	1	7.2	7.4	4.4(140)	13.8(440)	1.4(46)	3.3(104)
	2	7.2	7.3	7.5(240)	15.0(480)	2.5(79)	4.2(135)
	3	7.2	7.4	15.0(480)	20.0(640)	4.9(158)	4.8(152)
C-	1	7.1	7.2	3.8(120)	9.4(300)	1.4(46)	3.1(99)
	2	7.1	7.2	7.5(240)	9.4(300)	2.9(92)	3.1(99)
	3	7.1	7.2	21.3(680)	15.0(480)	8.1(260)	4.9(158)
D-	1	7.0	7.3	12.5(400)	21.3(680)	5.5(175)	6.0(191)
	2	7.0	7.2	16.3(520)	25.0(800)	7.1(228)	8.3(264)
	3	7.1	7.3	17.5(560)	30.0(960)	6.7(214)	8.4(270)
E-	1	7.0	7.2	13.8(440)	17.5(560)	6.0(193)	5.8(185)
	2	7.0	7.4	15.0(480)	25.0(800)	6.6(211)	5.9(190)
	3	7.1	7.3	22.5(720)	26.3(840)	8.6(276)	7.4(236)
F-	1	7.0	7.2	11.3(360)	15.0(480)	4.9(158)	4.9(158)
	2	7.1	7.2	13.8(440)	23.1(740)	5.3(168)	7.6(244)
	3	7.1	7.2	18.8(600)	26.3(840)	7.2(230)	2.4(76)
G-	1	8.2	8.0	12.5(400)	21.3(680)	0.6(19)	1.5(49)
	2	8.3	8.1	31.3(1000)	36.3(1160)	1.2(38)	2.1(68)
	3	8.3	8.1	62.5(2000)	62.5(2000)	2.3(75)	3.7(117)
H-	1	8.3	8.1	12.5(400)	30.0(960)	0.5(15)	1.8(56)
	2	8.3	8.1	31.3(1000)	38.8(1240)	1.2(38)	2.3(72)
	3	8.3	8.1	63.8(2040)	65.0(2080)	2.4(77)	3.8(122)

serum bottles at the start and end of the Phase 4 experimental run are presented in Table 4-9.

The serum bottle sets will be discussed one at a time. Each discussion will outline the objectives of the particular set and the relevant observations.

4.4.1 Low pH Control Set

Set A was the pH 7 control set, such that the conditions in these reactors were similar to the serum bottles from the R1 set in Phase 3 discussed in Section 4.3. The results were similar to those of the R1 set. The removal rates were higher due to the larger mass of inoculum used in Phase 4, as compared to Phase 3. These startup differences were outlined in Chapter 3.

The SRB and MPB responses were identical to that of Phase 3, i.e., MPB were more sensitive to sulfides than the SRB, no propionate buildup in the high UIS reactor, and sulfate reduction was proportional to lactate removal. The lactate removed to sulfate reduced molar ratio was 1:0.62±0.05 for these three reactors, while the lactate removed to acetate formed molar ratio was 1:1.18 in A3. The stoichiometry of equation 4-5 suggests molar ratios of 1:0.5:1 for lactate removed to sulfate reduced to acetate formed. These ratios approximate those predicted by Equation 4-5 more accurately than the Phase 3 results. This was probably due to the increased quality control utilized on the sulfate analysis during Phase 4.

The headspace hydrogen level was below 2.0 Pascals (Pa) or 20 parts per million (ppm) in reactor A1 during the entire experimental run (graph not shown - see Appendix C). This reactor had the lowest sulfide concentration. Reactor A2 had a pulse of H_2 (3.2 Pa or 31.5 ppm) after one day of operation, but was quickly lowered to below 2.0 Pa (20 ppm) by day 2. Reactor A3 had the highest sulfide concentration (Table 4-9) and also the highest recorded H_2 pulse of 8.1 Pa (80.1 ppm) (Figure 4-20). However, the H_2 was quickly removed and was down to 2.9 Pa (28.5 ppm) the next day. Mosey and Fernandes (1989) observed similar H_2 pulses in

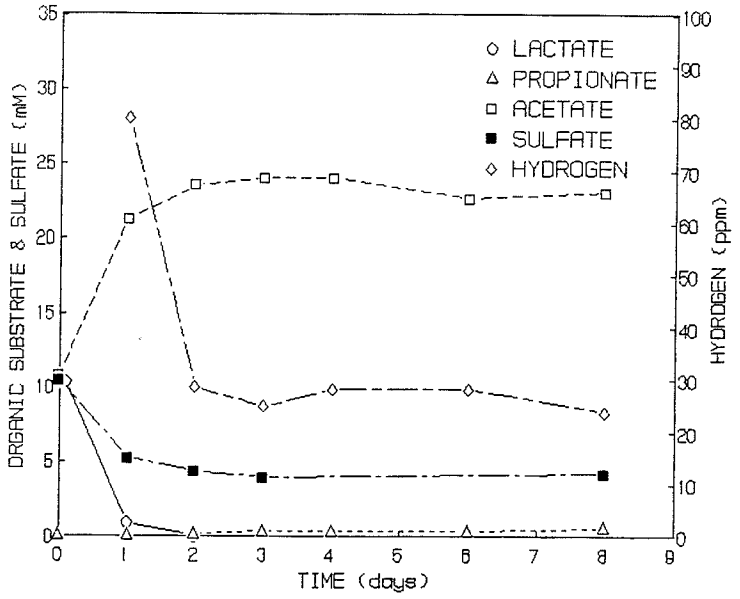


Figure 4-20. Concentration versus time profile of A3 during Phase 4.

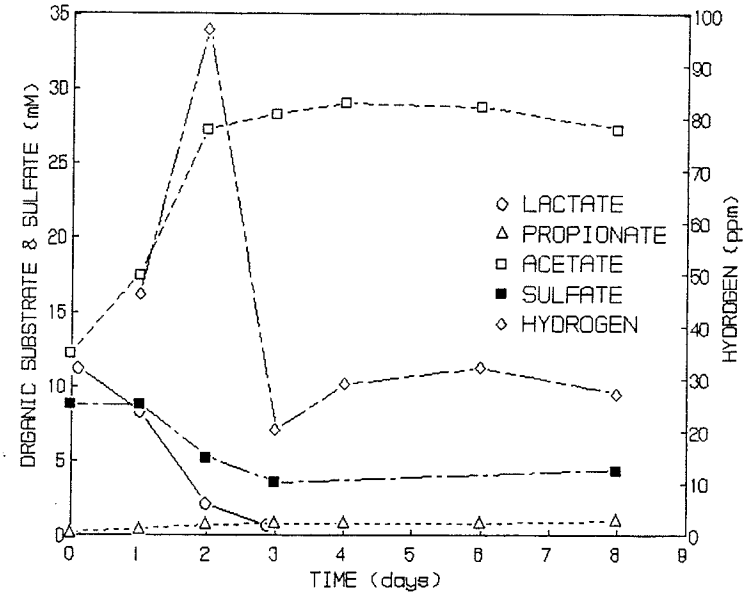


Figure 4-21. Concentration versus time profile of B3 during Phase 4.

response to feeding episodes. Using dried skim milk powder, they observed pulses of 6.1 to 14.2 Pa (60 to 140 ppm) after feeding, while the background H_2 concentration was 1.0 to 2.5 Pa (10 to 25 ppm).

4.4.2 Effect of pH Adjustment

Set B investigated the effect of adjusting the pH of the inoculum down to 7 from 8. The inoculum was taken from breeder 4, which was operated at pH 8. This pH adjustment was done in Phase 2 because there was no source of biomass acclimated to pH 7 conditions. No propionate buildup was observed in these reactors.

The lactate and sulfate removal rates (SRB activity) were lower than set A, but were still not as sulfide sensitive as the MPB (Figure 4-21). The H_2 profiles were similar to set A, except for B3, which peaked on the second day of operation rather than the first (Figure 4-21). This was probably due to the significant lactate removal which took place between the first and second days of operation.

The lactate removed to sulfate reduced molar ratio was 1:0.52±0.07 in set B. This relates well to Equation 4-5. The lactate removed to acetate formed molar ratio was 1.4. This ratio is very high, which suggests that the pH shock may cause the cells to release acetate into the bulk liquid through the lyzing of cells or other mechanisms.

4.4.3 Effect of Unacclimated Inoculum

Set C investigated the use of inoculum which had not been acclimated to the high sulfate conditions. The inoculum was taken from the municipal anaerobic digesters at the NEWPCC in Winnipeg. All other operating and environmental conditions were identical to set A. Propionate was observed as an intermediate of lactate degradation in all three serum bottles of the set. The lactate degraded to acetate and propionate, which was subsequently removed in reactors C1 and C2. These two reactors had the lowest sulfide concentrations (Table 4-9). A residual propionate concentration was observed in C3 (Figure 4-22), which operated at a high UIS concentration of 8.1 mM (260 mg L^{-1}). It was difficult to develop MPB and SRB activity relationships due to the limited number of serum bottles in the set (3). However, trends were evaluated such that comparisons could be made to Phases 2 and 3.

Sulfate removal proceeded proportionally to propionate removal in C1 (Figure 4-23), but did not proceed until significant propionate removal had already taken place in C2 (Figure 4.24). Sulfate reduction proceeded without significant propionate reduction in C3 (Figure 4-22). In an effort to separate the variations in sulfate reduction, two activity periods were investigated: Day 0 to 4 and Day 0 to 8. The effect of initial UIS on the activity is presented in Figure 4-25. The MPB activities were based on methane production (Day 0 to 8) and are presented in Figure 4-25. As shown in this figure, whether MPB were more or less affected by UIS was a matter of boundary conditions. The MPB were less sensitive when compared to the sulfate reduction to day 4, but were more sensitive when compared to the day 8 sulfate removals.

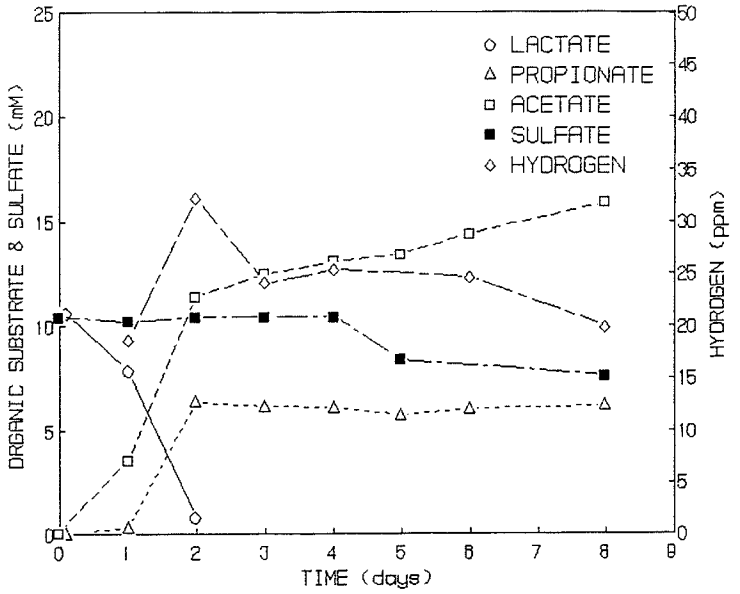


Figure 4-22. Concentration versus time profile of C3 during Phase 4.

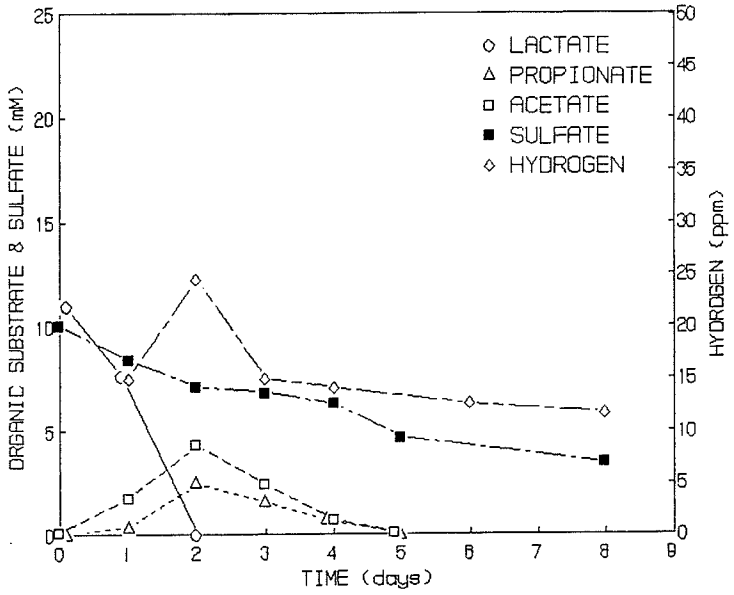


Figure 4-23. Concentration versus time profile of C1 during Phase 4.

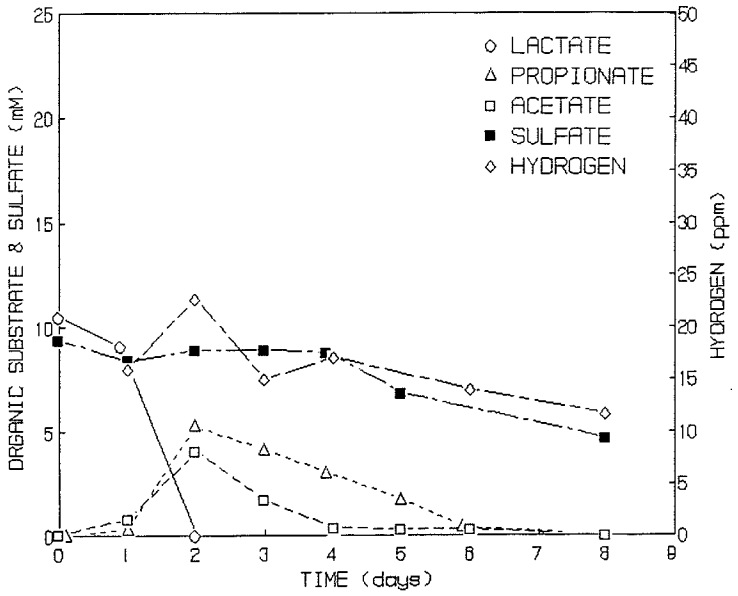


Figure 4-24. Concentration versus time profile of C2 during Phase 4.

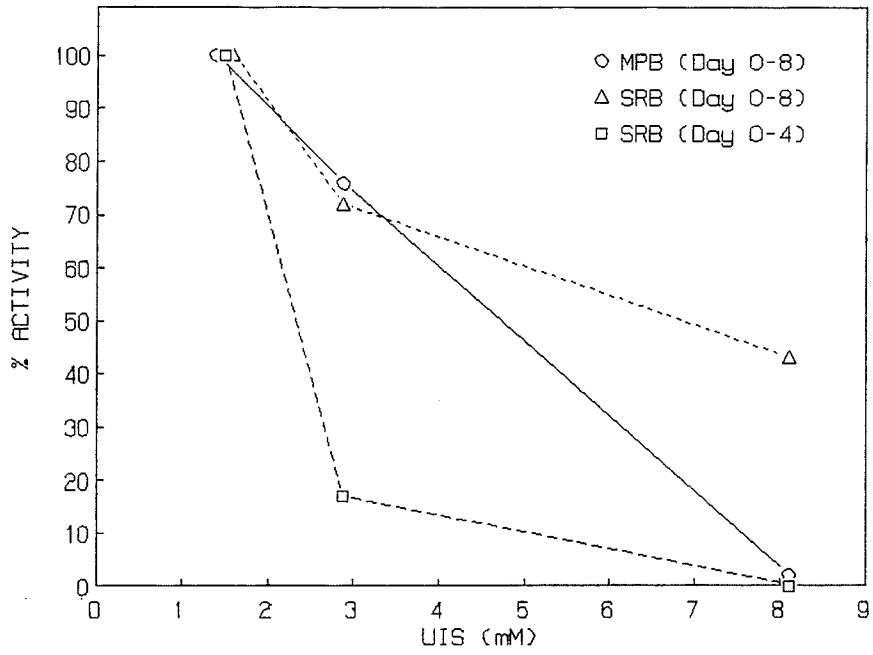


Figure 4-25. Effect of UIS on SRB and MPB activity on set C of Phase 4.

Since methane values for day 4 were not taken, the only fair comparison should be the removals to day 8. Based on this, the MPB were observed to be more sensitive to UIS than the SRB.

This does not support the observations of Phase 2 which found the SRB were more sensitive (Figures 4-9 and 4-10). Sulfate reduction and propionate removal were significantly inhibited at a UIS concentration of 3.61 mM (116 mg L^{-1}) in Phase 2. This response was similar to that observed during the first four days of Phase 4. The Phase 2 experimental run was 16 days long and did not experience the secondary sulfate removal observed in the latter days of Phase 4.

This secondary sulfate removal was considered to be real, after analyzing the sulfate samples a second time. The secondary sulfate removal was not related to H_2 removal (Figures 4-22 to 4-24); rather it may have been caused by the presence of other organic substrates which were not analyzed. Unlike the acclimated inoculum, which had only received the simple substrates lactate and acetate for the 306 days prior to the Phase 4 experimental run, the unacclimated inoculum came from full-scale municipal digesters. This inoculum source would contain many complex organics and since the inoculum made up 90% of the serum bottle contents, the concentration of these organics would have been significant. The sulfate reduction may have been affected by the presence of these other substrates not analyzed. No other plausible explanation of this secondary sulfate removal was found. This explanation fits the observations made by Choi and Rim (1991) and Parkin et al. (1990). The former observed SRB to be much less sensitive than MPB when growing on complex substrate, while the latter observed the MPB to be less sulfide-sensitive

than SRB when growing on propionate and acetate. The secondary sulfate removals observed in this thesis investigation support the complex substrate observations of Choi and Rim (1991). The sulfate removals from day 0 to 4 are related to simpler substrates as investigated by Parkin et al. (1990).

Reactor C3 had no propionate or acetate removal and complete removal of lactate, therefore should yield a picture of stoichiometry of lactate degradation. Upon complete removal of lactate, acetate production continued (Figure 4-22), which indicates that acetate was being produced from other sources. This observation confirms the previous speculation that more complex organics were continuing to degrade in these reactors, due to inoculum obtained from the municipal anaerobic digester. The acetate production rate from day 3 on was 0.76 mM d^{-1}. The acetate production was corrected using this value in an effort to eliminate sources of acetate other than lactate.

The lactate removed to propionate formed to acetate formed molar ratio for C3 was 1:0.57:0.96. Equation 4-3 predicts a molar ratio of 1:0.50:0.63, therefore approximates propionate production for C3, but not acetate production. The higher acetate production may again be attributed to the presence of more complex substrate. Pankhania et al. (1986) observed growth of *Desulfovibrio vulgaris* on lactate and hydrogen yielded two times as much acetate as growth on lactate alone. No sulfate reduction was observed for either condition. These ratios for C3 approximate those observed by Widdel and Pfennig (1982) for a pure culture of *Desulfobulbus propionicus* (1:0.67:0.75), which also did not have any sulfate reduction.

To estimate the propionate utilized to sulfate reduced ratio, reactor C1 was used. It was assumed that the lactate degradation to propionate produced ratio determined for C3 occurred and that all sulfate reduction was related to propionate utilization. The molar ratio of propionate utilized to sulfate reduced was then calculated after each day from day 1 to day 4 inclusive. This was done to exclude the secondary sulfate reduction phenomena. The mean for the four days was 1:0.76±0.09. This indicates a strong correlation with equation 4-4, which predicts a ratio of 1:0.75. Widdel and Pfennig (1982) also reported a molar ratio of 1:0.76 for a pure culture of *Desulfobulbus propionicus*. The correlations to equations 4-3 and 4-4 conform to similar observations of Phase 2.

The H_2 profiles were similar in C1 and C2 with both peaking on day 2 at < 2.5 Pa (< 25 ppm) and leaving a residual of < 1.5 Pa (< 15 ppm). The H_2 levels were slightly higher in C3, which peaked at 3.2 Pa (32 ppm) on day 2 of the experimental run and left a residual of approximately 2.0 Pa (20 ppm) (Figure 4-22).

To study the effects of H_2 and UIS on propionate degradation, the average propionate removal rates were calculated. The starting propionate concentrations were estimated using equation 4-3 and the total lactate removed in the reactors. The starting concentrations were then divided by the number of days required to completely remove the propionate. The periods used were days 0 to 4 and 0 to 6 for C1 and C2, respectively. There was no propionate removal in C3, therefore the rate was taken as zero. The calculated rates were plotted versus initial UIS and H_2 in Figure 4-26. The UIS values used were the starting concentrations, while the H_2 values were the averages during propionate degradation.

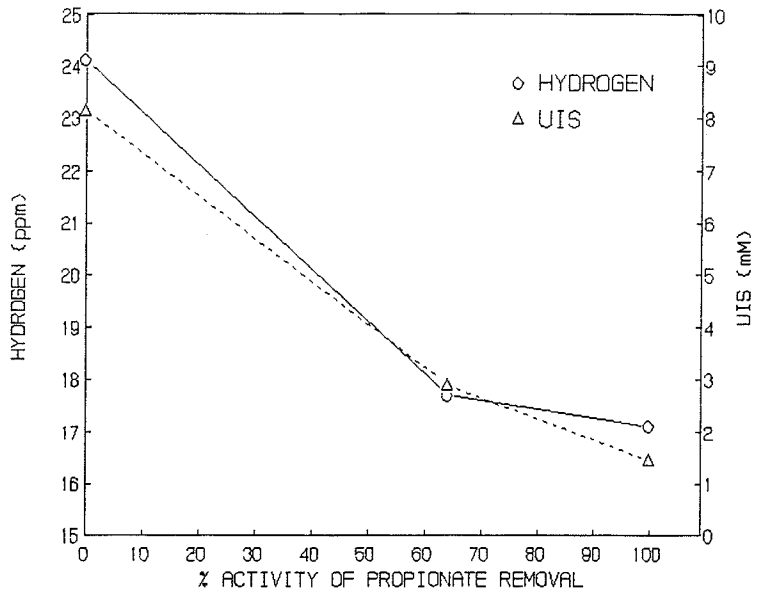


Figure 4-26. Effect of UIS and H_2 on propionate removal rates in set C.

The sensitivity of propionate degradation to UIS was again demonstrated (Figure 4.26). The degradation of propionate also appeared to be sensitive to elevated H_2 concentrations. However, this was not supported by the H_2 degradation profiles in the reactors. Propionate degradation in C1 proceeded when H_2 was at 2.5 Pa (24.4 ppm) (Figure 4.23), but did not proceed in C3 when H_2 level was down to 2.0 Pa (19.8 ppm) (Figure 4-22). This indicates that H_2 was not inhibiting propionate degradation in C3. Add to this the observation that H_2 degradation proceeded even though propionate degradation was completely inhibited in C3 (Figure 4-22). These observations suggest that H_2 buildup was not the cause of propionate inhibition, therefore mechanism B of Figure 3-2 was responsible for propionate buildup. This conforms to the thermodynamics of H_2 inhibition of propionate degradation

presented in Figure 2-3. This figure suggested that H_2 partial pressures below 10.1 Pa (100 ppm) will not inhibit propionate reduction.

McCarty and Smith (1984) reported the theoretical window (no H_2 inhibition of propionate utilization) will occur at headspace H_2 partial pressures of 0.3 to 12.8 Pa (3.2 to 125.9 ppm). Hayes and Hall (1983) reported headspace H_2 partial pressures of 4.0 to 6.4 Pa (39.8 to 63.1 ppm) during methanogenic conditions with propionate as the sole carbon source. Another group observed background H_2 levels of 7 to 20 Pa (69.1 to 197.4 ppm) during propionate utilization by a methanogenic culture (Boone and Xun, 1987). Harper and Pohland (1986) suggest that at a headspace H_2 partial pressure of > 10.1 Pa (> 100 ppm) will lead to inhibition of propionate utilization. During anaerobic digestion of milk sugars Mosey and Fernandes (1989) observed background H_2 levels of 1.0 to 2.5 Pa (10 to 25 ppm). These same researchers suggested H_2 monitoring should be advantageous for detecting shock loads. Hickey et al. (1987) observed rapid accumulation of H_2 in headspace when 70 percent inhibition of methanogenesis occurred, however, when inhibition was less severe, H_2 accumulated to levels only slightly above controls. Based on their observations, Hickey et al. (1987) suggested that there may be some limits on the potential for using H_2 as an early warning indicator of process upsets.

It would appear that H_2 monitoring will not offer the global process monitoring coverage most researchers had hoped for. The monitoring of H_2 can detect shock loads, but did not correlate to propionate inhibition in this thesis investigation or to mild inhibition episodes using various toxicants (Hickey et al., 1987).

4.4.4 Effect of H_2 on Propionate Removal

Set D investigated the effect of flushing the serum bottle headspace with 100 percent H_2 gas. The serum bottles were started identically to the pH 7 control set A. However, on day 1, the headspace of the serum bottles were flushed with H_2 gas for several minutes. The inoculum was taken from breeder 1 and the serum bottles were started with 1 g L^{-1} lactate plus nutrients (including sulfate) on day 0 and day 1.

No propionate buildup was observed in this set. Lactate and sulfate were removed quickly on day 0 and day 1 (Figure 4-27). Therefore, the introduction of H_2 did not affect lactate or sulfate reduction. The lack of significant propionate buildup suggests that propionate was not an intermediate during the degradation of lactate using the acclimated biomass.

Set E investigated the effect of elevated H_2 on propionate and lactate degradation. The serum bottles were started with a 1:1 mixture (COD basis) of lactate and propionate. The headspaces of the serum bottles were flushed with H_2 gas at startup. All other conditions were identical to the pH 7 control set A. Lactate and sulfate were removed quickly; however, propionate was not removed in any of the serum bottles (Figure 4-28). Kaspar and Wuhrmann (1987b) also demonstrated the inhibition of propionate degradation using H_2. Propionate degradation stopped at H_2 levels of 202.6 Pa (2000 ppm). The lack of propionate degradation in the presence of elevated H_2 concentrations combined with the observations of set D, strengthens the conclusion that propionate was not an intermediate during lactate degradation using the acclimated inoculum.

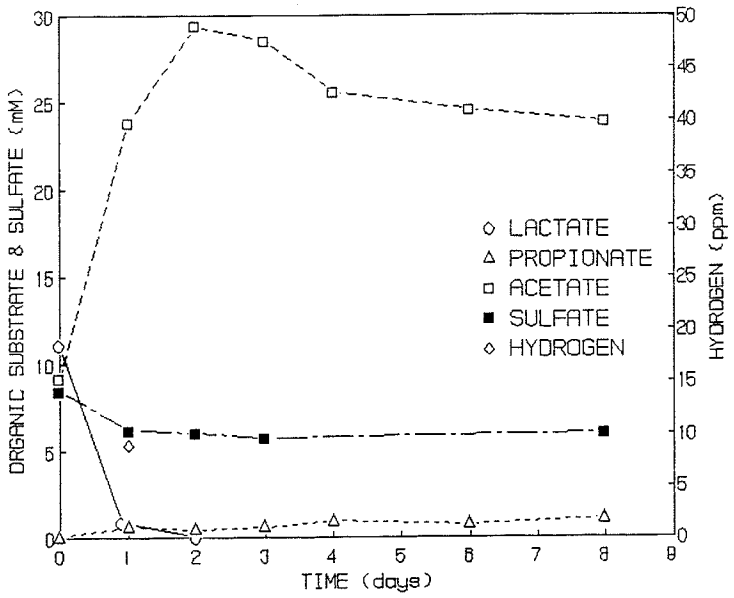


Figure 4-27. Concentration versus time profile of D1 during Phase 4.

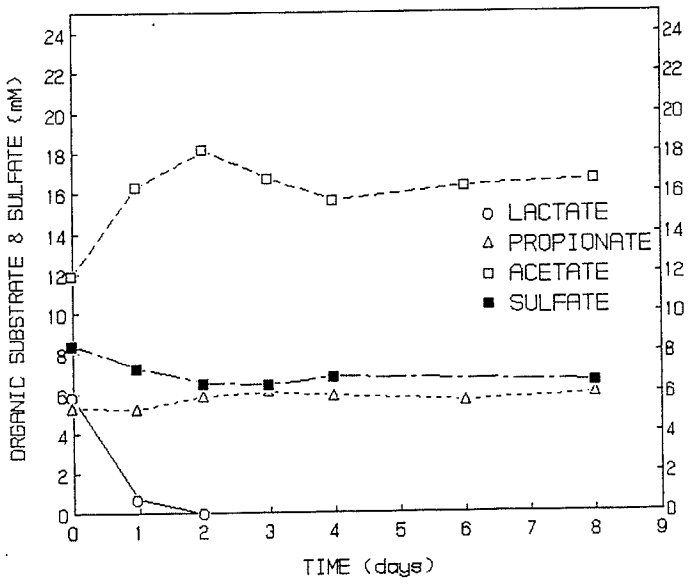


Figure 4-28. Concentration versus time profile of E1 during Phase 4.

4.4.5 Effect of Racemic Lactate

Set F investigated the effect of using racemic lactate (Phase 2) rather than L(+) lactate (Phase 3). All conditions were identical to the pH 7 control set A, except 1 gL^{-1} (COD) of racemic lactate was added at startup. Propionate was not observed as an intermediate (Appendix C), in spite of the high UIS concentrations in the serum bottles (Table 4-9). This indicates that the use of racemic lactate in Phase 2 was not the cause of propionate formation.

4.4.6 High pH control Set and Effects of High Sulfate

Set G was the pH 8 control set, such that the conditions in these serum bottles were similar to those of the Phase 3 R4 set discussed in Section 4.3. The following general observations were made: The MPB were more sensitive to sulfides when compared to the SRB; no propionate buildup occurred; and sulfate was proportional to lactate removal. The lactate removed to sulfate removed molar ratio was 1:0.41±0.04, for these three reactors, while the lactate removed to acetate formed molar ratio was 1:1.06 in G3. The H_2 levels increased dramatically in G1 during the experimental run. The H_2 partial pressure was 2.3 Pa (22.7 ppm) on day 2 and gradually increased to 5.9 Pa (57.9 ppm) by day 8. These H_2 levels were the highest measured in any of the serum bottles of Phase 4. The H_2 levels in the G and H sets did not correlate with the sulfide levels. It was not clear what caused these high H_2 levels.

Set H investigated the use of high initial sulfate concentrations. There were no relevant observations to report from this reactor set.

4.4.7 Effect of COD:Sulfate Ratio and Acclimation

The net metabolism of the mixed-culture inoculum was dependent upon the relative quantities of substrate and sulfate, as well as acclimation time. The various substrate ratios and acclimation periods for Phases 2 to 4 are presented in Table 4-10. The sulfate requirement of equation 4-5 was satisfied with the molar ratio of Phase 2, but may not have been sufficient to become the dominant thermodynamic and kinetic reaction. The sulfate to lactate molar ratio was increased by a factor of five in Phase 3. This increase may have resulted in equation 4-5 becoming dominant over equation 4-3.

Table 4-10. Substrate to sulfate ratios and acclimation times used in the breeder reactors and serum bottles during the four experimental phases

Phase	Lactate:Acetate Sulfate (molar ratio) Breeders	Lactate:Sulfate (molar ratio) Serum Bottles	COD:Sulfate ($g\ g^{-1}$) Breeders	Acclimation Period (days)
2	1:1.39:0.50	1:0.75	3.70	90
3	1:1.67:2.83	1:4.0	0.79	214
4	1:1.67:1.42	1:1	1.59	306[1]
NEWPCC[2]	NA	1:1	> 50	-

NA = Not Available

1. Cumulative time which includes Phase 3 period
2. COD estimated from operating records of plant, while sulfate estimated from City water supply values and typical concentrations reported in Metcalf and Eddy (1979).

The length of acclimation to the high sulfate and high lactate conditions would also affect the process. Inoculum used in Phases 1 and 2 was taken from a 20 L breeder reactor, which was batch fed once per week. The acclimated biomass used in Phases 3 and 4 was taken from 2 L breeder reactors which were batch fed three

times per week. The 20 and 2 L reactors received the same amount of mixing energy, which suggests the 2 L reactors would be more efficiently mixed. The NEWPCC municipal digester biomass served as startup seed for the 20 L breeder. The 20 L breeder biomass and NEWPCC biomass served as seed for the 2 L breeders. The 2 L breeders were then operated for 214 and 306 days before the Phase 3 and Phase 4 experimental runs, respectively.

The combination of the more efficient mixing, extended acclimation time,and increased sulfate to lactate ratio resulted in a change in the net metabolic pathways. The prolonged exposure to high sulfate, high lactate and elevated sulfide concentrations resulted in the selection of a different type of SRB species or the stimulation of a different metabolic pathway. Both pathways were that of incomplete-oxidizing SRB (SRB_i). This supports the conclusions of Widdel (1988) who stated that SRB_i grow faster than complete-oxidizing SRB, so competition for substrates would be expected to select for SRB_i.

A summary of the molar ratios observed in selected serum bottles from Phases 2, 3, and 4, as well as equations 4-2 to 4-5, inclusive, are presented in Table 4-11. The two different lactate pathways observed, stoichiometrically represented by equations 4-3 and 4-5, dramatically altered the response of the mixed cultures to sulfide. The acclimated biomass (equation 4-5) did not produce propionate as a product, while the unacclimated biomass (equation 4-3) did produce propionate. The oxidation of propionate to acetate was coupled to sulfate reduction (equation 4-4). This reduction was observed to be the rate limiting step of lactate degradation to methane under elevated UIS concentrations. In contrast, the bypassing of propionate as an

Table 4-11 Various stoichiometric molar ratios observed in Phases 2, 3, and 4 and equations 4-2, 4-3, 4-4, and 4-5.

Description	Molar ratio of lactate removed to sulfate reduced to propionate formed to acetate formed (Lactate:Sulfate:Propionate: Acetate) ($HLa:SO_4^{2-}:HPr:HAc$)	Molar ratio of propionate removed to sulfate reduced (propionate:sulfate) ($HPr:SO_4^{2-}$)
Phase 2:		
Serum bottle:		
S_L5	1:0.00:0.46:0.64	-
S_L1	-	1:1.53
Phase 3:		
Serum bottle:		
All	1:0.80:0.00:1.00	-
Phase 4:		
Serum bottle:		
C3	1:0.00:0.57:0.96	-
C1	-	1:0.76
A3	1:0.62:0.00:1.18	-
Equations:		
(4-2) $HLa \rightarrow HPr+HAc$	1:0.00:0.67:0.33	-
(4-3) $HLa \rightarrow HPr+HAc$	1:0.00:0.50:0.63	-
(4-4) $HPr+SO_4^{2-} \rightarrow HAc$	-	1:0.75
(4-5) $HLa+SO_4^{2-} \rightarrow HAc$	1:0.50:0.00:1.00	-

intermediate by the acclimated biomass resulted in acetoclastic methanogenesis being the rate limiting step. This change in the rate limiting step under elevated UIS conditions represented a shift from the SRB pathways being more sulfide sensitive than methanogenesis (unacclimated) to the methanogenic pathways being more sulfide sensitive than the SRB (acclimated). These observations support the earlier hypothesis (Section 4.4.3) that SRB growing on more complex substrate (lactate in this investigation) would be less sulfide-sensitive than SRB grown on propionate. The

hypothesis was based on the observations of Choi and Rim (1991) and Parkin et al. (1990).

Parkin et al. (1990) observed SRB to be slightly more sulfide sensitive than MPB when growing on propionate. In contrast, Choi and Rim (1991) observed SRB to be much less sulfide sensitive than MPB when growing on a complex substrate. Pichon et al. (1988) reported a buildup of propionate using pulp and paper wastewater at a COD:sulfate ratio of 4.5 (g g^{-1}). These researchers stated that the COD:BOD_5 ratio was approximately 3, and therefore a comparison to any theoretical COD values used in this thesis would best be done using the BOD_5:sulfate (g g^{-1}) ratio. This ratio was 1.5, which does not relate well to the COD:sulfate ratios of this thesis investigation. At a COD:sulfate (g g^{-1}) ratio of 3.7 propionate was observed as an intermediate, but not at a ratio of 1.6 (Table 4-11 and Table 4-10).

Mendez et al. (1989) investigated COD:sulfate ratios of 100 and 5 using a synthetic feed consisting of 95% sucrose, 2.3% acetate and 2.7% propionate. They observed propionate in the effluent of the reactor fed a COD:sulfate ratio of 100, which was five times higher than that of the reactor fed a ratio of 5. They attributed this difference to the increased H_2 scavenging of the SRB at the lower COD:sulfate ratio. It may also have been caused by the majority of the substrate being degraded via equation 4-3 at the higher COD:sulfate ratio, while the majority of the substrate was degraded via equation 4-5 at the lower COD:sulfate ratio. This would have resulted in more propionate being formed in the higher COD:sulfate ratio reactor.

Ueki et al. (1986) reported that lactate was oxidized rapidly with or without the presence of sulfate. These researchers also observed lactate degrading to

propionate and acetate in the presence of excess sulfate when the cultures were not acclimated to high sulfate. The addition of sulfate also significantly aided the degradation of propionate (Ueki et al., 1986; Figure 2-7). Lactate degradation with and without a MPB was investigated by Soubes et al. (1989). They reported two different stoichiometries, which both produced propionate under low sulfate conditions. The molar ratios (lactate removed to propionate formed) were 1:0.23 and 1:0.54 for cultures with and without MPB enrichment. The latter ratio relates well to the stoichiometry of this thesis investigation (Table 4-11). Other researchers reported that a pure culture of *Selenomonas ruminantium* degraded lactate to propionate and acetate, but in co-culture with a MPB lactate was degraded to acetate and methane (Chen and Wolin, 1977).

All of these investigations, including this thesis, point to the complicated pathways which exist given even the simpler substrates. A pathway taken will be highly dependent upon the environmental conditions which exist, and if the environmental conditions change, the pathway can be expected to change as well.

4.4.8 Phase 4--Observation Summary

The following observations of the unacclimated serum bottle set C were made: lactate degraded to propionate and acetate; the propionate utilization rates were sensitive to increased sulfide concentrations; propionate buildup occurred in the high sulfide serum bottles of this set; H_2 buildup did not cause propionate buildup; and it was difficult to establish relative MPB and SRB sensitivities to sulfide due to the interferences from organic substrates not monitored.

The following observations of the acclimated serum bottle sets were made: lactate degraded directly to acetate with no propionate as an intermediate; propionate buildup did not occur in any of these serum bottle sets; and the MPB were much more sensitive to sulfides than SRB in all the acclimated serum bottle sets.

Chapter 5.

RESEARCH OVERVIEW

The important aspects of this thesis investigation are discussed in this chapter. The objectives and outcomes of each phase are presented, along with an overall summary which highlights the critical observations of the work. Conclusions are summarized and the engineering relevance of the work is given. The chapter concludes with directions for future investigations.

5.1 SUMMARY

Section 5.1 summarizes the objectives, observations, and discussions of each experimental phase.

5.1.1 Phase 1--Summary

Phase 1 was designed as a screening experiment to investigate the progress reported in the literature. The simple organic substrates; lactate, butyrate, propionate, and acetate were used. The objective was to observe the effects of TS and UIS on the batch degradation kinetics of these organics in a matrix of serum bottle reactors.

In general, a higher TS and/or a lower pH resulted in a lower removal activity for all of the substrates investigated. The decrease in activity correlated to the increase in UIS concentration. Propionate degradation was the most sensitive to increasing UIS concentrations, followed by acetate, then butyrate, and lactate was the least sensitive to sulfide. The exponential inhibition constants calculated for

propionate and acetate removal (Equation 4-1) showed that propionate removal was twice as sensitive to increased UIS levels when compared to acetate removal. The observed sensitivities of propionate degradation corroborated the work of Rinzema and Lettinga (1988). These researchers concluded that propionate breakdown was the rate-limiting step during UIS inhibition of MPB.

5.1.2 Phase 2--Summary

Phase 2 was designed to investigate the high sensitivity of propionate degradation observed in Phase 1. Lactate was used as a substrate, since its degradation produced both acetate and propionate in Phase 1. Acetate was also used as a substrate. The objective was to increase the understanding of propionate and acetate sensitivities to sulfide and to study the sulfide inhibition of SRB and MPB. The COD:sulfate ratio fed to the inoculum was 3.7 g g^{-1}, which resulted in a lactate degradation pathway best represented by equations 4-3 and 4-4 (Table 4-11).

Acetate utilization followed zero-order kinetics above a concentration of 2 mM (120 mg L^{-1}). Acetate utilization was found to fit the exponential inhibition model, which was developed in Phase 1 over the UIS range of 2 to 8 mM (68 to 272 mg L^{-1}).

Significant propionate residuals occurred at UIS concentrations of ≥2.9 mM (100 mg L^{-1}). The propionate residuals were correlated to UIS concentration and to sulfate reduction. This observation pointed to an important link between the inhibition of propionate utilization and SRB by UIS. The propionate residuals could have been due to the buildup of H_2, which consequently caused propionate removal to stop. Weigant et al. (1986) have shown that syntrophic propionate utilizers have

extremely low growth rates and are very sensitive to an increase in H_2 concentration, which is a reaction product of propionate degradation. The SRB responsible for H_2 removal may have been inhibited by the UIS. Another possible explanation was the inhibition of the SRB group which utilizes propionate directly. Since H_2 was not measured in this investigation, it was not known whether SRB participated in H_2 removal.

The MPB outcompeted the SRB for the available acetate. The MPB were also found to be less sensitive to TS and UIS when compared to the SRB.

5.1.3 Phase 3 Summary

Phase 3 was designed to eliminate the effects of pH adjustment which was used in Phases 1 and 2. The objective was to repeat the experiment conducted in Phase 2 using two sources of inoculum which had been acclimated to pH 7 and pH 8 conditions. Daily H_2 and sulfate monitoring allowed the roles of H_2 and SRB to be investigated further. The COD:sulfate ratio fed to the inoculum was 0.8 g g^{-1}, which resulted in a lactate degradation pathway best represented by equation 4-5 (Table 4-11). This stoichiometric relationship represented a significant change in metabolic pathways, since lactate and sulfate removals were observed to be independent in Phase 2.

A surprising observation was that no propionate was observed as an intermediate or residual in any of the reactors, regardless of pH, TS, or UIS concentration. Just as surprising was the observation that MPB were much more

sensitive to TS and UIS when compared to SRB. The SRB were observed to be more sensitive to TS than UIS in the pH 8 reactors.

Acetate removal was not related to SRB activity. This observation was also made in Phases 1 and 2.

5.1.4 Phase 4 Summary

Phase 4 investigated the experimental variables which differed between Phases 2 and 3. This was done in an effort to isolate the cause of the contradictory observations of the two phases. Four different variables were investigated, as follows: adjustment of inoculum pH from 8 to 7 at startup; unacclimated inoculum substituted for acclimated inoculum; racemic lactate as substrate instead of L(+) lactate; and a high sulfate concentration. Phase 4 also investigated the effect of elevated H_2 concentrations on propionate degradation. Two sets were run, with one fed lactate and the other lactate and propionate, while both sets had the headspaces of the serum bottles flushed with 100 percent H_2 gas.

The objective was to reproduce the observations made during Phase 2, namely, propionate as an intermediate and residual. A secondary objective was to observe the effect of H_2 on propionate degradation.

Propionate was observed as an intermediate in the serum bottle set inoculated with biomass not acclimated to high lactate and sulfate conditions. Propionate removal was inhibited by UIS, but not H_2. In fact, propionate removal was observed to be more sensitive to UIS when compared to H_2 removal.

The MPB were observed to be slightly more sensitive than SRB to UIS. This observation was clouded by secondary sulfate removal which occurred after the substrates being monitored were utilized. The secondary removals were attributed to the complex organics which were included with the inoculum.

The stoichiometry of lactate degradation in the unacclimated reactors was found to approximate equations 4-3 and 4-4, while in the acclimated reactors, equation 4-5 was approximated (Table 4-11).

The three other variables investigated used acclimated biomass and had similar results as observed in Phase 3. The higher degree of acclimation to high sulfate conditions of the Phase 3 inoculum compared to the Phase 2 inoculum was the key to the different observations during the two experimental phases. The prolonged exposure to high sulfate, high lactate, and elevated sulfide concentrations resulted in the selection of a different type of SRB species or the stimulation of a different metabolic pathway. The various metabolic pathways of *Desulfobulbus propionicus* (Widdel and Pfennig, 1982) were similar to those observed in this thesis investigation. The net result was a change in the rate limiting step under elevated UIS conditions, which resulted in the shift from the SRB pathways being slightly more sulfide sensitive than MPB, to the MPB being much more sulfide sensitive than the SRB.

Flushing the serum bottle headspace with the H_2 gas completely inhibited propionate degradation. Lactate and sulfate removal proceeded, uninhibited by H_2 saturation, forming acetate with no propionate buildup occurring. These

observations led to the conclusion that propionate was not an intermediate during the degradation of lactate using the acclimated biomass.

5.1.5 Overall Observation Summary

The utilization rates of propionate, acetate, and butyrate were more sensitive to UIS as compared to TS. Lactate degradation that proceeded without sulfate reduction (equation 4-3) was also more sensitive to UIS, but lactate degradation that proceeded concurrently with sulfate reduction (equation 4-5) was observed to be tolerant of much higher UIS concentrations and sensitive to relatively high concentrations of TS (Table 5-1). Propionate and acetate utilization rates were the most sensitive to UIS concentrations, followed by butyrate utilization (Table 5-1). The exponential inhibition constant calculated for propionate and acetate showed that propionate rates were twice as sulfide sensitive compared to acetate rates. Lactate utilization with or without concurrent sulfate reduction was by far the least sensitive pathway (Table 5-1).

The net metabolic pathways of the mixed-culture biomass inoculums were dependent upon acclimation to the sulfate, lactate, and sulfide concentrations in the breeder reactors. The acclimation times and substrate to sulfate ratios used in this investigation were presented in Table 4-10. The breeder inoculum from Phase 2 and NEWPCC inoculum were considered unacclimated, while Phases 3 and 4 breeder inoculum were acclimated to the higher sulfate conditions.

Lactate degradation for the acclimated and the unacclimated inoculums were represented by stoichiometric equations 4-5 and 4-3, respectively (Table 4-11). The

Table 5-1. Summary of sulfide concentrations resulting in fifty percent inhibition of pertinent reactions

	Reaction (Equation number)	Sulfide concentration resulting in 50% inhibition of reaction, mM(mgS L^{-1}) TS	UIS
1.	Lactate + sulfate → acetate (4-5)	66(2100)	NI
2.	Lactate → propionate + acetate (4-3)	NI	11.1 (355)
3.	Butyrate → acetate (1, Table 2-2)	NI	7.3 (235)
4.	Acetate → methane (6, Table 2-2)	NI	3.4±0.4(110±15)†
5.	Propionate + sulfate → acetate (4-4)	NI	2.5 (80)

NI = Inhibitory concentrations not reached, i.e., not the cause of sulfide inhibition at the concentrations and pH values investigated.

† Inhibition of methanogenesis was determined in three phases, while the other reactions were only analyzed in one phase each.

acclimated biomass did not produce propionate as an intermediate, while the unacclimated biomass did produce propionate (Figure 5-1). Propionate utilization was coupled to sulfate reduction as represented by equation 4-4 (Table 4-11).

The following is a summary of the unacclimated inoculum observations. Acetate was not utilized by SRB. Propionate utilization by SRB was slightly more sulfide sensitive than acetoclastic methanogenesis (Table 5-1). Both of these reactions were more sensitive to UIS as compared to TS. Propionate utilization was more sulfide sensitive than H_2 utilization, therefore H_2 was not inhibiting propionate degradation.

The following is a summary of the acclimated inoculum observations. Acetate was not utilized by SRB. The SRB utilization of lactate was not significantly inhibited

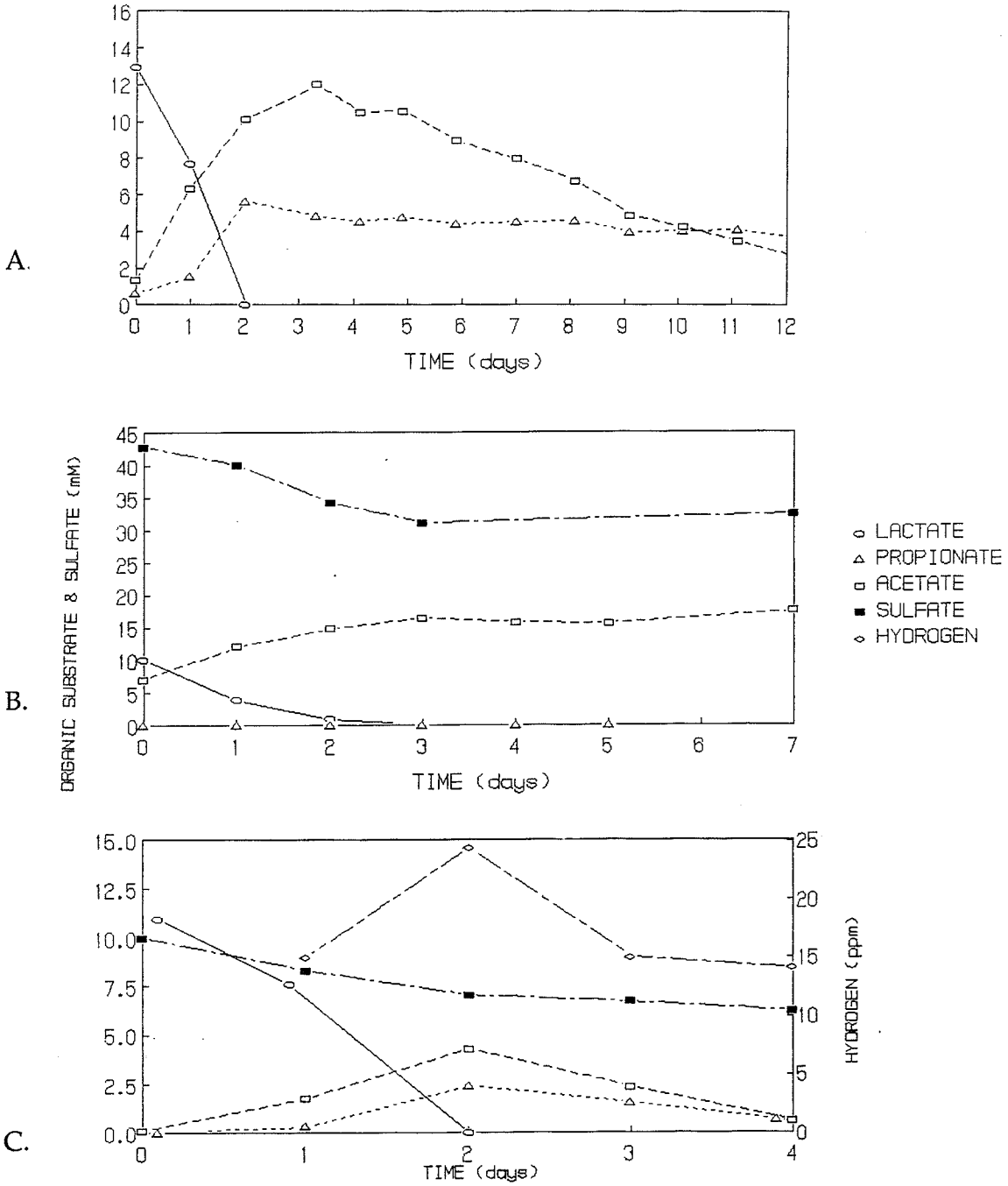


Figure 5-1. Concentration versus time profiles for the acclimated and unacclimated inoculum from Phase 2, 3, and 4. A. Phase 2 - S_L 3 (12 d). B. Phase 3 - R12 (7 d). C. Phase 4 - C1 (4 d). The progressively higher removal rates in Phases 2, 3, and 4 were due to larger inoculum concentrations in each successive Phase.

by UIS concentrations below 8.6 mM (275 mg L^{-1}), but reached 50 percent inhibition at a TS concentration of 65.6 mM (2100 mg L^{-1}, Table 5-1). The acetoclastic methanogens were much more sensitive to UIS than TS, with 50 percent inhibition occurring at 3.4±0.44 mM (108±14 mg L^{-1}) concentration of UIS (Table 5-1). The MPB were much more sensitive to sulfide than the SRB.

5.2 CONCLUSIONS

The following conclusions were based on the results obtained in all four phases of this experimental investigation.

1. Acetate removal was a first-order reaction facilitated by MPB not SRB at all COD:SO_4^{2-} ratios investigated. The scrubbing of H_2S from the headspace of the breeder reactors allowed the MPB to remain active, even at a very low COD:SO_4^{2-} ratio (0.8 g g^{-1}).
2. Acetate, propionate, and butyrate utilization were more sensitive to UIS rather than TS.
3. Sulfide inhibition of acetate and propionate utilization was modelled using an exponential inhibition constant.
4. Propionate utilization was the most sensitive to sulfide inhibition, followed closely by acetate utilization, and butyrate utilization was the least sensitive of the three reactions. The UIS concentrations resulting in 50 percent inhibition were 2.5, 3.4, and 7.3 mM (80, 110, and 235 mg L^{-1}), respectively.
5. The lactate degradation pathway was dependent upon the COD:sulfate ratio (g g^{-1}) used in the feed. A ratio of 3.7 g g^{-1} resulted in a pathway which had

propionate and acetate as products (equation 4-3, Table 4-11), but did not result in significant sulfate reduction. A ratio of ≤ 1.6 g g^{-1} resulted in an SRB pathway which had acetate as a product and proceeded concurrently with sulfate reduction (equation 4-5, Table 4-11).

6. The lactate utilization pathways were by far the least sensitive to sulfide inhibition relative to other organics investigated (Table 5-1).
7. Propionate utilization was always directly correlated to sulfate reduction.
8. Hydrogen utilization was less sulfide sensitive than propionate degradation.
9. Sulfate reduction associated with propionate degradation was more sensitive to sulfide than acetotrophic MPB.
10. Sulfate reduction associated with lactate degradation was much less sensitive to sulfide than acetotrophic MPB.
11. The sensitivity of sulfate reduction processes to sulfide was dependent upon the metabolic pathway being utilized, which depended upon the lactate:sulfate ratio.

5.3 ENGINEERING SIGNIFICANCE AND RECOMMENDATIONS

The batch serum bottle experimental arrays used in this investigation allowed an enormous number of observations to be accomplished in a relatively short time. In total, 162 reactors were operated, of which 10 were semi-continuous. However, when interpreting these results, we must keep in mind the "snapshot" nature of batch studies.

A suitable but somewhat morbid analogy would be if an assassin could freeze a group of dynamic people using a snapshot, he or she could easily pick out an individual target and fire a bullet at the immobilized person. However, a bullet following the same trajectory a few moments later would have a completely different effect as the people will have moved. Of course, an assassin cannot freeze time in a snapshot, and therefore must wait for the target to become stable within the surrounding environment.

This same principle should be applied to single-run batch studies. The literature is replete with studies where inoculum has been taken from a given set of environmental conditions and tested for responses to another set of conditions without acclimating the inoculum to the new conditions. In fact, these techniques were used in this thesis investigation. Therefore, when making recommendations from these results, it is imperative to remember that we are bound to the environmental conditions from where the inoculum came, not just those we impose in our experiments. Anaerobic microflora, with their relatively slow growth rates, are quite sensitive to acclimation periods.

When trying to understand life, we must be aware of its dynamic nature, even at the microscopic level. The single-run batch study allows us to take a snapshot of inoculum and observe its response to various conditions, but we must remember that, given time to acclimate to those conditions, the responses observed may be completely different. Do you think your observations of the people in the assassin's snapshot would change after the shot had been fired and the target was struck?

Sorry for the image, but with the snapshot principle in mind, the engineering significance of the observations of this thesis investigation will be discussed.

Ideally, biological reactor engineers strive to use real waste combined with practical and economic operating strategies to assess various parameters. However, sulfide sensitivity was already linked to the simple substrates directly preceding methanogenesis, which provided the focus for this investigation. To achieve this focus, extreme artificial environments were used. For this reason, the direct engineering benefits are hard to measure, but some general suggestions and recommendations can be made.

The results suggest that the type of organic substrate used and the COD:sulfate ratio are important considerations when assessing SRB-MPB competition and sulfide inhibition. For example, a waste which largely ferments to lactate under high sulfate conditions will be expected to degrade to acetate without significant inhibition by sulfide. If the amount of COD removed is significant, the process may be considered viable even though methane is not produced. An investigation into landfill leachate treatment at a COD:sulfate ($g\ g^{-1}$) ratio of less than 1 by Prasad et al. (1991) concluded that the SRB were predominant and that COD removal efficiency was equivalent to methanogenic pathways, i.e., SRB were able to stabilize the waste equally as well as MPB. The residuals from this unit process will include sulfide and acetate. The economics of recovering sulfur and treating and/or recovering the acetate would also have to be investigated. The issues regarding the anaerobic stabilization of wastes under high sulfate conditions and the various ways to recover sulfur have been presented by many researchers (Olthof et al., 1985; Lebel et al., 1985;

Obsrsky et al., 1978; Maree and Hill, 1987; Couillard et al., 1988; McFarland and Jewell, 1989; Maree and Hill, 1989; Sarner, 1990). Sulfur recovery was not the topic of this thesis, and therefore has not been discussed in detail. Those interested are referred to the aforementioned references for more detailed information. However, wastes that largely degrade to propionate may stop there, leaving a mixture of propionate and acetate which reduces the amount of COD removal achieved.

The point is, so what if we don't achieve significant methanogenesis? Maybe SRB can achieve the job practically and economically. We can't know until the evaluations are done for the waste. We must not run when we hear the words "high sulfate".

Reactors operating at high COD:sulfate ratios should not have serious competition or inhibition problems. If conditions exist or develop causing sulfide buildup to levels that are inhibitory, immediate remedial action may involve raising the pH to lower the UIS concentration in the reactor. This would be particularly true for industries that may be subject to sulfuric acid or sulfide solution spills. McFarland and Jewell (1989), however, found that pH adjustment resulted in TS buildup. The merits of this technique must be determined on a case by case basis.

Some wastes may not have significant sulfate concentrations, but high organic sulfur loads. The control of pH in these reactors may also increase reaction rates or mitigate process instabilities.

Reactors that are subject to sulfide or sulfate shock loads can be monitored reliably. Propionate and acetate concentrations can be reliably monitored. The buildup of propionate and/or acetate may signal sulfide inhibition and offer early

warnings of pending reactor upsets. However, based on the observations of this investigation, H_2 monitoring will not detect sulfide inhibition episodes.

The MPB were able to outcompete the SRB for acetate in the various serum bottle reactors operated in this investigation. This included the breeder reactors, which operated at COD:sulfate ($g\ g^{-1}$) ratios of 0.8. However, sulfide was still produced due to the SRB degrading lactate. The sulfide was removed from the headspace of the reactors using a gas scrubbing system. This could also be done in full-scale, but economic evaluations would determine the feasibility of the process as an option. This economic analysis would be sensitive to local energy costs.

Based on this investigation, the following recommendations are made:

1. One should keep in mind the snapshot principle when reviewing the results reported in the literature. Always ask yourself if the conditions of the experiment simulate real conditions. If not, further testing is necessary to simulate the real conditions before any conclusions can be made for a certain application.
2. We must not immediately discard anaerobic treatment when wastewater contains high sulfate concentrations. Higher SRB kinetics may allow a reduction in reactor volume. At the very least, treat the option to a fair paper economic analysis; don't discard on the basis of fear alone.
3. In process streams that are subject to sulfide toxicity episodes, the best routine monitoring techniques recommended are propionate and acetate analysis. Once the original investment is made into a gas chromatograph equipped with a flame ionization detector, the analysis is simple, accurate, and painless.

Hydrogen concentrations did not correlate with sulfide inhibition in this investigation. Aqueous sulfide and/or gaseous hydrogen sulfide can also be used, but are not as reliable as propionate and acetate analysis.

4. Increasing the pH to lower the aqueous UIS concentration may help mitigate short-term toxicity episodes. However, inhibition can also result from a buildup of TS, so we must exercise caution when utilizing this temporary UIS control technique.

5. Scrubbing sulfide from the biogas allowed MPB to remain active and actually outcompete SRB for acetate at a COD:sulfate (g g^{-1}) ratio of down to 0.8 (Section 3.3.2). Therefore, methanogenic reactor operations can be maintained by keeping sulfide concentrations artificially low using scrubbing systems. The economic advantages of this system would have to be investigated.

5.4 SUGGESTIONS FOR FURTHER STUDY

The further study suggestions have been divided into two categories as follows: Applied research and basic research.

5.4.1 Applied Research

There are two areas where the practical implications of this research may be investigated further. The first deals with studies using real wastes, while the other strives to compare attached biomass to suspended growth biomass.

Determining the anaerobic degradation profiles of various industrial wastes would be of interest. The presence of propionate as an intermediate was shown to

have a significant effect on sulfide sensitivity of the process in this research. Using various industrial wastewaters and two sets of inoculum that had been bred on high and low COD:sulfate ratios, a serum bottle screening test could be done on the wastes. The screen would assess the COD:lactate:propionate ratios in the specific wastes. Further investigations could be done on wastes that may bypass propionate as an intermediate. Chemostat or semi-continuous reactors could then establish the process kinetics and potential process operational needs. An economic analysis would then determine the relative feasibility of the process in the marketplace.

The work of others has suggested that attached growth reactors are resistant to sulfide inhibition. Isa et al. (1986a) reported that a UIS concentration of 35.3 mM (1200 mg L^{-1}) only caused a fifty percent inhibition of MPB. They attributed this lack of sensitivity to acclimation; however, similar results could not be achieved in this suspended growth thesis investigation using long acclimation times and high biological solids retention times. Solids were never intentionally wasted from the breeder reactors (Section 3.3.2). A side-by-side comparison of attached and suspended growth systems should be conducted with startup at high COD:sulfate ratios, followed by incremental increases in sulfate resulting in decreasing COD:S ratios. A suitable real waste or practical simulations of a waste should be used for this investigation.

5.4.2 Basic Research

The effect of the lactate:sulfate ratio on lactate degradation was demonstrated in this investigation. A continuous reactor investigation using four molar ratios

between 1.42 and 0.5 would focus on the changeover of the metabolic pathways of lactate degradation. More complex organics that are known to degrade via lactate may also be used in serum bottle studies run concurrently with the continuous studies.

An investigation into the competition between SRB and MPB for hydrogen would be of interest. This investigation has shown that hydrogen was not rate limiting when subjected to elevated sulfide concentrations, but did not focus on the roles of SRB and MPB in hydrogen removal. This could be accomplished by controlling the amount of hydrogen available and correlating to SRB and MPB.

The observations of this thesis investigation may offer some hints as to the mechanism of UIS inhibition. The acetyl coenzyme A and the coenzyme M both have an active sulfide group, which indicates a potential for sulfide inhibition. High sulfide conditions may prevent the binding of this active group, by reacting with all potential binding sites. Coenzyme M was reported as being unique to MPB (Jones et al., 1987). The acetyl coenzyme A pathway for CO_2 fixation is common to SRB and MPB (Struthamer, 1988), and therefore the propionate-utilizing SRB and acetotrophic MPB may both be utilizing this pathway.

A broader investigation into the relationship between propionate, hydrogen, and UIS would also be beneficial. Using inoculum from the NEWPCC, two sets of ten serum bottles could be run. All reactors would be run at a pH of 7, with one set fed 1 g COD L^{-1} of propionate at startup, and lactate in the other set. Starting UIS concentrations would vary from controls at 0.63 mM (20 mg L^{-1}) to high

concentrations of 5 mM (150 mg L^{-1}) and 14.1 mM (450 mg L^{-1}) for the propionate and lactate sets, respectively.

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APPENDIX A.

PHASE 2 RAW DATA

		SULFATES AS S		SULFIDES AS S		pH		METHANE	CO2
		START (mg/L)	END (mg/L)	START (mg/L)	END (mg/L)	START	END	(mL)	(mL)
PHASE 2 RESULTS									
PHASE 2 RESULTS									
PHASE 2 RESULTS	A1	262	261	101	200	7.20	7.20	27.3	25.2
PHASE 2 RESULTS	A2	279	295	321	300	7.60	7.50	24.4	17.1
PHASE 2 RESULTS	A3	301	334	357	340	7.50	7.30	30.5	24.7
PHASE 2 RESULTS	A4	301	334	441	600	7.40	7.30	23.2	20.5
PHASE 2 RESULTS	A5	301	323	762	980	7.40	7.25	11.8	20.4
PHASE 2 RESULTS	A6	271	240	201	160	8.10	7.70	20.5	7.8
PHASE 2 RESULTS	A7	288	263	442	380	8.10	7.80	21.3	7.1
PHASE 2 RESULTS	A8	292	268	362	300	8.20	7.80	20.6	5.1
PHASE 2 RESULTS	A9	301	297	522	540	8.35	7.90	18.3	4.0
PHASE 2 RESULTS	A10	301	297	902	900	8.20	7.80	24.1	6.2
PHASE 2 RESULTS									
PHASE 2 RESULTS	L1	275	38	162	330	7.25	7.05	25.5	37.2
PHASE 2 RESULTS	L2	275	57	322	440	7.65	7.50	26.9	18.7
PHASE 2 RESULTS	13	301	215	322	420	7.35	7.30	20.4	30.8
PHASE 2 RESULTS	L4	309	300	502	620	7.25	7.30	8.6	30.7
PHASE 2 RESULTS	L5	314	317	762	940	7.35	7.30	5.5	26.9
PHASE 2 RESULTS	L6	275	51	282	400	8.05	7.50	16.4	9.1
PHASE 2 RESULTS	L7	301	57	442	580	8.10	7.60	18.1	9.6
PHASE 2 RESULTS	L8	301	57	362	520	8.10	7.70	15.8	7.6
PHASE 2 RESULTS	L9	305	97	561	670	8.25	7.80	14.1	5.2
PHASE 2 RESULTS	L10	309	261	962	1040	8.10	7.70	29.4	14.2
PHASE 2 RESULTS									
PHASE 2 RESULTS	F1	284	119	102	240	7.20	7.20	28.6	33.3
PHASE 2 RESULTS	F2	271	170	282	380	7.60	7.50	30.9	23.0
PHASE 2 RESULTS	F3	296	279	362	460	7.30	7.30	25.6	35.9
PHASE 2 RESULTS	F4	305	323	362	580	7.40	7.20	23.7	32.9
PHASE 2 RESULTS	F5	305	334	762	960	7.35	7.20	6.4	23.6
PHASE 2 RESULTS	F6	282	126	322	300	8.00	7.75	17.9	7.8
PHASE 2 RESULTS	F7	299	160	402	480	8.10	7.70	18.7	7.6
PHASE 2 RESULTS	F8	316	171	482	530	8.10	7.70	15.4	7.3
PHASE 2 RESULTS	F9	311	205	522	590	8.20	7.70	17.7	5.1
PHASE 2 RESULTS	F10	328	263	882	1060	8.10	7.50	17.6	7.7
PHASE 2 RESULTS									
PHASE 2 RESULTS									

Ac-1

1.07

	TIME (h)	TIME (d)	HLA (mg/L)	HPR (mg/L)	HAC (mg/L)	HLA (mM)	HPR (mM)	HAC (mM)
A1	0.0	0.0	0	51	1143	0.00	0.69	19.05
	23.0	1.0	0	6	1141	0.00	0.08	19.02
	47.0	2.0	0	20	1240	0.00	0.27	20.67
	73.0	3.3	0	6	1138	0.00	0.08	18.97
	98.0	4.1	0	0	976	0.00	0.00	16.27
	118.5	4.9	0		1018	0.00	0.00	16.97
	142.5	5.9	0		950	0.00	0.00	15.83
	168.5	7.0	0		738	0.00	0.00	12.30
	194.5	8.1	0		710	0.00	0.00	11.83
	217.3	9.1	0		561	0.00	0.00	9.35
	241.5	10.1	0		456	0.00	0.00	7.60
	266.5	11.1	0		412	0.00	0.00	6.87
	290.5	12.1	0		318	0.00	0.00	5.30
	314.5	13.1	0		202	0.00	0.00	3.37
	338.5	14.1	0		114	0.00	0.00	1.90
	360.5	15.0	0		73	0.00	0.00	1.22
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
A2	0.0	0.0	0	91	1262	0.00	1.23	21.03
	23.0	1.0	0	0	1220	0.00	0.00	20.33
	47.0	2.0	0		1149	0.00	0.00	19.15
	73.0	3.3	0		872	0.00	0.00	14.53
	98.0	4.1	0		802	0.00	0.00	13.37
	118.5	4.9	0		636	0.00	0.00	10.60
	142.5	5.9	0		471	0.00	0.00	7.85
	168.5	7.0	0		282	0.00	0.00	4.70
	194.5	8.1	0		190	0.00	0.00	3.17
	217.3	9.1	0		77	0.00	0.00	1.28
	241.5	10.1	0		48	0.00	0.00	0.80
	266.5	11.1	0		26	0.00	0.00	0.43
	290.5	12.1	0		31	0.00	0.00	0.52
	314.5	13.1	0		23	0.00	0.00	0.38
	338.5	14.1	0		17	0.00	0.00	0.28
	360.5	15.0	0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
A3	0.0	0.0	0	45	1205	0.00	0.61	20.08
	23.0	1.0	0	0	1345	0.00	0.00	22.42
	47.0	2.0	0		1159	0.00	0.00	19.32
	73.0	3.3	0		1127	0.00	0.00	18.78
	98.0	4.1	0		981	0.00	0.00	16.35
	118.5	4.9	0		832	0.00	0.00	11.70
	142.5	5.9	0		808	0.00	0.00	13.47
	168.5	7.0	0		625	0.00	0.00	10.42
	194.5	8.1	0		529	0.00	0.00	8.80
	217.3	9.1	0		335	0.00	0.00	5.58

	241.5	10.	0			251	0.00	0.00	4.18
	265.5	11.1	0			172	0.00	0.00	2.87
	290.5	12.1	0			95	0.00	0.00	1.58
	314.5	13.1	0			37	0.00	0.00	0.62
	338.5	14.1	0			31	0.00	0.00	0.52
	360.5	15.0	0				0.00	0.00	0.00
			0				0.00	0.00	0.00
			0				0.00	0.00	0.00
			0				0.00	0.00	0.00
			0				0.00	0.00	#VALUE
A4	0.0	0.0	0	48	1232	0.00	0.65	20.53	
	23.0	1.0	0	59	1264	0.00	0.80	21.07	
	47.0	2.0	0	8	1240	0.00	0.11	20.67	
	78.0	3.3	0	12	1211	0.00	0.16	20.18	
	98.0	4.1	0	20	1164	0.00	0.27	19.40	
	118.5	4.9	0	0	1020	0.00	0.00	17.00	
	142.5	5.9	0	17	1004	0.00	0.23	16.73	
	168.5	7.0	0	14	876	0.00	0.19	14.60	
	194.5	8.1	0	15	769	0.00	0.20	12.82	
	217.3	9.1	0	30	578	0.00	0.41	9.63	
	241.5	10.1	0	27	525	0.00	0.38	8.75	
	266.5	11.1	0	35	399	0.00	0.47	6.65	
	290.5	12.1	0	25	326	0.00	0.34	5.43	
	314.5	13.1	0	46	213	0.00	0.62	3.55	
	338.5	14.1	0	43	108	0.00	0.58	1.80	
	360.5	15.0	0	40	74	0.00	0.54	1.23	
			0				0.00	0.00	0.00
			0				0.00	0.00	0.00
			0				0.00	0.00	0.00
			0				0.00	0.00	0.00
A5	0.0	0.0	0	64	1256	0.00	0.86	20.93	
	23.0	1.0	0	76	1246	0.00	1.03	20.77	
	47.0	2.0	0	62	1367	0.00	0.84	22.78	
	78.0	3.3	0	61	1256	0.00	0.82	20.93	
	98.0	4.1	0	59	1223	0.00	0.80	20.38	
	118.5	4.9	0	63	1175	0.00	0.85	19.58	
	142.5	5.9	0	63	1136	0.00	0.85	18.93	
	168.5	7.0	0	63	1103	0.00	0.85	18.38	
	194.5	8.1	0	66	1001	0.00	0.89	16.68	
	217.3	9.1	0	72	862	0.00	0.97	14.37	
	241.5	10.1	0	85	883	0.00	1.15	14.72	
	266.5	11.1	0	86	834	0.00	1.16	13.90	
	290.5	12.1	0	73	759	0.00	0.99	12.65	
	314.5	13.1	0	76	718	0.00	1.03	11.97	
	338.5	14.1	0	85	649	0.00	1.15	10.82	
	360.5	15.0	0	80	572	0.00	1.08	9.53	
			0			0.00	0.00	0.00	
			0			0.00	0.00	0.00	
			0			0.00	0.00	0.00	
			0			0.00	0.00	0.00	
A6	0.0	0.0	0	5	1184	0.00	0.07	19.75	
	23.0	1.0	0	2	1003	0.00	0.03	16.72	
	47.0	2.0	0	3	747	0.00	0.01	12.45	
	78.0	3.3	0	4	507	0.00	0.03	8.45	

	98.0	4.1	0		321	0.00	0.00	5.35
	118.5	4.9	0		173	0.00	0.00	2.88
	142.5	5.9	0		69	0.00	0.00	1.15
	168.5	7.0	0		61	0.00	0.00	1.02
	194.5	8.1	0		97	0.00	0.00	1.62
	217.5	9.1	0			0.00	0.00	0.00
	241.5	10.1	0			0.00	0.00	0.00
	266.5	11.1	0			0.00	0.00	0.00
	290.5	12.1	0			0.00	0.00	0.00
	314.5	13.1	0			0.00	0.00	0.00
	338.5	14.1	0			0.00	0.00	0.00
	360.5	15.0	0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
A7	0.0	0.0	0	0	1238	0.00	0.00	20.65
	23.0	1.0	0		1030	0.00	0.00	17.17
	47.0	2.0	0		802	0.00	0.00	13.37
	78.0	3.3	0		463	0.00	0.00	7.72
	98.0	4.1	0		287	0.00	0.00	4.78
	118.5	4.9	0		144	0.00	0.00	2.40
	142.5	5.9	0		44	0.00	0.00	0.73
	168.5	7.0	0		18	0.00	0.00	0.30
	194.5	8.1	0		9	0.00	0.00	0.15
	217.5	9.1	0			0.00	0.00	0.00
	241.5	10.1	0			0.00	0.00	0.00
	266.5	11.1	0			0.00	0.00	0.00
	290.5	12.1	0			0.00	0.00	0.00
	314.5	13.1	0			0.00	0.00	0.00
	338.5	14.1	0			0.00	0.00	0.00
	360.5	15.0	0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
A8	0.0	0.0	0	0	1251	0.00	0.00	20.85
	23.0	1.0	0		1165	0.00	0.00	19.42
	47.0	2.0	0		868	0.00	0.00	14.47
	78.0	3.3	0		484	0.00	0.00	8.07
	98.0	4.1	0		289	0.00	0.00	4.82
	118.5	4.9	0		152	0.00	0.00	2.53
	142.5	5.9	0		44	0.00	0.00	0.73
	168.5	7.0	0		12	0.00	0.00	0.20
	194.5	8.1	0		0	0.00	0.00	0.00
	217.5	9.1	0			0.00	0.00	0.00
	241.5	10.1	0			0.00	0.00	0.00
	266.5	11.1	0			0.00	0.00	0.00
	290.5	12.1	0			0.00	0.00	0.00
	314.5	13.1	0			0.00	0.00	0.00
	338.5	14.1	0			0.00	0.00	0.00
	360.5	15.0	0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00

			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
A9	0.0	0.0	0	0	1259	0.00	0.00	20.98
	23.0	1.0	0		1242	0.00	0.00	20.70
	47.0	2.0	0		881	0.00	0.00	14.68
	78.0	3.3	0		558	0.00	0.00	9.30
	98.0	4.1	0		450	0.00	0.00	7.50
	118.5	4.9	0		232	0.00	0.00	3.87
	142.5	5.9	0		100	0.00	0.00	1.67
	168.5	7.0	0		22	0.00	0.00	0.37
	194.5	8.1	0		0	0.00	0.00	0.00
	217.3	9.1	0			0.00	0.00	0.00
	241.5	10.1	0			0.00	0.00	0.00
	266.5	11.1	0			0.00	0.00	0.00
	290.5	12.1	0			0.00	0.00	0.00
	314.5	13.1	0			0.00	0.00	0.00
	338.5	14.1	0			0.00	0.00	0.00
	360.5	15.0	0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
A10	0.0	0.0	0	0	1275	0.00	0.00	21.25
	23.0	1.0	0		1224	0.00	0.00	20.40
	47.0	2.0	0		887	0.00	0.00	14.78
	78.0	3.3	0		624	0.00	0.00	10.40
	98.0	4.1	0		548	0.00	0.00	9.13
	118.5	4.9	0		399	0.00	0.00	6.65
	142.5	5.9	0		210	0.00	0.00	3.50
	168.5	7.0	0		84	0.00	0.00	1.40
	194.5	8.1	0		34	0.00	0.00	0.57
	217.3	9.1	0			0.00	0.00	0.00
	241.5	10.1	0			0.00	0.00	0.00
	266.5	11.1	0			0.00	0.00	0.00
	290.5	12.1	0			0.00	0.00	0.00
	314.5	13.1	0			0.00	0.00	0.00
	338.5	14.1	0			0.00	0.00	0.00
	360.5	15.0	0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
			0			0.00	0.00	0.00
				HLa	Hlr	Hlc.	0.00	0.00	0.00
L1	0.0	0.0	1131	34	141	12.57	0.46	2.35
	23.0	1.0	225	289	441	2.50	3.91	7.35
	47.0	2.0	0	184	749	0.00	2.49	12.48
	78.0	3.3		60	990	0.00	0.81	16.50
	98.0	4.1		52	907	0.00	0.70	15.12
	118.5	4.9		60	887	0.00	0.81	14.78
	142.5	5.9		63	815	0.00	0.85	13.58
	168.5	7.0		57	623	0.00	0.77	10.38
	194.5	8.1		71	667	0.00	0.96	11.12
	217.3	9.1		76	595	0.00	1.03	9.92
	241.5	10.1		67	581	0.00	0.89	9.68
	266.5	11.1		71	491	0.00	0.96	8.18

	290.5	12.1		67	426	0.00	0.91	7.10
	314.5	13.1		74	403	0.00	1.00	6.72
	338.5	14.1		86	276	0.00	1.16	4.60
	360.5	15.0		89	214	0.00	1.20	3.57
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
L2	0.0	0.0	1120	20	110	12.44	0.27	1.83
	23.0	1.0	58	195	618	0.64	2.64	10.30
	47.0	2.0	0	33	655	0.00	0.45	10.92
	78.0	3.3		32	679	0.00	0.43	11.32
	98.0	4.1		24	549	0.00	0.32	9.15
	118.5	4.9		26	470	0.00	0.35	7.83
	142.5	5.9		23	362	0.00	0.31	6.03
	168.5	7.0		25	248	0.00	0.34	4.13
	194.5	8.1		24	140	0.00	0.32	2.33
	217.3	9.1		22	91	0.00	0.30	1.52
	241.5	10.1		30	54	0.00	0.41	0.90
	266.5	11.1		19	57	0.00	0.26	0.95
	290.5	12.1		16	67	0.00	0.22	1.12
	314.5	13.1		6	32	0.00	0.08	0.53
	338.5	14.1		6	19	0.00	0.08	0.32
	360.5	15.0				0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
L3	0.0	0.0	1166	48	79	12.96	0.65	1.32
	23.0	1.0	692	112	384	7.69	1.51	6.40
	47.0	2.0	0	416	611	0.00	5.62	10.18
	78.0	3.3		357	723	0.00	4.82	12.05
	98.0	4.1		335	631	0.00	4.53	10.52
	118.5	4.9		353	635	0.00	4.77	10.58
	142.5	5.9		326	538	0.00	4.41	8.97
	168.5	7.0		335	481	0.00	4.53	8.02
	194.5	8.1		341	407	0.00	4.61	6.78
	217.3	9.1		294	294	0.00	3.97	4.90
	241.5	10.1		299	255	0.00	4.04	4.25
	266.5	11.1		302	210	0.00	4.08	3.50
	290.5	12.1		275	163	0.00	3.72	2.72
	314.5	13.1		316	101	0.00	4.27	1.68
	338.5	14.1		315	61	0.00	4.26	1.02
	360.5	15.0		299	77	0.00	4.04	1.28
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
L4	0.0	0.0	1405	67	76	15.61	0.91	1.27
	23.0	1.0	1056	88	246	11.73	1.19	4.10
	47.0	2.0	0	543	515	0.00	7.34	8.58
	78.0	3.3		527	613	0.00	7.12	10.22
	98.0	4.1		497	576	0.00	6.72	9.60
	118.5	4.9		541	622	0.00	7.31	10.37

							(m/h²)	
						Hla	Pr	Re
	141.5	5.9		592	694	0.00	8.00	10.90
	168.5	7.0		565	576	0.00	7.64	9.60
	194.5	8.1		542	558	0.00	7.32	9.30
	217.5	9.1		497	435	0.00	6.72	7.25
	241.5	10.1		516	438	0.00	6.96	7.30
	266.5	11.1		487	400	0.00	6.58	6.67
	290.5	12.1		470	349	0.00	6.35	5.82
	314.5	13.1		534	341	0.00	7.22	5.68
	338.5	14.1		542	304	0.00	7.32	5.07
	360.5	15.0		531	263	0.00	7.18	4.38
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
L5	0.0	0.0	1208	85	74	13.42	1.15	1.23
	23.0	1.0	996	70	211	11.07	0.95	3.52
	47.0	2.0	0	543	471	0.00	7.34	7.85
	78.0	3.3		560	616	0.00	7.57	10.27
	98.0	4.1		541	555	0.00	7.31	9.25
	118.5	4.9		569	629	0.00	7.69	10.48
	142.5	5.9		573	579	0.00	7.74	9.65
	168.5	7.0		555	566	0.00	7.50	9.43
	194.5	8.1		583	577	0.00	7.91	9.62
	217.5	9.1		524	477	0.00	7.08	7.95
	241.5	10.1		520	464	0.00	7.03	7.73
	266.5	11.1		534	450	0.00	7.22	7.50
	290.5	12.1		522	441	0.00	7.05	7.35
	314.5	13.1		552	436	0.00	7.46	7.27
	338.5	14.1		572	419	0.00	7.73	6.98
	360.5	15.0		541	389	0.00	7.31	6.48
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
L6	0.0	0.0	1154	5	75	12.82	0.04	1.25
	23.0	1.0	76	0	650	0.84	0.00	10.83
	47.0	2.0	0	0	692	0.00	0.00	11.53
	78.0	3.3		37	395	0.00	0.50	6.42
	98.0	4.1		23	209	0.00	0.31	3.48
	118.5	4.9		39	135	0.00	0.53	2.25
	142.5	5.9		56	90	0.00	0.76	1.50
	168.5	7.0			74	0.00	0.00	1.23
	194.5	8.1			73	0.00	0.00	1.22
	217.5	9.1				0.00	0.00	0.00
	241.5	10.1				0.00	0.00	0.00
	266.5	11.1				0.00	0.00	0.00
	290.5	12.1				0.00	0.00	0.00
	314.5	13.1				0.00	0.00	0.00
	338.5	14.1				0.00	0.00	0.00
	360.5	15.0				0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00

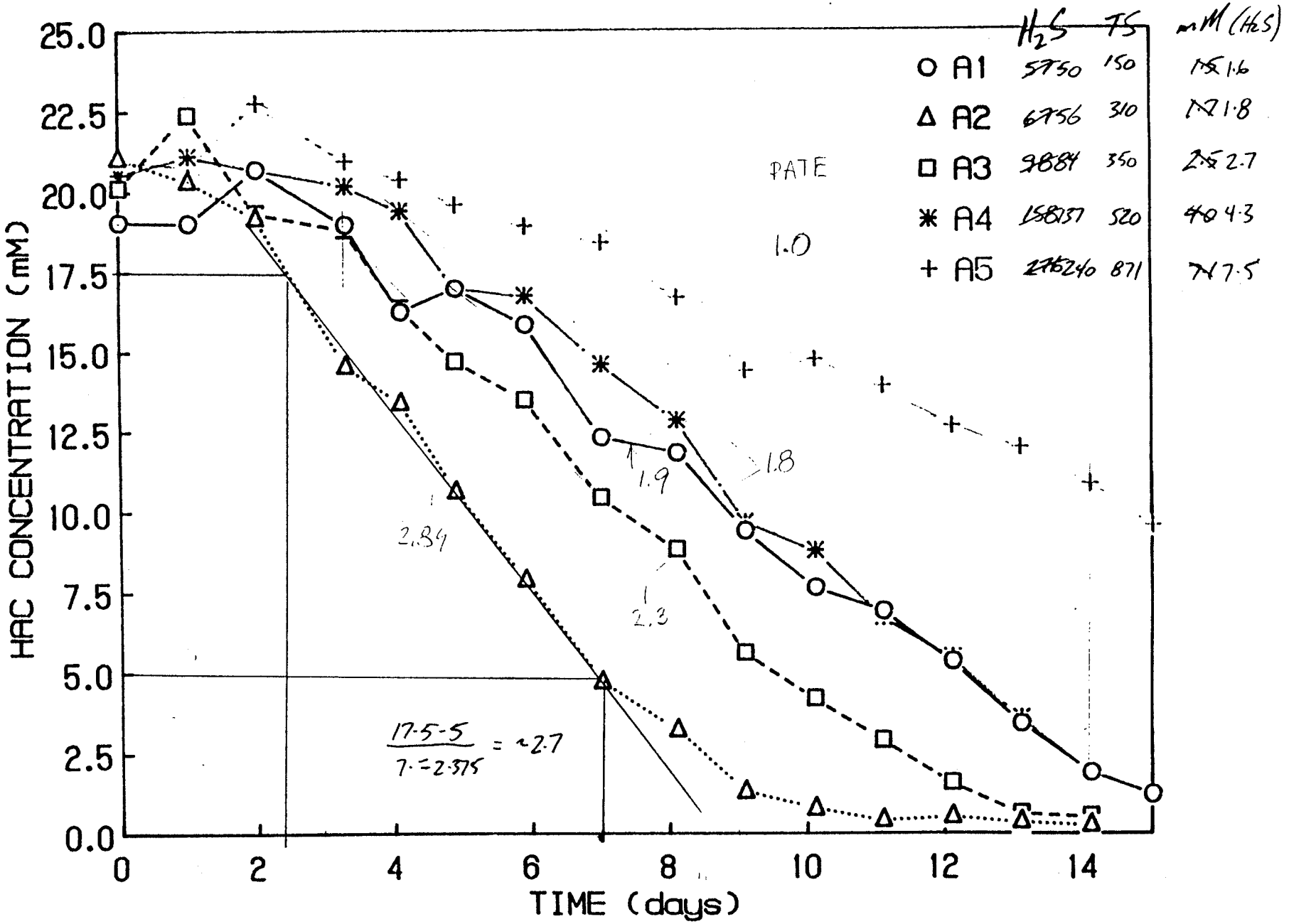
L7	0.0	0.0	1148	5	105	12.76	0.04	1.75
	23.0	1.0	47	66	733	0.52	0.89	12.22
	47.0	2.0	0	6	566	0.00	0.08	9.43
	78.0	3.3		13	402	0.00	0.18	6.70
	98.0	4.1		12	258	0.00	0.16	4.30
	118.5	4.9		2	128	0.00	0.08	2.13
	142.5	5.9		9	66	0.00	0.12	1.10
	168.5	7.0			24	0.00	0.00	0.40
	194.5	8.1			14	0.00	0.00	0.23
	217.3	9.1				0.00	0.00	0.00
	241.5	10.1				0.00	0.00	0.00
	266.5	11.1				0.00	0.00	0.00
	290.5	12.1				0.00	0.00	0.00
	314.5	13.1				0.00	0.00	0.00
	338.5	14.1				0.00	0.00	0.00
	360.5	15.0				0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
L8	0.0	0.0	1159	4	105	12.88	0.05	1.75
	23.0	1.0	48	64	728	0.53	0.86	12.13
	47.0	2.0	0	7	642	0.00	0.09	10.70
	78.0	3.3		3	427	0.00	0.04	7.12
	98.0	4.1		15	280	0.00	0.20	4.67
	118.5	4.9		9	175	0.00	0.12	2.92
	142.5	5.9		0	53	0.00	0.00	0.88
	168.5	7.0			18	0.00	0.00	0.30
	194.5	8.1			11	0.00	0.00	0.18
	217.3	9.1				0.00	0.00	0.00
	241.5	10.1				0.00	0.00	0.00
	266.5	11.1				0.00	0.00	0.00
	290.5	12.1				0.00	0.00	0.00
	314.5	13.1				0.00	0.00	0.00
	338.5	14.1				0.00	0.00	0.00
	360.5	15.0				0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
L9	0.0	0.0	1137	10	165	12.63	0.14	2.75
	23.0	1.0	50	50	677	0.56	0.68	11.28
	47.0	2.0	0	33	575	0.00	0.43	9.58
	78.0	3.3		9	484	0.00	0.12	8.07
	98.0	4.1		8	327	0.00	0.11	5.45
	118.5	4.9		4	199	0.00	0.05	3.32
	142.5	5.9		5	70	0.00	0.07	1.17
	168.5	7.0			26	0.00	0.00	0.43
	194.5	8.1			7	0.00	0.00	0.12
	217.3	9.1				0.00	0.00	0.00
	241.5	10.1				0.00	0.00	0.00
	266.5	11.1				0.00	0.00	0.00
	290.5	12.1				0.00	0.00	0.00
	314.5	13.1				0.00	0.00	0.00

						L	I	A
	338.5	14.1				0.00	0.00	0.00
	360.5	15.0				0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
	0.0	0.0	1126	10	91	12.51	0.14	1.52
	23.0	1.0	62	236	602	0.69	3.19	10.03
	47.0	2.0	0	264	524	0.00	3.57	8.73
	73.0	3.0		181	512	0.00	2.45	8.53
	98.0	4.1		152	380	0.00	2.05	6.33
	118.5	4.9		162	356	0.00	2.19	5.93
	142.5	5.9		142	232	0.00	1.92	3.87
	168.5	7.0		113	157	0.00	1.53	2.62
	194.5	8.1		97	111	0.00	1.31	1.85
	217.5	9.1		112	121	0.00	1.51	2.02
	241.5	10.1		68	102	0.00	0.92	1.70
	266.5	11.1		47	97	0.00	0.64	1.62
	290.5	12.1		35	80	0.00	0.47	1.33
	314.5	13.1		30	34	0.00	0.41	0.57
	338.5	14.1		48	49	0.00	0.65	0.82
	360.5	15.0				0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
Sm1								
	0.0	0.0	599		648	6.66	0.00	10.80
	23.0	1.0	0	175	966	0.00	2.36	16.10
	47.0	2.0		26	1136	0.00	0.35	18.93
	73.0	3.0		9	1133	0.00	0.12	18.88
	98.0	4.1		18	962	0.00	0.24	16.03
	118.5	4.9		19	1040	0.00	0.26	17.33
	142.5	5.9		19	911	0.00	0.26	15.18
	168.5	7.0		11	887	0.00	0.15	14.78
	194.5	8.1		22	744	0.00	0.30	12.40
	217.5	9.1		35	655	0.00	0.47	10.92
	241.5	10.1		30	496	0.00	0.41	8.27
	266.5	11.1		34	410	0.00	0.46	6.83
	290.5	12.1		25	352	0.00	0.34	5.87
	314.5	13.1		17	248	0.00	0.23	4.13
	338.5	14.1			165	0.00	#VALUE	2.75
	360.5	15.0		29	76	0.00	0.39	1.27
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
						0.00	0.00	0.00
F2	0.0	0.0	557	30	683	6.19	0.41	11.38
	23.0	1.0	0	91	928	0.00	1.23	15.47
	47.0	2.0		8	929	0.00	0.11	15.48
	73.0	3.0		12	941	0.00	0.16	15.68
	98.0	4.1		9	651	0.00	0.12	10.85
	118.5	4.9		11	583	0.00	0.15	9.72
	142.5	5.9		14	917	0.00	0.19	15.28
	168.5	7.0		14	329	0.00	0.19	5.48

	194.5	8.1		10	171	0.00	0.14	2.39	
	217.3	9.1		17	99	0.00	0.23	1.68	
	241.3	10.1		9	43	0.00	0.11	0.72	
	266.3	11.1			58	0.00		0.97	
	290.3	12.1		10	55	0.00	0.14	0.92	
	314.5	13.1		7	44	0.00	0.09	0.73	
	338.5	14.1			8	0.00	0.00	0.13	
	360.5	15.0				0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
F3	0.0	0.0	568	65	640	6.31	0.88	10.67	
	23.0	1.0	358	77	853	3.98	1.04	14.22	
	47.0	2.0	0	171	954	0.00	2.31	15.90	
	78.0	3.3		154	924	0.00	2.08	15.40	
	98.0	4.1		149	860	0.00	2.01	14.33	
	118.5	4.9		167	894	0.00	2.26	14.90	
	142.5	5.9		145	722	0.00	1.96	12.03	
	168.5	7.0		160	717	0.00	2.16	11.95	
	194.5	8.1		161	593	0.00	2.18	9.88	
	217.3	9.1		150	421	0.00	2.03	7.02	
	241.5	10.1		148	350	0.00	2.00	5.83	
	266.5	11.1		149	297	0.00	2.01	4.95	
	290.5	12.1		150	224	0.00	2.03	3.73	
	314.5	13.1		156	119	0.00	2.11	1.98	
	338.5	14.1		145	61	0.00	1.96	1.02	
	360.5	15.0		131	73	0.00	1.77	1.22	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
F4	0.0	0.0	747	50	685	8.30	0.68	11.42	
	23.0	1.0	325	80	871	3.61	1.08	14.52	
	47.0	2.0	0	232	1030	0.00	3.14	17.17	
	78.0	3.3		220	1088	0.00	2.97	18.13	
	98.0	4.1		190	939	0.00	2.57	15.65	
	118.5	4.9		212	960	0.00	2.86	16.00	
	142.5	5.9		209	890	0.00	2.82	14.83	
	168.5	7.0		217	837	0.00	2.93	13.95	
	194.5	8.1		213	787	0.00	2.88	13.12	
	217.3	9.1		171	528	0.00	2.31	8.80	
	241.5	10.1		206	532	0.00	2.78	8.87	
	266.5	11.1		185	458	0.00	2.50	7.63	
	290.5	12.1		187	396	0.00	2.53	6.60	
	314.5	13.1		196	322	0.00	2.65	5.37	
	338.5	14.1		211	231	0.00	2.86	3.85	
	360.5	15.0		197	178	0.00	2.66	2.97	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	Hz
F5	0.0	0.0	734	97	661	8.16	1.31	11.02	O
	23.0	1.0	421	83	709	4.68	1.12	12.15	↑

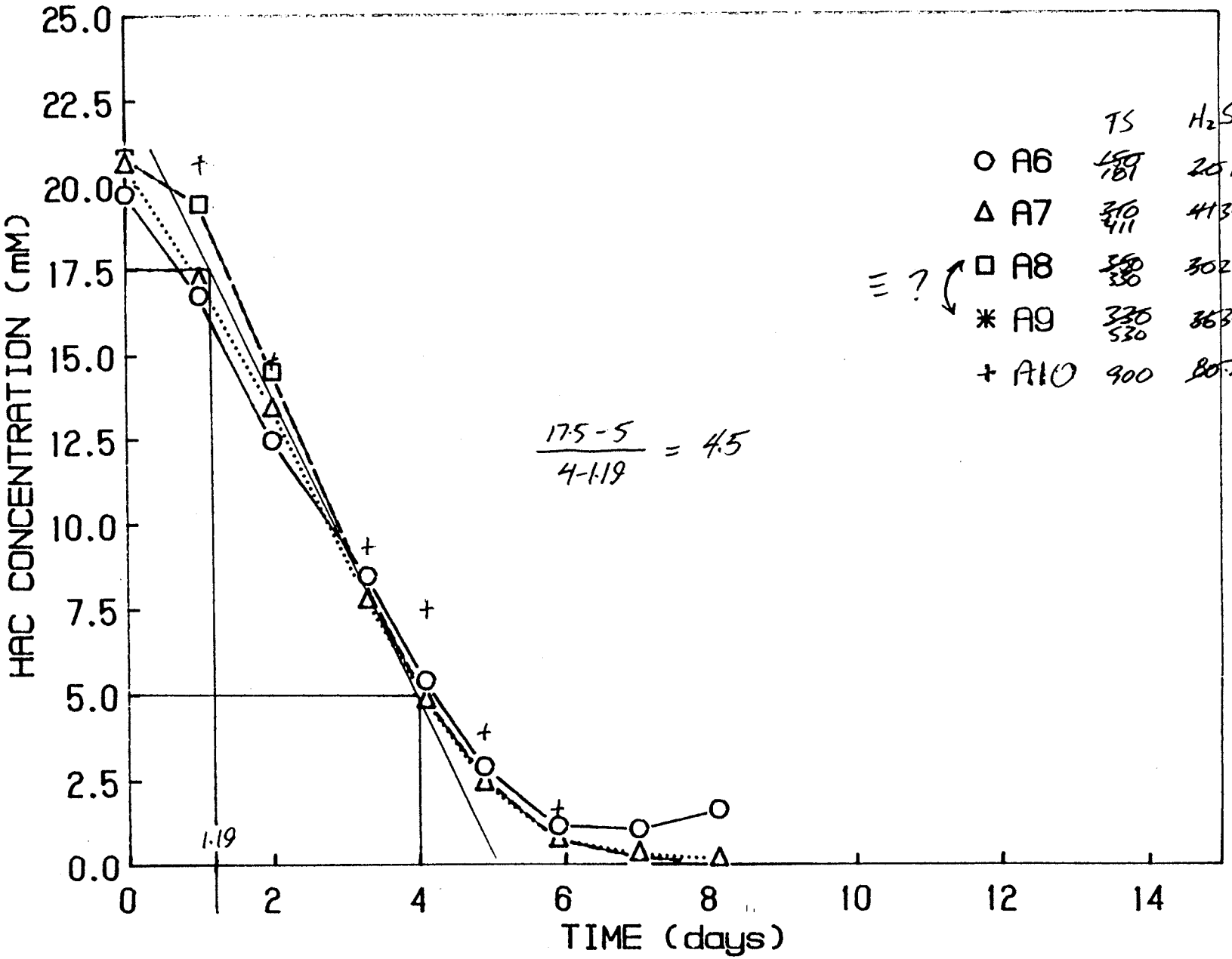
	47.0	2.0	0	276	990	0.00	3.75	16.50	↑
	78.0	3.3		300	1040	0.00	4.05	17.55	
	98.0	4.1		272	803	0.00	3.68	13.42	
	118.5	4.9		265	851	0.00	3.58	14.18	
	142.5	5.9		300	974	0.00	4.05	16.23	
	168.5	7.0		304	951	0.00	4.11	15.85	
	194.5	8.1		301	894	0.00	4.07	14.90	
	217.3	9.1		265	711	0.00	3.58	11.85	
	241.5	10.1		259	663	0.00	3.50	11.05	
	266.5	11.1		249	639	0.00	3.36	10.65	
	290.5	12.1		278	691	0.00	3.76	11 52	
	314.5	13.1		277	655	0.00	3.74	10.92	
	338.5	14.1		294	642	0.00	3.97	10.70	
	360.5	15.0		290	592	0.00	3.92	9.87	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
F6	0.0	0.0	480	80	612	5.33	1.08	10.20	
	23.0	1.0	25	0	943	0.28	0.00	15.72	
	47.0	2.0	0	21	720	0.00	0.28	12.00	
	78.0	3.3		28	507	0.00	0.38	8.45	
	98.0	4.1		14	315	0.00	0.19	5.25	
	118.5	4.9		3	197	0.00	0.04	3.28	
	142.5	5.9		14	82	0.00	0.19	1.37	
	168.5	7.0			116	0.00	0.00	1.93	
	194.5	8.1			50	0.00	0.00	0.83	
	217.3	9.1				0.00	0.00	0.00	
	241.5	10.1				0.00	0.00	0.00	
	266.5	11.1				0.00	0.00	0.00	
	290.5	12.1				0.00	0.00	0.00	
	314.5	13.1				0.00	0.00	0.00	
	338.5	14.1				0.00	0.00	0.00	
	360.5	15.0				0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
						0.00	0.00	0.00	
F7	0.0	0.0	531	50	653	5.90	0.68	10.88	
	23.0	1.0	19	0	858	0.21	0.00	14.30	
	47.0	2.0	0	0	675	0.00	0.00	11.25	
	78.0	3.3		5	436	0.00	0.07	7.27	
	98.0	4.1		3	280	0.00	0.04	4.67	
	118.5	4.9		9	154	0.00	0.12	2.57	
	142.5	5.9		0	53	0.00	0.00	0.88	
	168.5	7.0			34	0.00	0.00	0.57	
	194.5	8.1			24	0.00	0.00	0.40	
	217.3	9.1				0.00	0.00	0.00	
	241.5	10.1				0.00	0.00	0.00	
	266.5	11.1				0.00	0.00	0.00	
	290.5	12.1				0.00	0.00	0.00	
	314.5	13.1				0.00	0.00	0.00	
	338.5	14.1				0.00	0.00	0.00	
	360.5	15.0				0.00	0.00	0.00	

241.5	10.1	0.00	0.00	0.00
266.5	11.1	0.00	0.00	0.00
290.5	12.1	0.00	0.00	0.00
314.5	13.1	0.00	0.00	0.00
338.5	14.1	0.00	0.00	0.00
360.5	15.0	0.00	0.00	0.00


HAC CONCENTRATION (mM)
TIME (days)
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0
2
4
6
8
10
12
14
A1
A2
A3
A4
A5
PATE
1.0
1.9
>1.8
2.84
2.3
17.5-5 / 7-=2.375 = ~2.7

RATE = 2.75 mM/d

Common



		TS	H_2S	mM/(l·s)	
○	A6	~~450~~ 181	2017	0.5	0.5
△	A7	~~310~~ 411	4134	NQ	1.1
□	A8	~~530~~ 330	3025	0.2	0.7
*	A9	~~330~~ 530	3633	NQ	1.1
+	A10	900	8071	2.1	2.2

≡ ?

$\frac{17.5 - 5}{4 - 1.19} = 4.5$

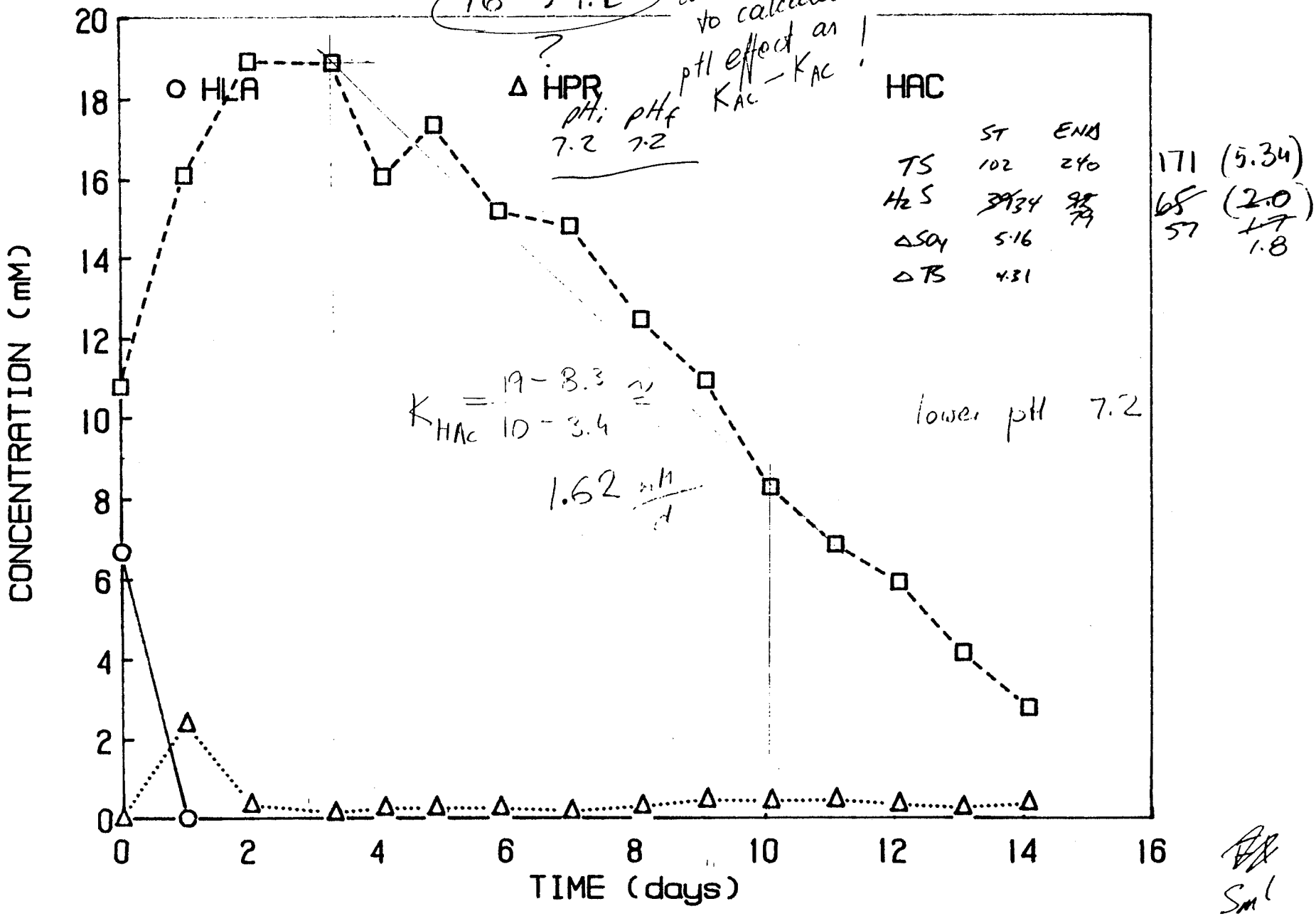
avg = 1.2

hi pH

F1

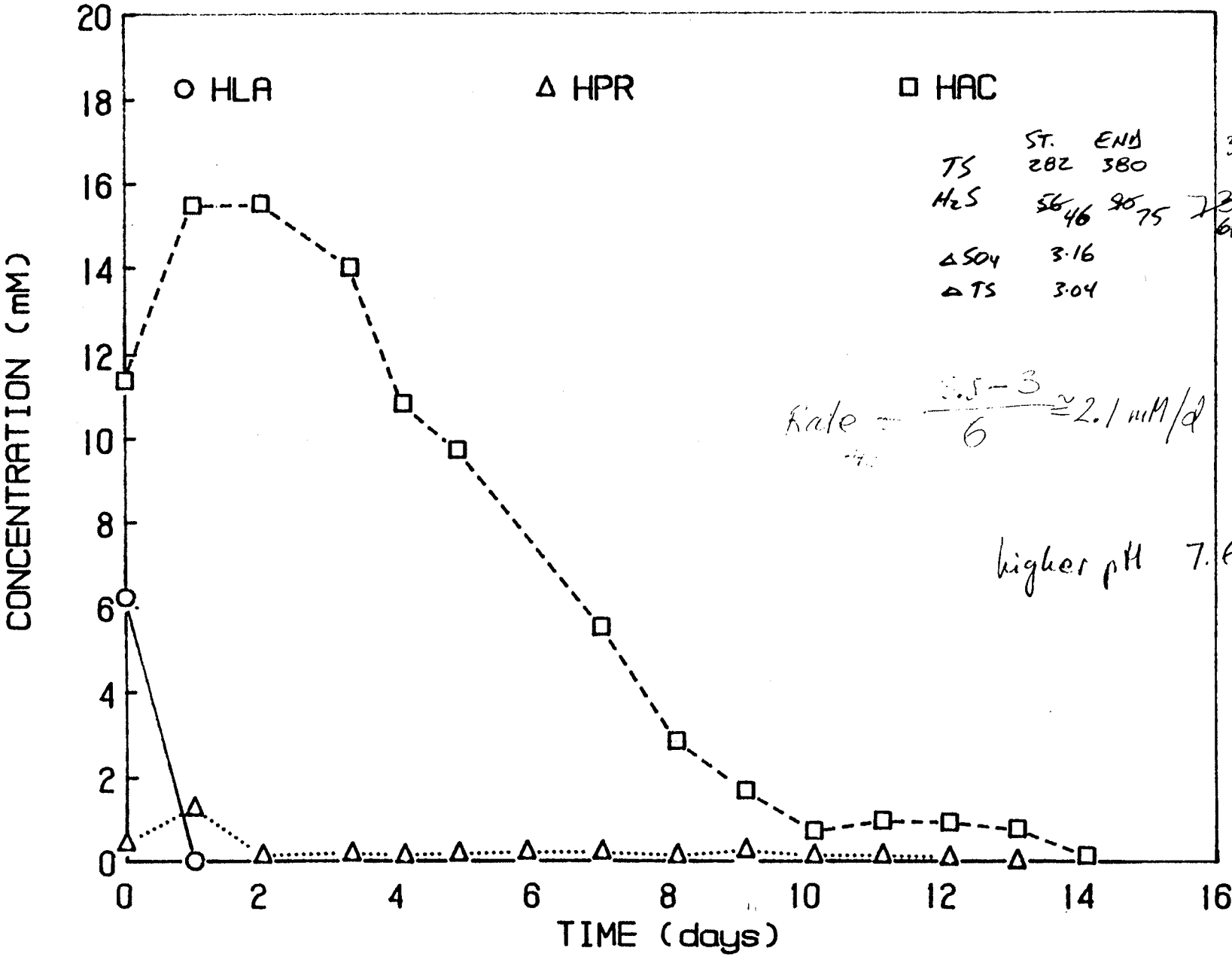
Anomaly – caused by serious pH difference 1.6 → 7.2 ?

Anomaly ? could serve to calculate pH effect on K_{AC} – K_{AC} !



Sm

F2



	ST.	END
TS	282	380
H_2S	~~56~~ 46	~~90~~ 75
ΔSO_4	3.16	
ΔTS	3.04	

331 (10.34

~~73~~ 61 (~~2.3~~ 1.9

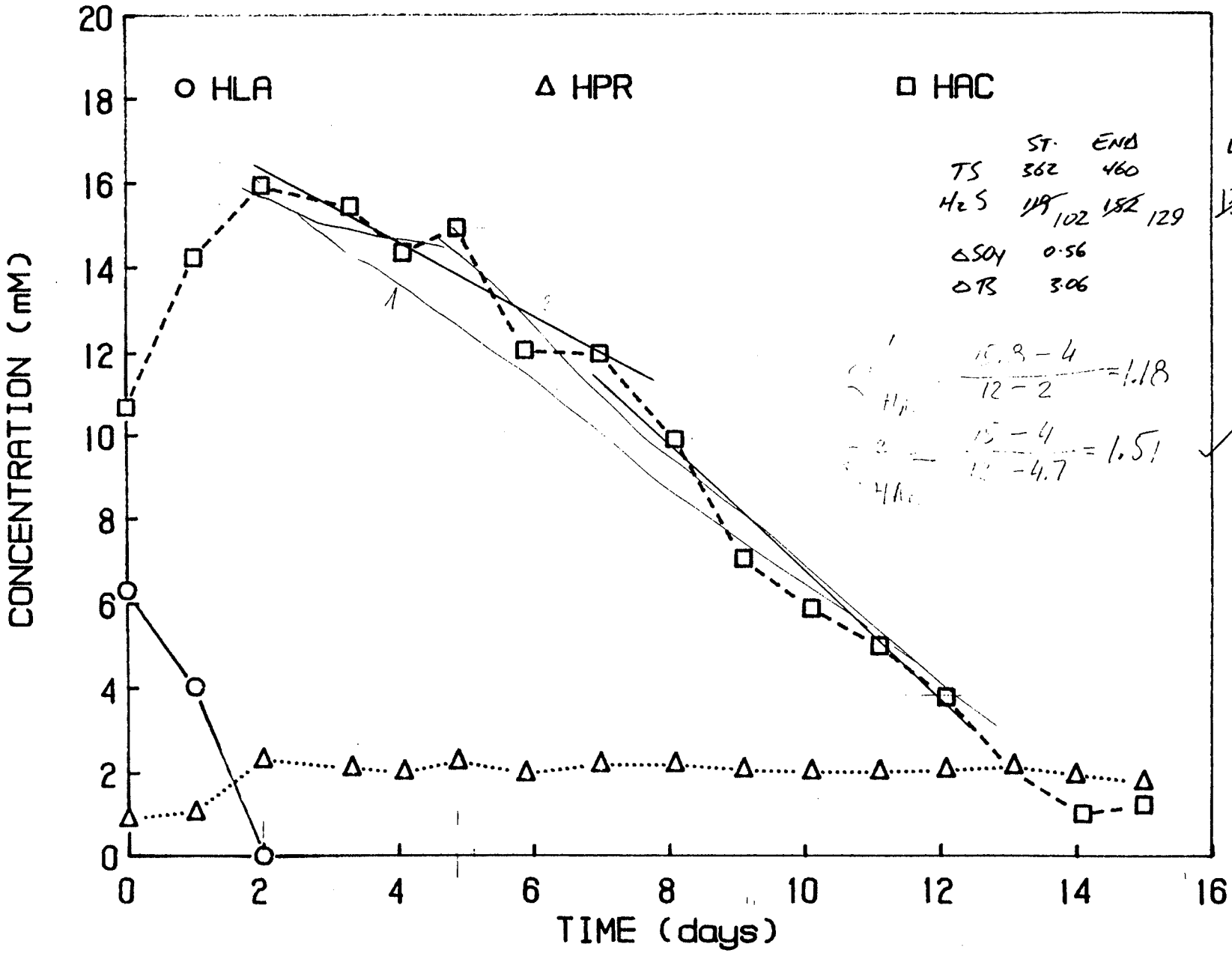
~~48~~

$$\text{Rate} = \frac{8.5 - 3}{6} \approx 2.1 \text{ mM/d}$$

higher pH 7.6

SM2

F3



	ST.	END
TS	362	460
H_2S	~~119~~ 102	~~152~~ 129
ΔSO4	0.56	
OTS	3.06	

411 (12.8)

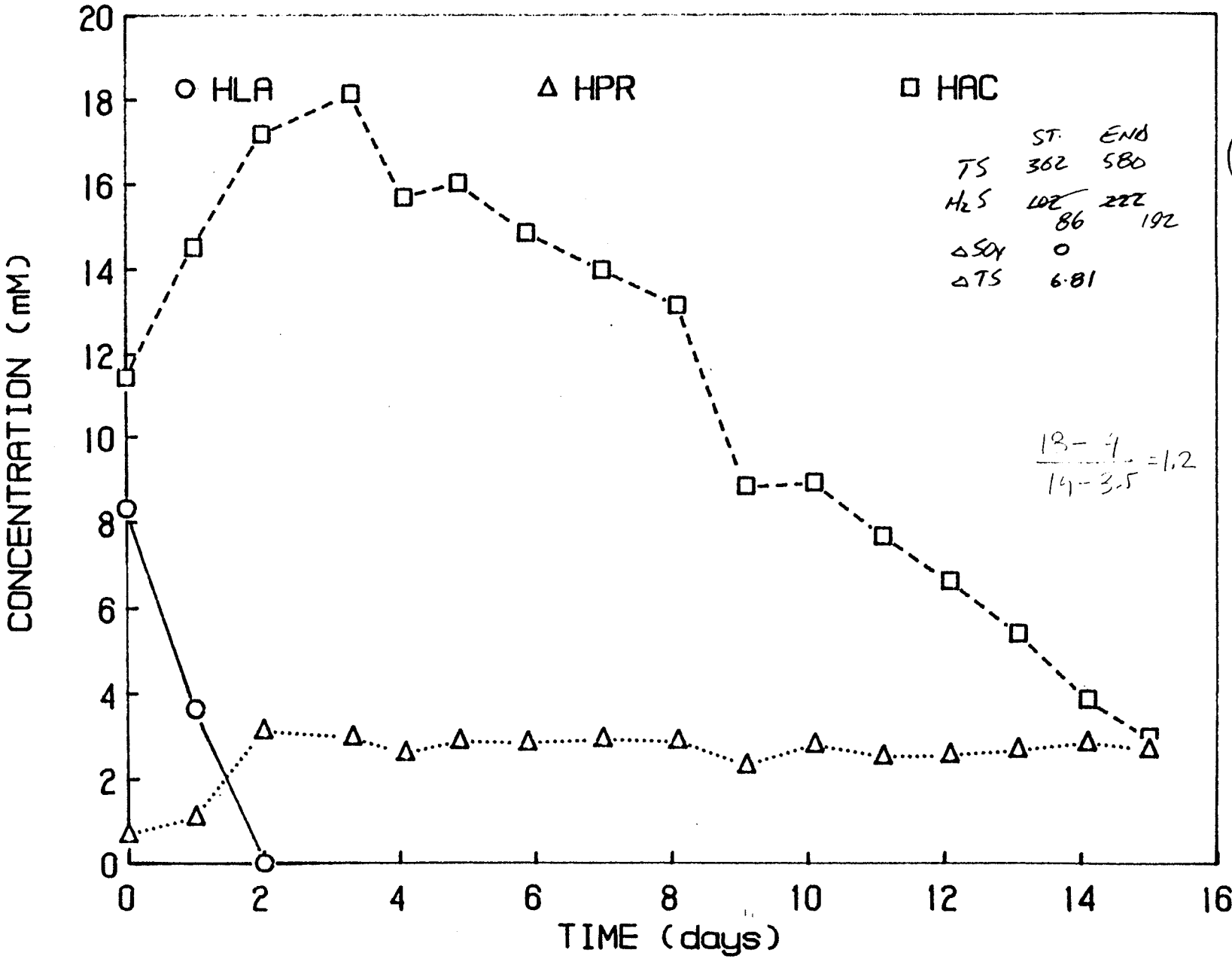
~~135~~ 116 (~~4.2~~ 3.4 3.6)

$\frac{15.8-4}{12-2} = 1.18$

$\frac{15-4}{12-4.7} = 1.51$ ✓

Sm3

F4



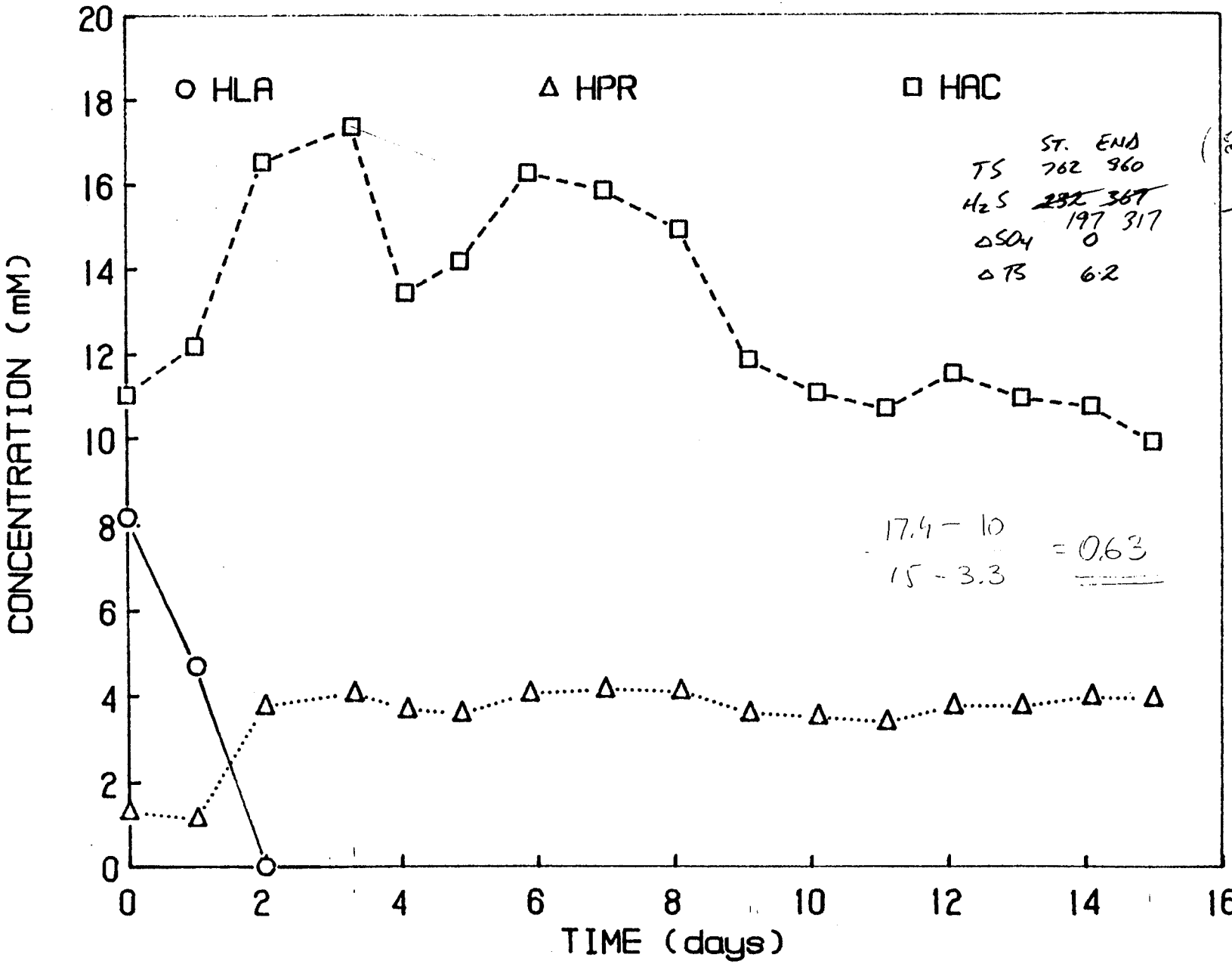
	ST.	END
TS	362	580
H_2S	~~102~~ 86	222 ~~192~~
ΔSOx	0	
ΔTS	6.81	

(471 14.72
~~162~~ 139 ~~5.1~~ 4.4

$\frac{13-1}{14-3.5} = 1.2$

Smt

F5



	ST.	END
TS	762	960
H_2S	~~282~~	~~367~~ 197 317
ΔSO_4		0
ΔTS		6.2

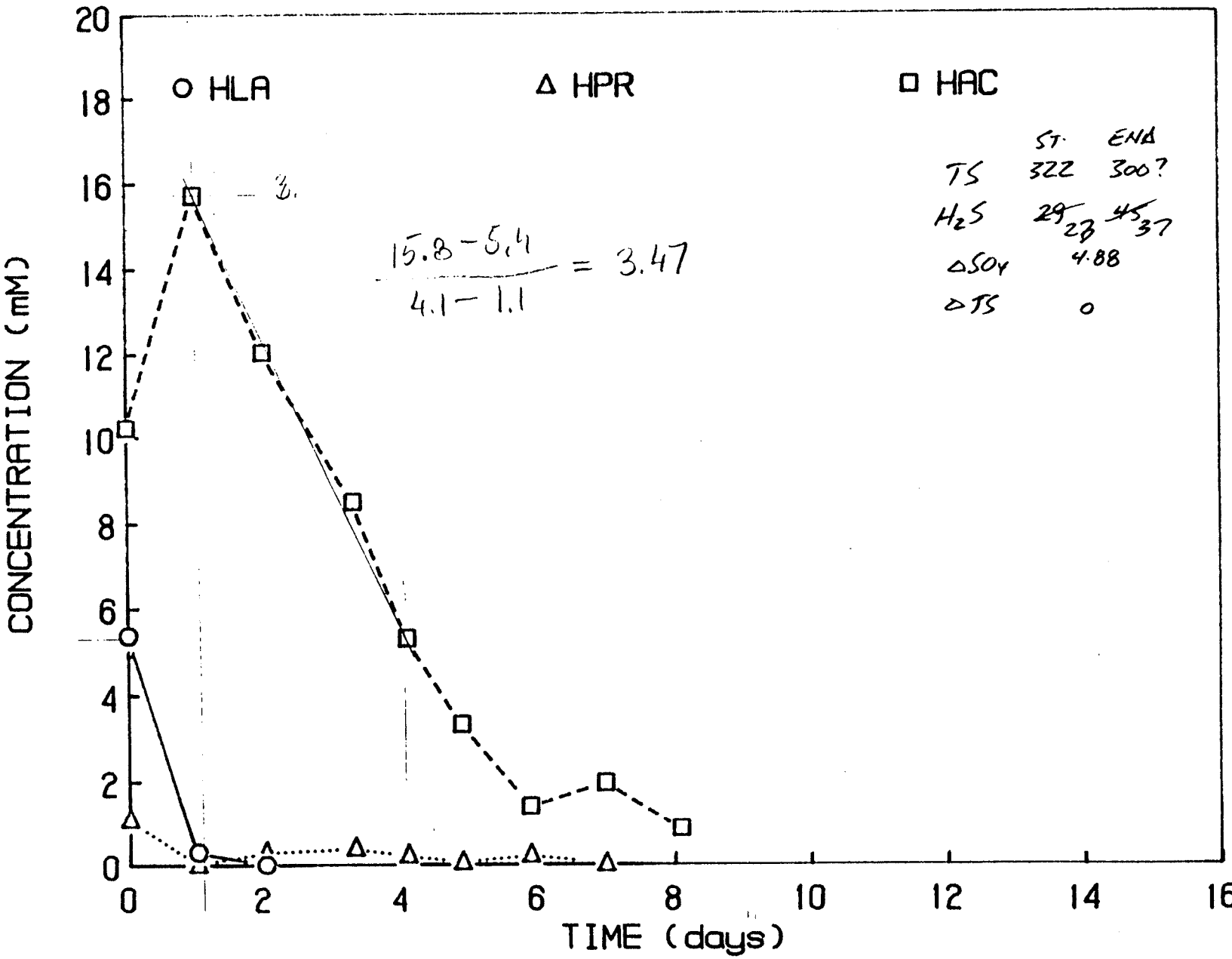
(861) 26.9

~~300~~ ~~336~~ 297 716 257 8.1

17.4 − 10 / 15 − 3.3 = 0.63

Sm 5

F6



3.

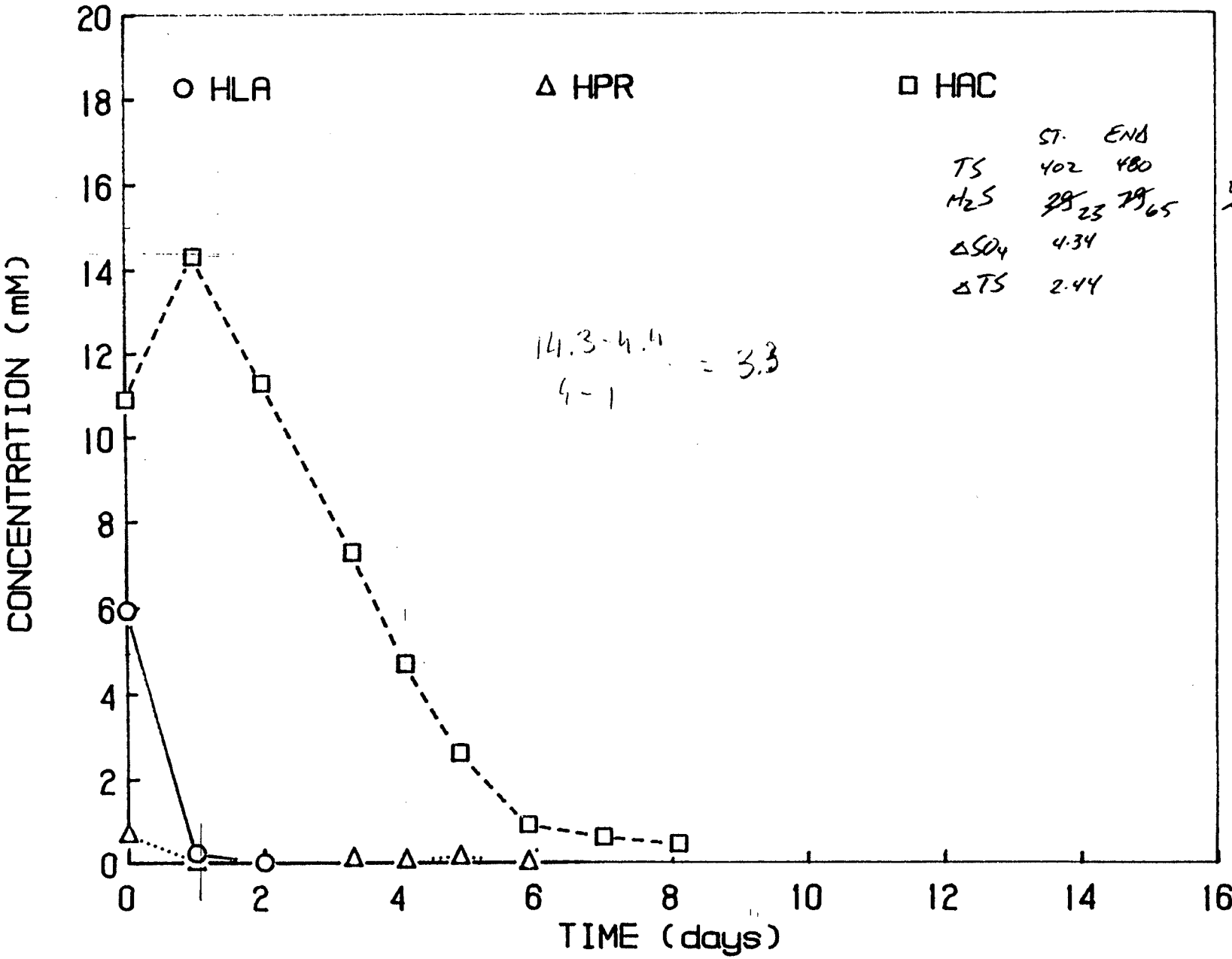
$$\frac{15.8 - 5.4}{4.1 - 1.1} = 3.47$$

	ST.	END
TS	322	300?
H_2S	~~29~~ 28	~~45~~ 37
ΔSO_4	4.88	
ΔTS	0	

311 9.7
~~37~~ 30 (~~1.6~~ 0.9 1.0)

SM6

F7



	ST.	END
TS	402	480
H_2S	~~29~~ 23	~~79~~ 65
ΔSO_4	4.34	
ΔTS	2.44	

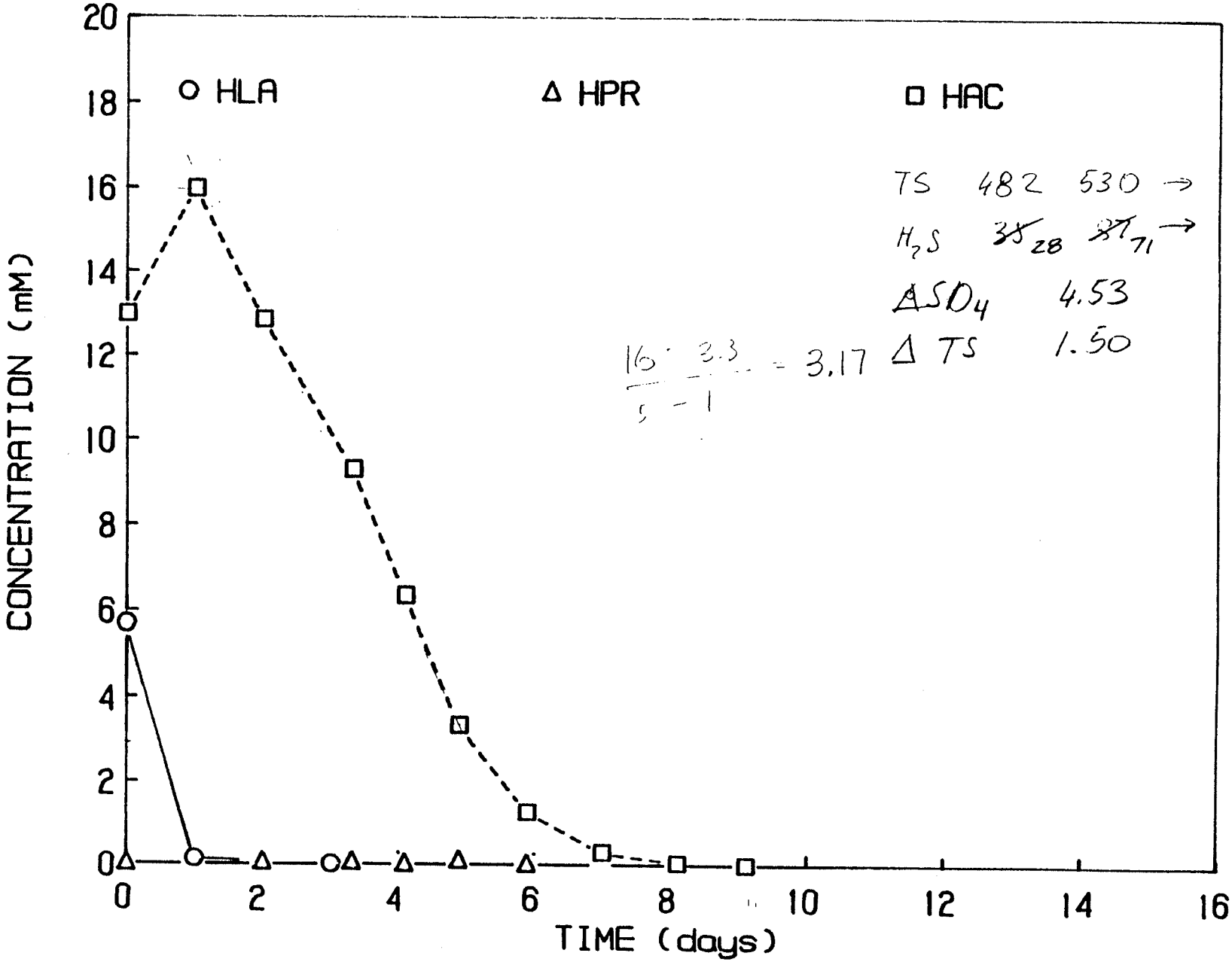
14.3 - 4.4 / 4 - 1 = 3.3

441 13.8

54/44 1.69 1.5 1.4

SM7

FB corrected.



TS 482 530 → 606 ~~530~~ (15.8)

H_2S ~~35~~ 28 ~~27~~ 71 → ~~61~~ 60 (~~1.7~~ 1.6)

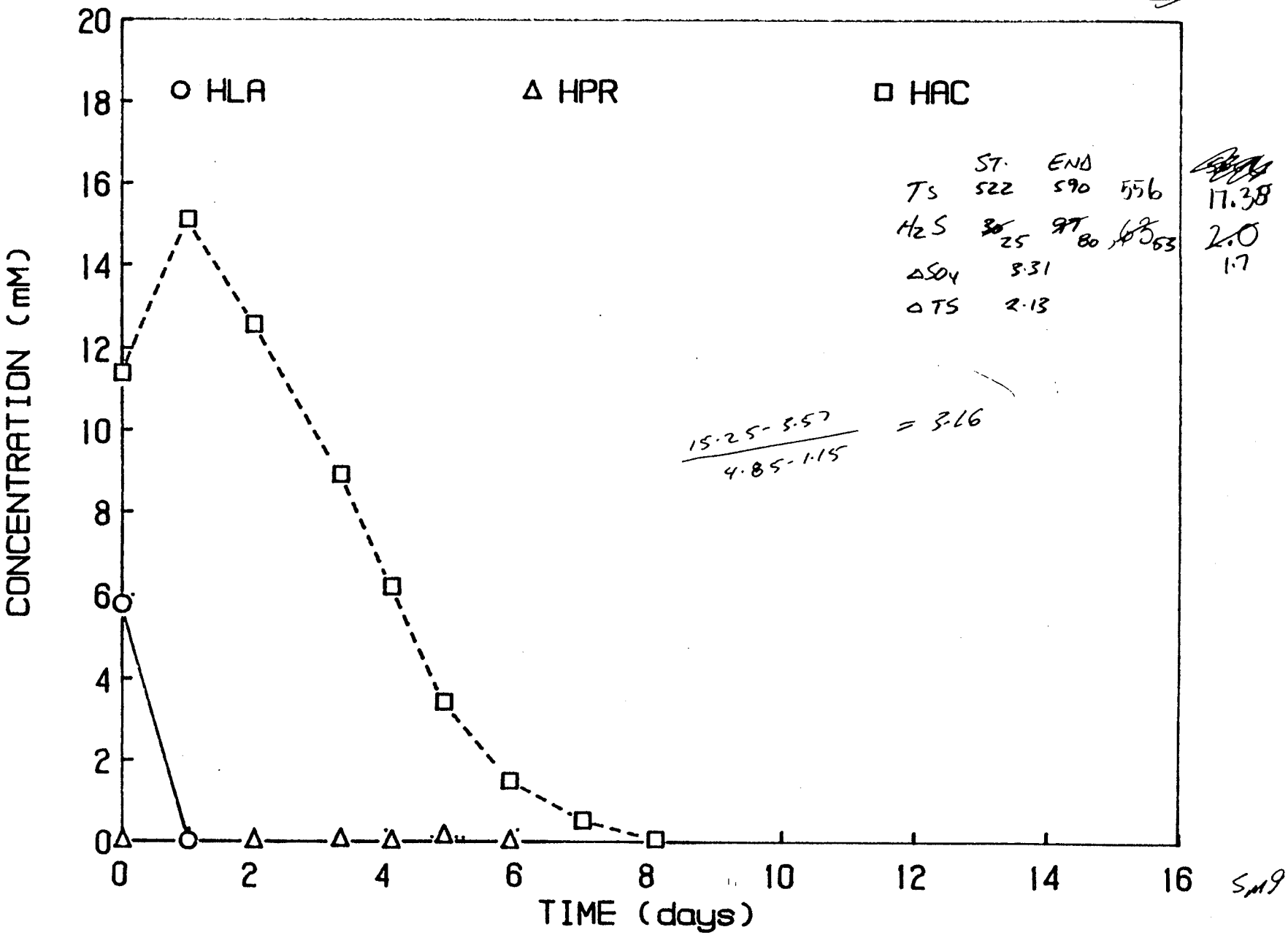
ΔSO_4 4.53

Δ TS 1.50

$\frac{16 \cdot 3.3}{5 - 1} = 3.17$

SmB

F9 correct

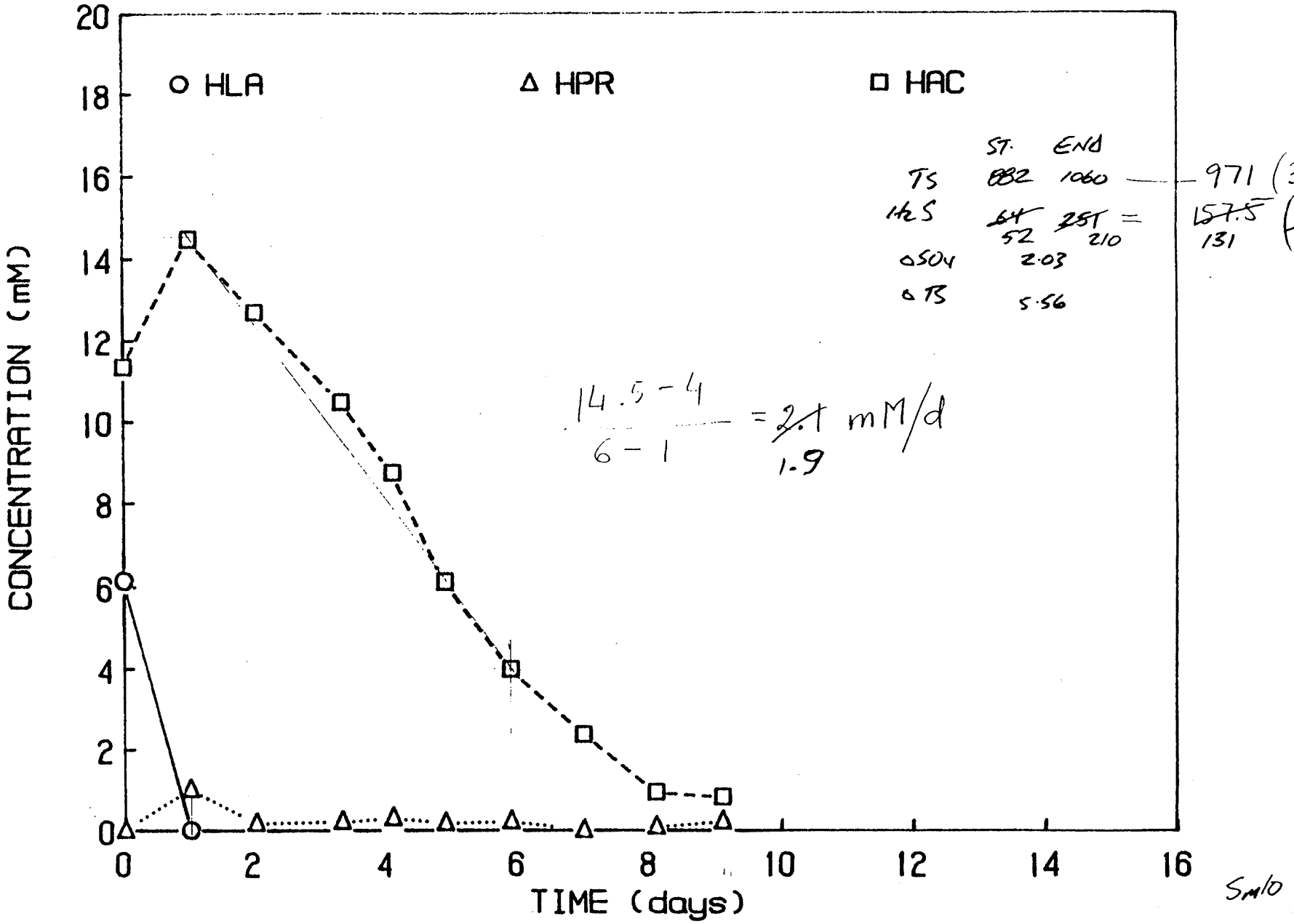


	ST.	END		
Ts	522	590	556	17.38
H_2S	~~30~~ 25	~~97~~ 80	~~68~~ 63	2.0
				1.7
ΔSO_4	3.31			
ΔTS	2.13			

$\frac{15.25 - 5.57}{4.85 - 1.15} = 3.16$

5 of 9

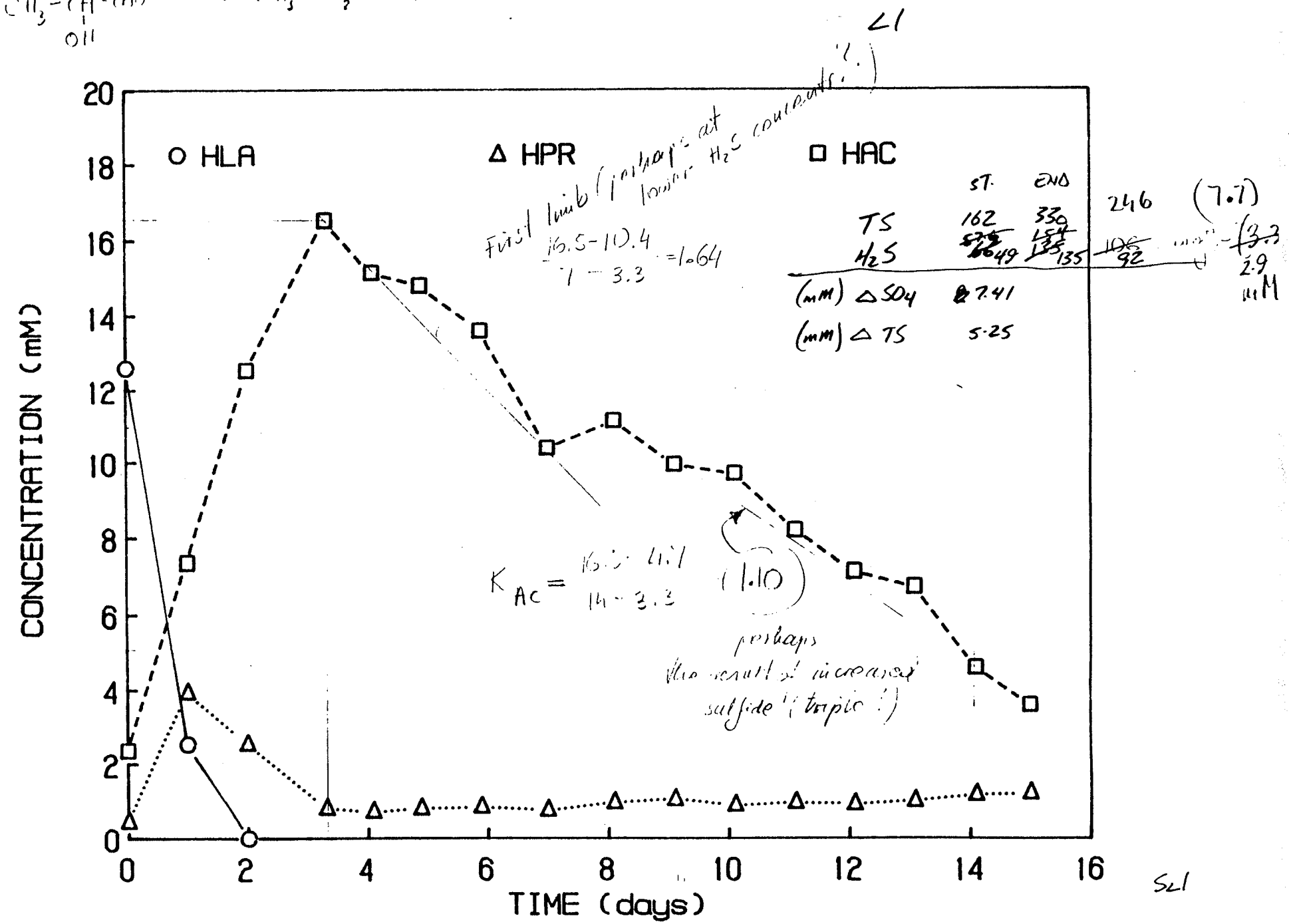
F10

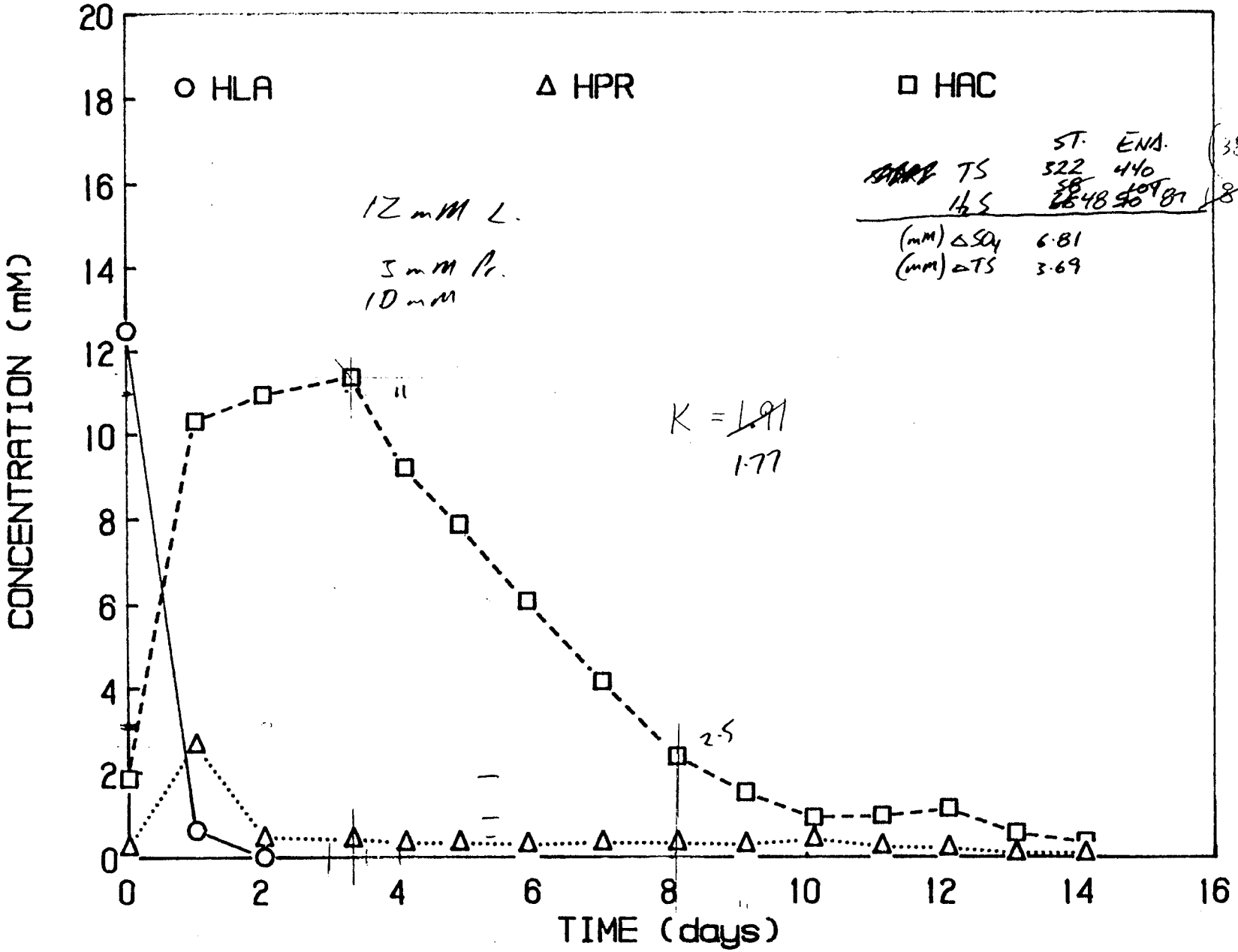


	ST.	END	
TS	~~882~~	1060	~~971~~ (30.34 mM)
H_2S	~~64~~ 52	~~251~~ 210	= ~~157.5~~ 131 (~~4.92~~ 4.1)
ΔSO_4	2.03		
ΔTS	5.56		

$$\frac{14.5-4}{6-1} = \frac{10.5}{5} = \text{~~2.1~~ } 1.9 \text{ mM/d}$$

SM10


CONCENTRATION (mM)
TIME (days)
0
2
4
6
8
10
12
14
16
18
20
O HLA
Δ HPR
□ HAC
ST.
END
TS
162
330
H2S
649
135
(mM) Δ SO4
7.41
(mM) Δ TS
5.25
246
(7.7)
92
2.9
mM
First limb (primary) at lower H2S concentrations
16.5-10.4 / 1 - 3.3 = 1.64
K Ac = 16.5 - 4.1 / 14 - 3.3
(1.10)
perhaps the result of increased sulfide toxicity



mM

ST. ENA. (381) 11.9

TS 522 440

H_2S 48 87 68 2.5 2.1

(mM) ΔSO_4 6.81

(mM) ΔTS 3.69

12 mM L.

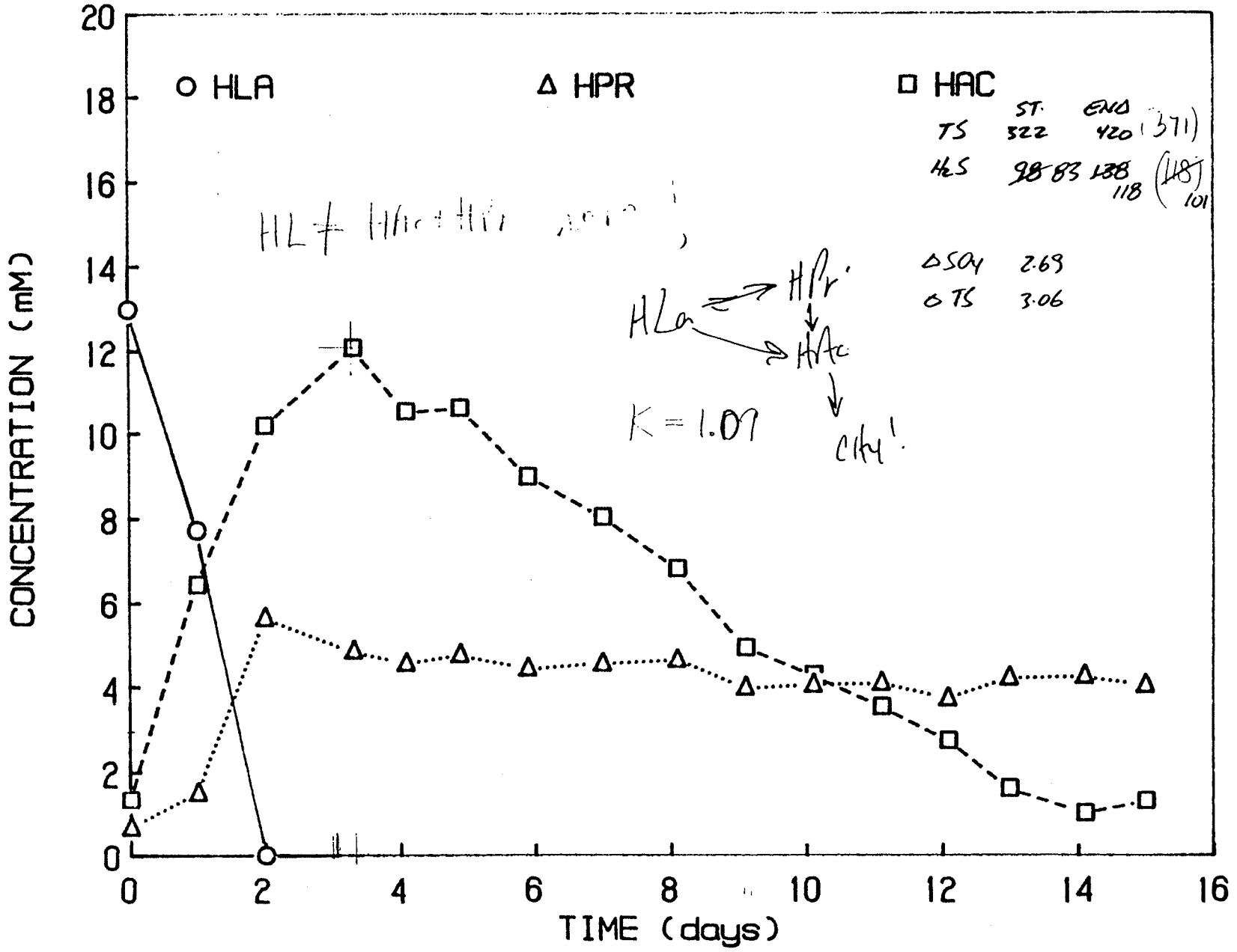
5 mM R.

10 mM

$K = 1.77$

2.5

S42



mM

	ST.	END	
TS	522	420	(371)
H_2S	~~98~~ 83	~~138~~ 118	(~~118~~ 101)

11.6

3.7 → 3.2

ΔSO4 2.69

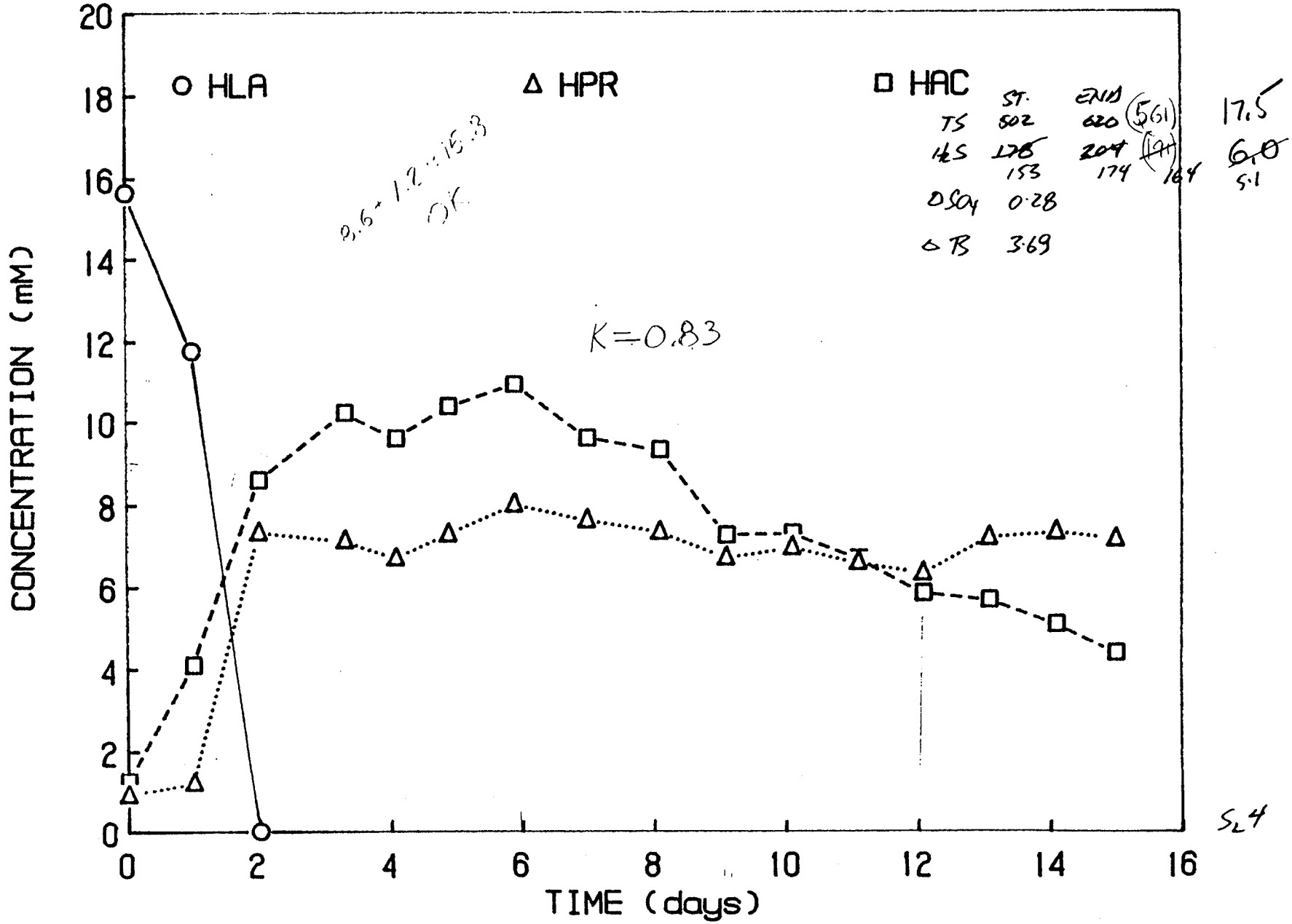
Δ TS 3.06

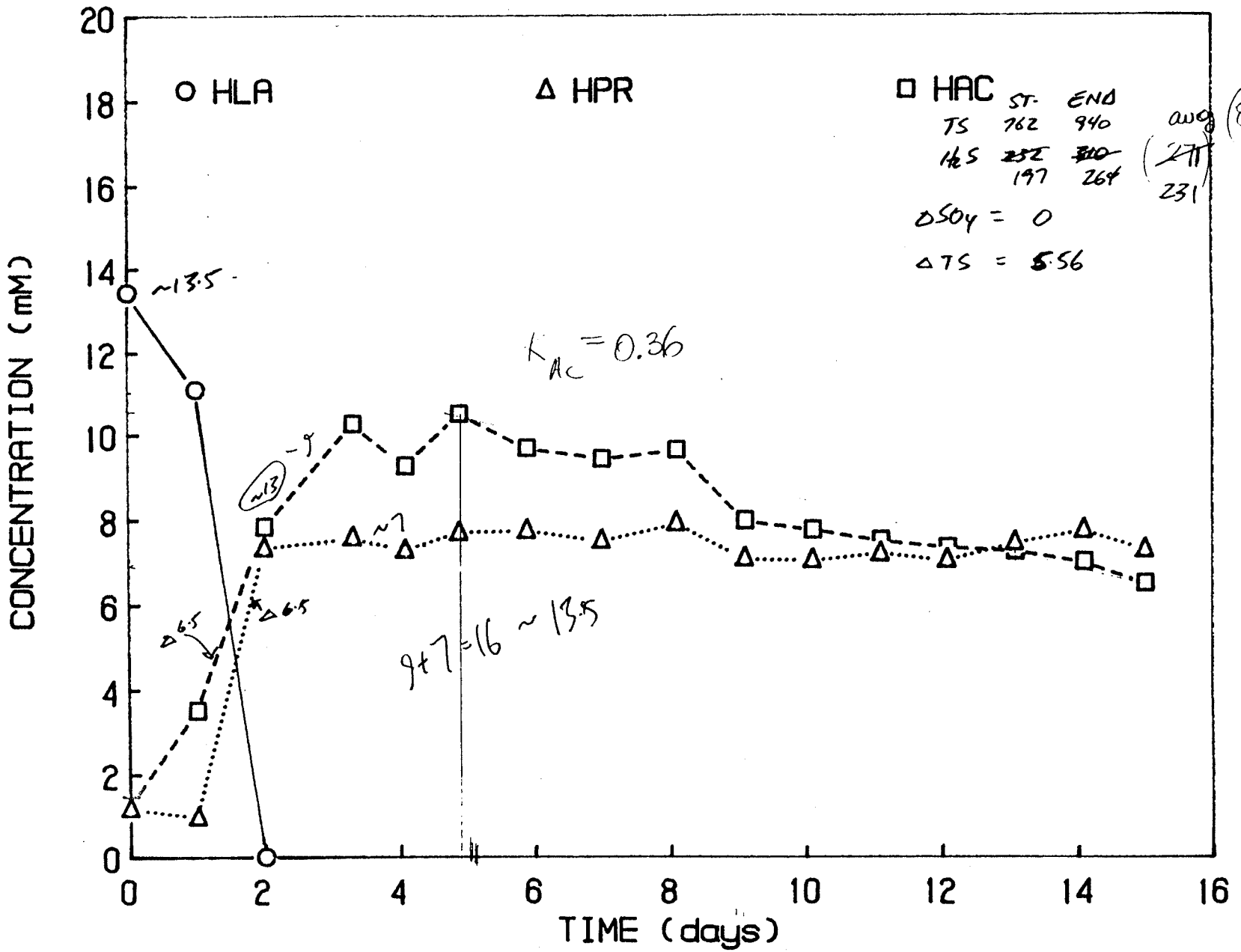
HL + HAc + HPr

HLa → HPr → HAc → CH4

K = 1.07

SC3


HLA
HPR
HAC
CONCENTRATION (mM)
TIME (days)
K=0.83
0
2
4
6
8
10
12
14
16
18
20



	ST.	END
TS	762	940
H_2S	~~252~~ 197	~~310~~ 264

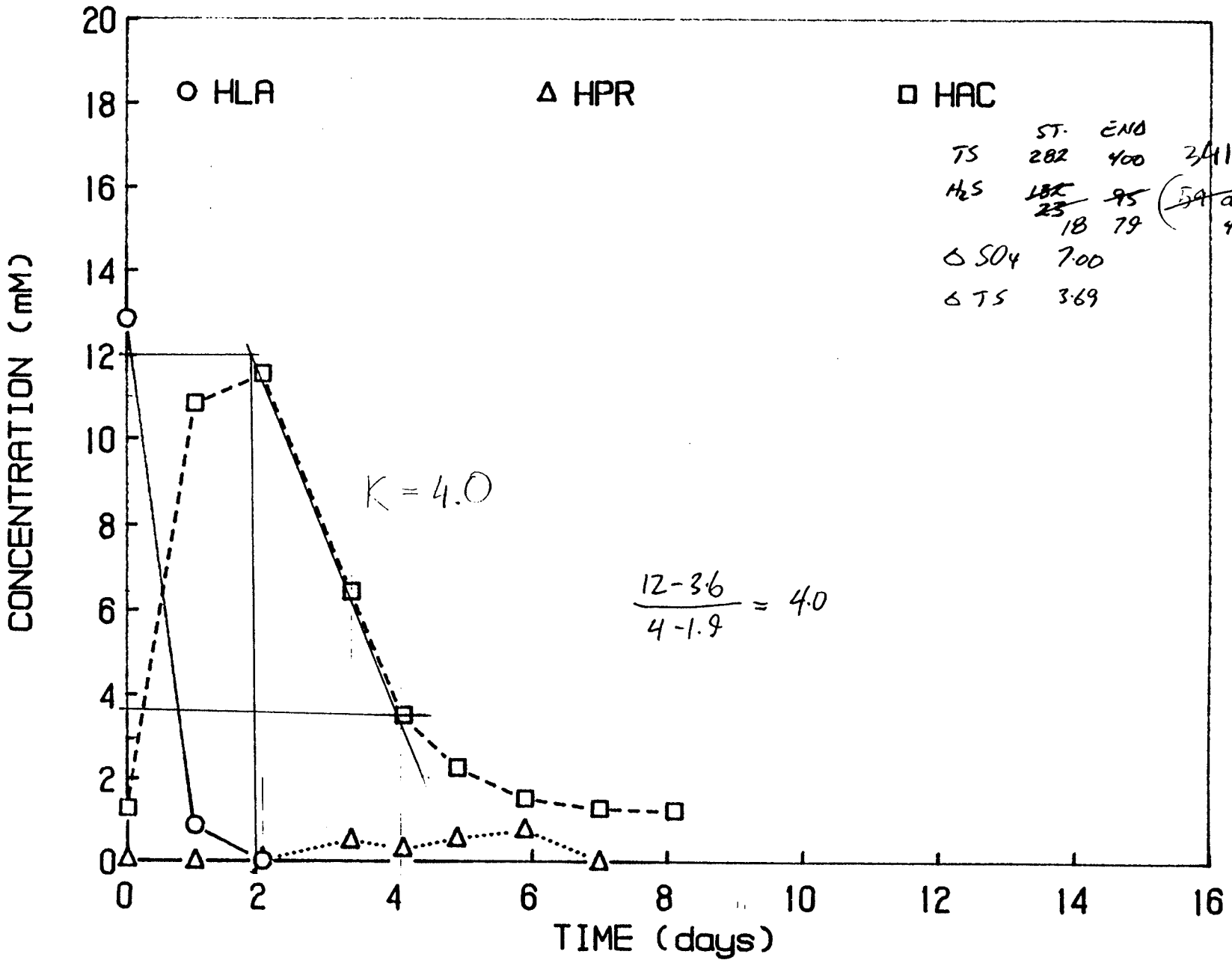
avg (85:1) 26.6 (~~27~~ 23)

~~8.5~~ 7.2

$\Delta SO_4 = 0$

$\Delta TS = 556$

S45

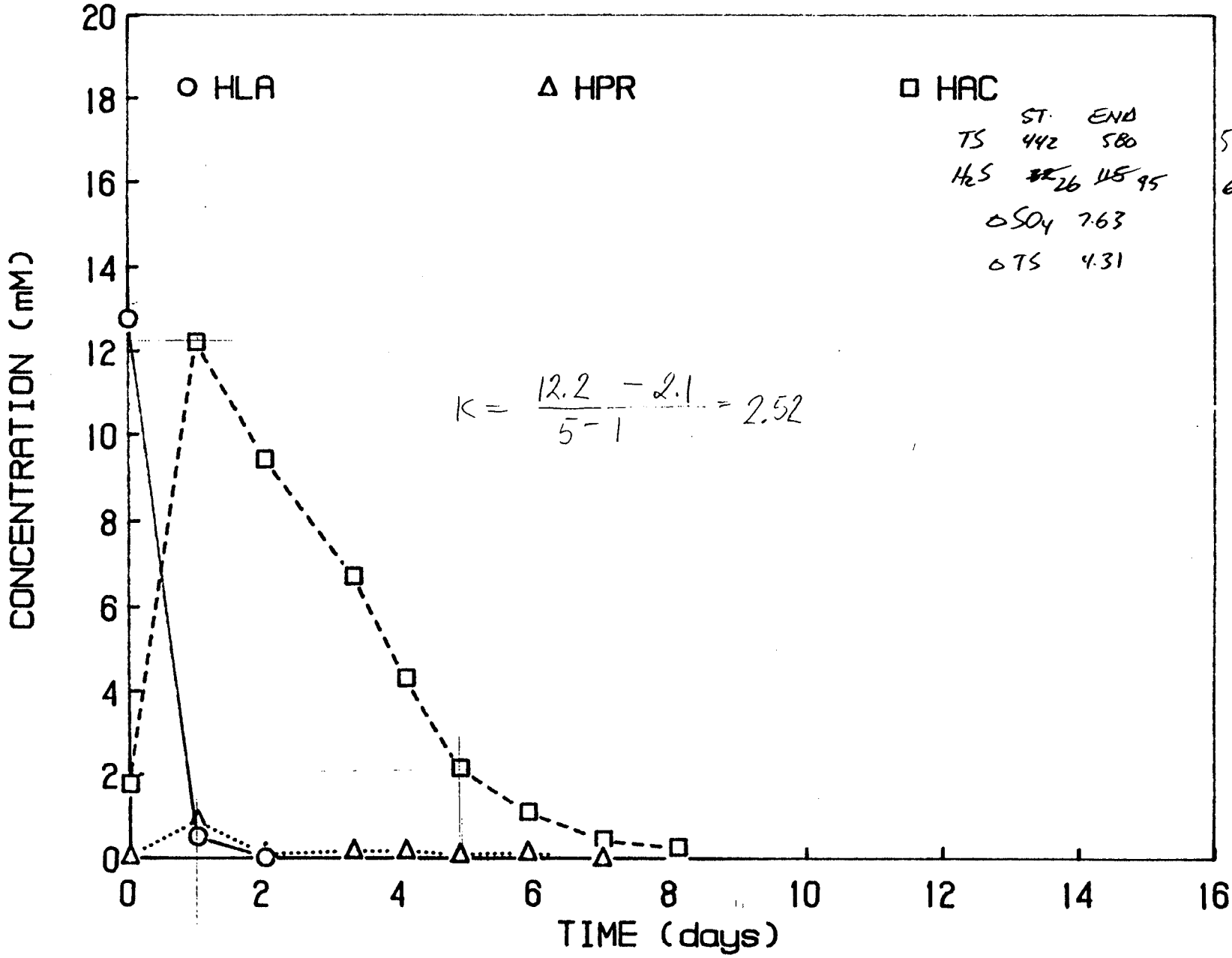


	ST.	END		
TS	282	400	341	(10.6)
H_2S	~~LOST 23~~ 18	~~95~~ 79	(~~54~~ avg 49)	(~~1.8~~ 1.5)
Δ SO_4	7.00			
Δ TS	3.69			

K = 4.0

$\frac{12-3.6}{4-1.9} = 4.0$

S26



	ST.	END
TS	442	580
H_2S	~~22~~ 26	~~45~~ 95
SO_4	7.63	
TS	4.31	

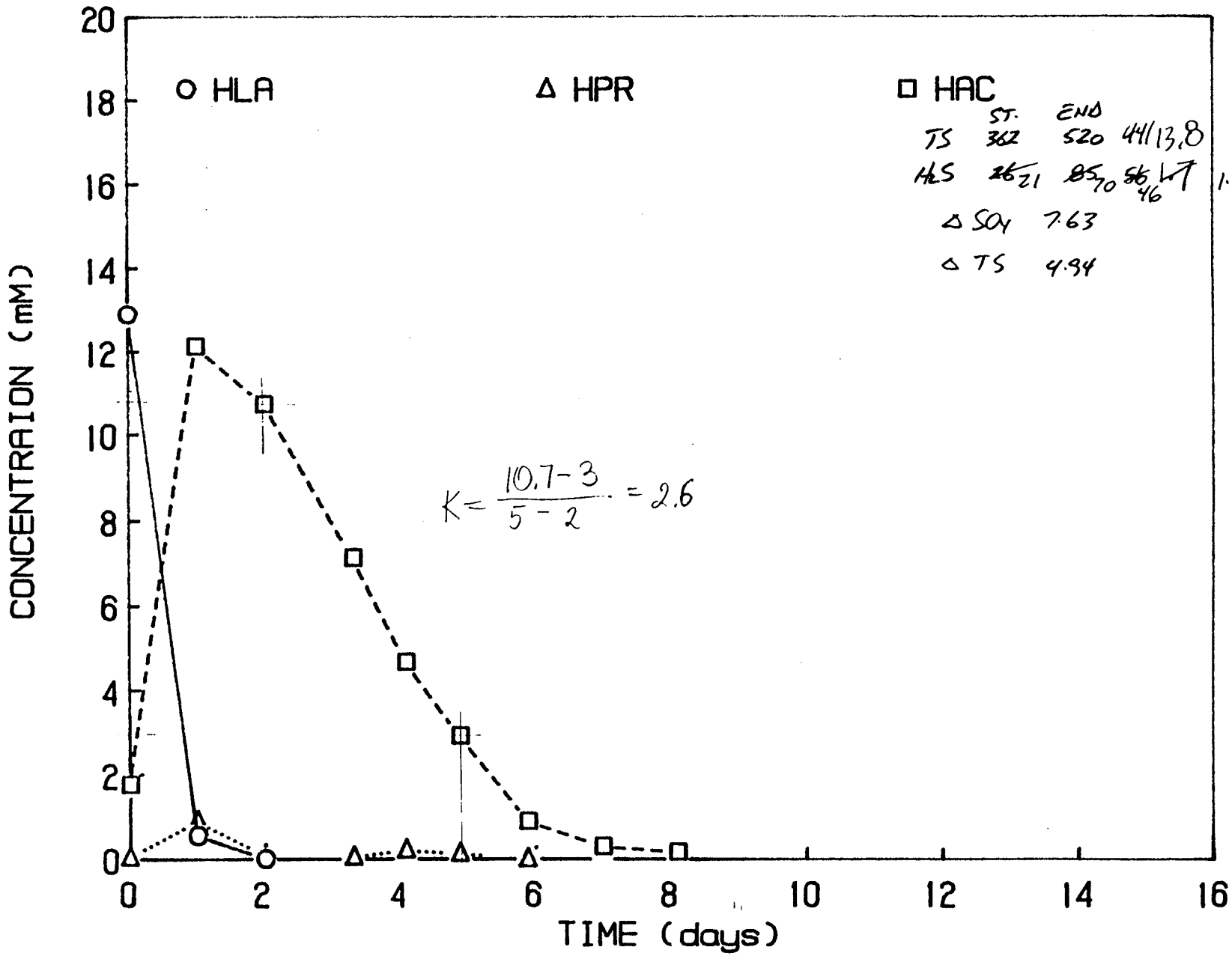
$$K = \frac{12.2 - 2.1}{5 - 1} = 2.52$$

mM

511 (16)

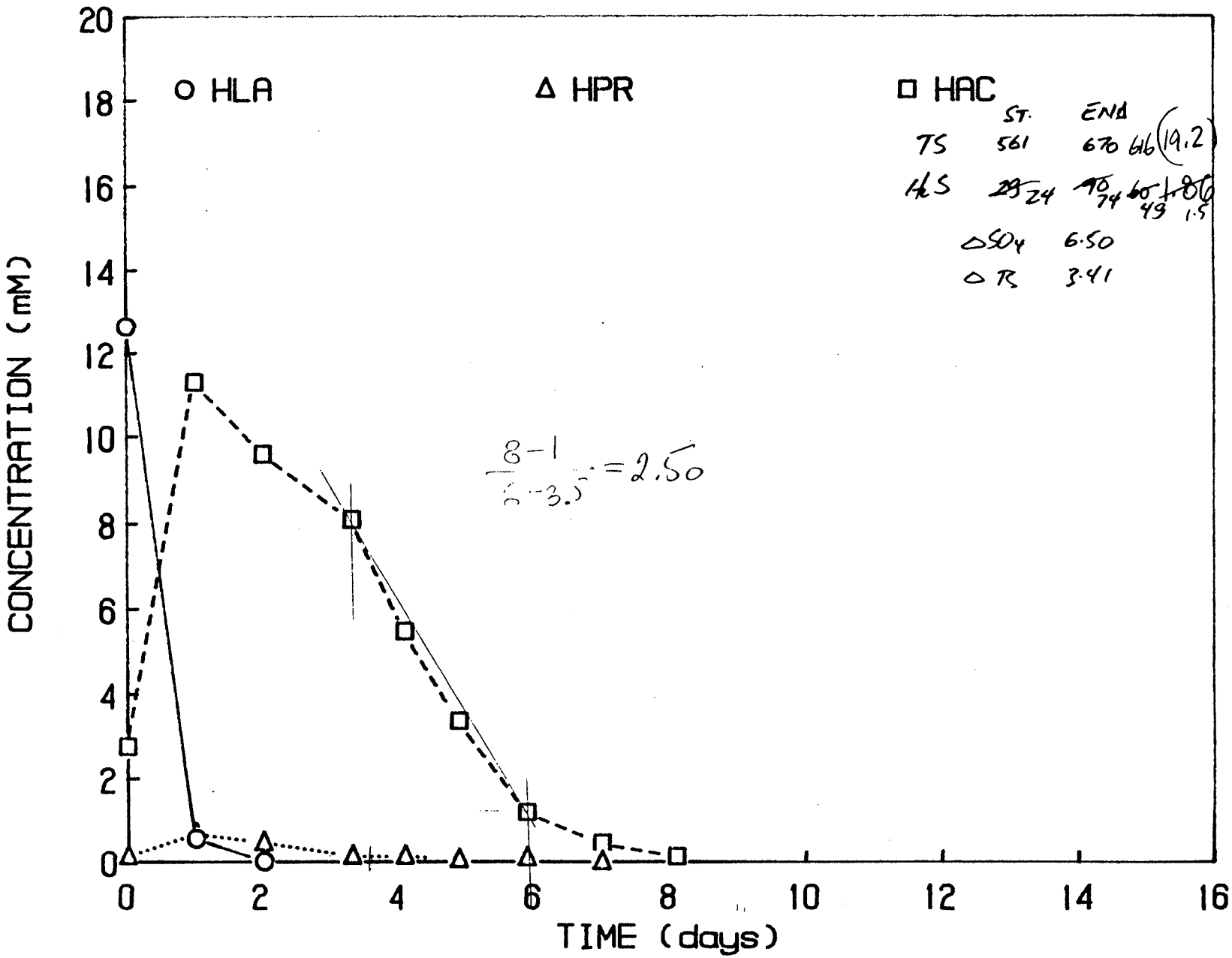
61 (~~2.3~~ 1.9)

S27



	ST.	END	
TS	362	520	44/13,8
H_2S	~~26~~ 21	~~85~~ 70	~~56~~ 46 ↓↑ 1.4
Δ SO_4	7.63		
Δ TS	4.94		

$$K = \frac{10.7 - 3}{5 - 2} = 2.6$$



ST. END

TS 561 670 616 (19.2)

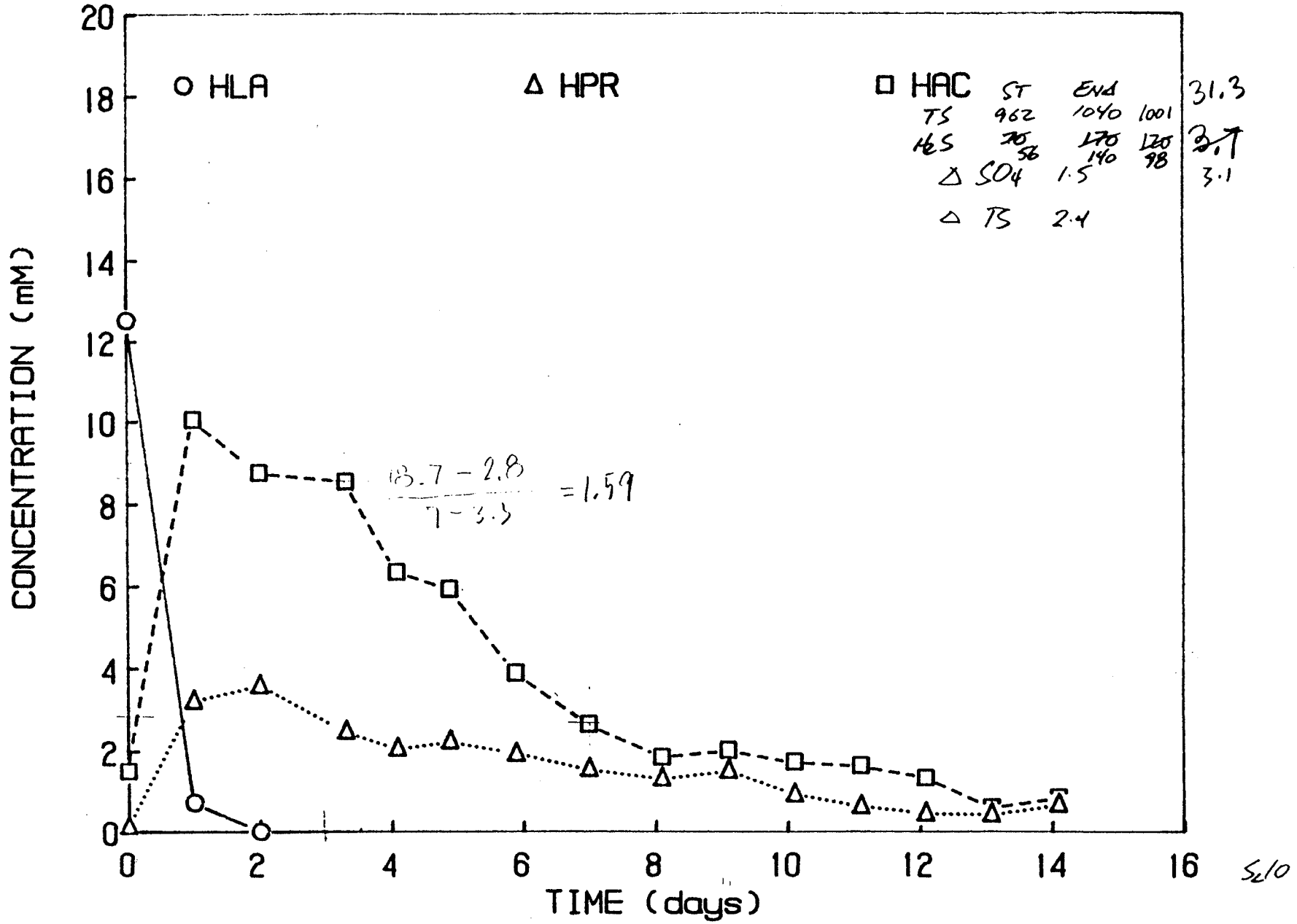
H2S ~~29~~ 24 ~~90~~ 74 ~~60~~ 49 ~~+86~~ 1.5

ΔSO4 6.50

Δ R 3.41

$\frac{8-1}{5-3.5} = 2.50$

S L9


20
18
16
14
12
10
8
6
4
2
0
CONCENTRATION (mM)
O HLA
Δ HPR
□ HAC
ST
END
TS
962
1040
1001
HES
70
56
170
140
120
98
Δ SO4
1.5
Δ TS
2.4
31.3
2.7
3.1
18.7 − 2.8 / 7 − 3.3 = 1.59
0
2
4
6
8
10
12
14
16
TIME (days)

APPENDIX B.

PHASE 3 RAW DATA

PHASE 3											R11S
PHASE 3 REACTOR 11										REACTOR	R12S
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY	R13S
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM		R14S
PHASE 3	0	914.70	10.16	0.00	0.00	22.00	0.37	4000.00	41.67	0	R15
PHASE 3	1	500.90	5.57		0.00	219.00	3.65	3600.00	37.50	1	R16
PHASE 3	2	126.00	1.40		0.00	426.00	7.10	3040.00	31.67	2	R17
PHASE 3	3	20.00	0.22		0.00	395.00	6.58	3200.00	33.33	3	R21
PHASE 3	4		0.00		0.00	248.00	4.13			4	R22
PHASE 3	5		0.00		0.00	212.00	3.53			5	R23
PHASE 3	6									6	R24
PHASE 3	7		0.00		0.00	109.00	1.82	2760.00	28.75	7	R25
PHASE 3	8		0.00		0.00					8	R26
PHASE 3	9		0.00		0.00	43.00	0.72			9	R27
PHASE 3	10		0.00		0.00					10	R31
PHASE 3	11		0.00		0.00					11	R32
PHASE 3	12		0.00		0.00	43.00	0.72	2280.00	23.75	12	R33
PHASE 3	13		0.00		0.00					13	R34
PHASE 3	14		0.00		0.00					14	R35
PHASE 3	15		0.00		0.00					15	R36
PHASE 3	16		0.00		0.00	6.00	0.10			16	R37
PHASE 3	28.00		0.00		0.00			2100.00	21.88	28.00	R41
PHASE 3 REACTOR 12			0.00		0.00				0.00	REACTOR	R42
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY	R43
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM		R44
PHASE 3	0	906.90	10.08		0.00	72.00	1.20	4000.00	41.67	0	R45
PHASE 3	1	425.90	4.73		0.00	276.00	4.60	3200.00	33.33	1	R46
PHASE 3	2	126.00	1.40		0.00	399.00	6.65	3000.00	31.25	2	R47
PHASE 3	3	15.00	0.17		0.00	366.00	6.10	3000.00	31.25	3	R47S
PHASE 3	4		0.00		0.00	348.00	5.80			4	
PHASE 3	5		0.00		0.00	304.00	5.07			5	
PHASE 3	6									6	
PHASE 3	7		0.00		0.00	226.00	3.77	2900.00	30.21	7	
PHASE 3	8		0.00		0.00					8	
PHASE 3	9		0.00		0.00	167.00	2.78			9	
PHASE 3	10		0.00		0.00					10	
PHASE 3	11		0.00		0.00					11	
PHASE 3	12		0.00		0.00	86.00	1.43	2240.00	23.33	12	
PHASE 3	13		0.00		0.00					13	
PHASE 3	14		0.00		0.00					14	
PHASE 3	15		0.00		0.00					15	
PHASE 3	16.00		0.00		0.00	5.00	0.08				
PHASE 3	28.00		0.00		0.00			2160.00	22.50		
PHASE 3 REACTOR 13			0.00		0.00				0.00	REACTOR 13	

PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	914.30	10.16		0.00	30.00	0.50	4080.00	42.50	0
PHASE 3	1	532.70	5.92		0.00	255.00	4.25	3440.00	35.83	1
PHASE 3	2	206.00	2.29		0.00	304.00	5.07	3340.00	34.79	2
PHASE 3	3	2.00	0.02		0.00	503.00	8.38	3200.00	33.33	3
PHASE 3	4		0.00		0.00	395.00	6.58			4
PHASE 3	5		0.00		0.00	429.00	7.15			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	424.00	7.07	2900.00	30.21	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	377.00	6.28			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	349.00	5.82	2000.00	20.83	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	272.00	4.53			16
PHASE 3	28.00		0.00		0.00			2200.00	22.92	
PHASE 3	REACTOR 14		0.00		0.00				0.00	REACTOR 14
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	942.00	10.47	tr	0.00	24.00	0.40	4000.00	41.67	0
PHASE 3	1	476.70	5.30		0.00	268.00	4.47	3800.00	39.58	1
PHASE 3	2	189.00	2.10		0.00	355.00	5.92	3300.00	34.38	2
PHASE 3	3	13.00	0.14		0.00	531.00	8.85	3200.00	33.33	3
PHASE 3	4		0.00		0.00	518.00	8.63			4
PHASE 3	5		0.00		0.00	447.00	7.45			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	535.00	8.92	3150.00	32.81	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	472.00	7.87			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	475.00	7.92	2200.00	22.92	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	469.00	7.82			16
PHASE 3	28.00		0.00		0.00			2200.00	22.92	
PHASE 3			0.00		0.00				0.00	
PHASE 3	REACTOR 15		0.00		0.00				0.00	REACTOR 15
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY

PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	950.20	10.56		0.00	24.00	0.40	4200.00	43.75	0
PHASE 3	1	529.10	5.88		0.00	289.00	4.82	3800.00	39.58	1
PHASE 3	2	179.00	1.99		0.00	452.00	7.53	3300.00	34.38	2
PHASE 3	3	9.00	0.10		0.00	530.00	8.83	3240.00	33.75	3
PHASE 3	4		0.00		0.00	571.00	9.52			4
PHASE 3	5		0.00		0.00	549.00	9.15			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	611.00	10.18	3150.00	32.81	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	599.00	9.98			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	522.00	8.70	2300.00	23.96	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	613.00	10.22			16
PHASE 3	28.00		0.00		0.00			2200.00	22.92	
PHASE 3	REACTOR 16		0.00		0.00				0.00	REACTOR 16
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	902.00	10.02	tr	0.00	26.00	0.43	3960.00	41.25	0
PHASE 3	1	632.10	7.02		0.00	247.00	4.12	3880.00	40.42	1
PHASE 3	2	274.00	3.04		0.00	392.00	6.53	3240.00	33.75	2
PHASE 3	3	46.00	0.51		0.00	585.00	9.75	3300.00	34.38	3
PHASE 3	4		0.00		0.00	590.00	9.83		0.00	4
PHASE 3	5		0.00		0.00	616.00	10.27		0.00	5
PHASE 3	6								0.00	6
PHASE 3	7		0.00		0.00	658.00	10.97	3150.00	32.81	7
PHASE 3	8		0.00		0.00				0.00	8
PHASE 3	9		0.00		0.00	790.00	13.17		0.00	9
PHASE 3	10		0.00		0.00				0.00	10
PHASE 3	11		0.00		0.00				0.00	11
PHASE 3	12		0.00		0.00	646.00	10.77	2360.00	24.58	12
PHASE 3	13		0.00		0.00				0.00	13
PHASE 3	14		0.00		0.00				0.00	14
PHASE 3	15		0.00		0.00				0.00	15
PHASE 3	16.00		0.00		0.00	601.00	10.02		0.00	16.00
PHASE 3	28.00		0.00		0.00			2400.00	25.00	28.00
PHASE 3	REACTOR 17		0.00		0.00				0.00	REACTOR 17
PHASE 3	DAY	HLa	HLa	HPr	0.00	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	HPr	mg/L	mM	mg/L	mM	
PHASE 3	0	944.90	10.50	tr	mM	26.00	0.43	4040.00	42.08	0

PHASE 3	1	647.70	7.20		0.00	226.00	3.77	3880.00	40.42	1
PHASE 3	2	342.00	3.80		0.00	383.00	6.38	3400.00	35.42	2
PHASE 3	3	78.00	0.87		0.00	441.00	7.35	3400.00	35.42	3
PHASE 3	4	6.00	0.07		0.00	566.00	9.43	3400.00	35.42	4
PHASE 3	5		0.00		0.00	590.00	9.83			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	648.00	10.80	3040.00	31.67	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	660.00	11.00			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	705.00	11.75	2480.00	25.83	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	664.00	11.07			16
PHASE 3	28.00		0.00		0.00			2500.00	26.04	28.00
PHASE 3	REACTOR 21		0.00		0.00				0.00	REACTOR 21
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	925.50	10.28		0.00	97.00	1.62	4480.00	46.67	0
PHASE 3	1	29.80	0.33		0.00	665.00	11.08	3400.00	35.42	1
PHASE 3	2	6.00	0.07		0.00	621.00	10.35	3600.00	37.50	2
PHASE 3	3		0.00		0.00	521.00	8.68	3160.00	32.92	3
PHASE 3	4		0.00		0.00	530.00	8.83			4
PHASE 3	5		0.00		0.00	385.00	6.42			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	353.00	5.88	3120.00	32.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	301.00	5.02			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	149.00	2.48	2680.00	27.92	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	84.00	1.40			16
PHASE 3	28.00		0.00		0.00			2300.00	23.96	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3	REACTOR 22		0.00		0.00				0.00	REACTOR 22
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	917.00	10.19		0.00	108.00	1.80	4000.00	41.67	0
PHASE 3	1	48.20	0.54		0.00	739.00	12.32	3700.00	38.54	1

PHASE 3	2	15.00	0.17		0.00	703.00	11.72	3200.00	33.33	2
PHASE 3	3		0.00		0.00	651.00	10.85	3300.00	34.38	3
PHASE 3	4		0.00		0.00	643.00	10.72			4
PHASE 3	5		0.00			638.00	10.63			5
PHASE 3	6				0.00					6
PHASE 3	7		0.00		0.00	572.00	9.53	3200.00	33.33	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	512.00	8.53			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	402.00	6.70	2680.00	27.92	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00		0.00			15
PHASE 3	16		0.00		0.00	84.00	1.40			16
PHASE 3	28.00		0.00		0.00		0.00	2400.00	25.00	28.00
PHASE 3	REACTOR 23		0.00				0.00		0.00	REACTOR 23
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	886.90	9.85		0.00	101.00	1.68	4200.00	43.75	0
PHASE 3	1	136.60	1.52		0.00	719.00	11.98	3800.00	39.58	1
PHASE 3	2	2.00	0.02		0.00	785.00	13.08	3200.00	33.33	2
PHASE 3	3		0.00		0.00	750.00	12.50	3400.00	35.42	3
PHASE 3	4		0.00		0.00	797.00	13.28			4
PHASE 3	5		0.00		0.00	726.00	12.10			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	726.00	12.10	3300.00	34.38	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	784.00	13.07			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	789.00	13.15	2760.00	28.75	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16.00		0.00		0.00	791.00	13.18			16.00
PHASE 3	28.00		0.00		0.00			2360.00	24.58	28.00
PHASE 3	REACTOR 24		0.00		0.00				0.00	REACTOR 24
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	972.60	10.81	tr	0.00	93.00	1.55	4500.00	46.88	0
PHASE 3	1	230.90	2.57		0.00	674.00	11.23	4300.00	44.79	1
PHASE 3	2	1.00	0.01		0.00	752.00	12.53	3500.00	36.46	2
PHASE 3	3		0.00		0.00	771.00	12.85	3400.00	35.42	3

PHASE 3	4		0.00		0.00	782.00	13.03			4
PHASE 3	5		0.00		0.00	836.00	13.93			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	807.00	13.45	3640.00	37.92	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	795.00	13.25			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	846.00	14.10	2720.00	28.33	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	824.00	13.73			16
PHASE 3	28.00		0.00		0.00			2560.00	26.67	28.00
PHASE 3	REACTOR 25		0.00		0.00				0.00	REACTOR 25
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	930.00	10.33	0.00	0.00	95.00	1.58	4280.00	44.58	0
PHASE 3	1	746.50	8.29	tr	0.00	292.00	4.87	4200.00	43.75	1
PHASE 3	2	717.00	7.97	tr	0.00	353.00	5.88	4000.00	41.67	2
PHASE 3	3	571.00	6.34	tr	0.00	471.00	7.85	3800.00	39.58	3
PHASE 3	4	415.00	4.61	2.00	0.03	506.00	8.43	3700.00	38.54	4
PHASE 3	5	346.00	3.84	7.00	0.09	491.00	8.18			5
PHASE 3	6									6
PHASE 3	7	161.00	1.79	9.00	0.12	677.00	11.28	3760.00	39.17	7
PHASE 3	8				0.00					8
PHASE 3	9	13.00	0.14	10.00	0.14	798.00	13.30			9
PHASE 3	10				0.00					10
PHASE 3	11				0.00					11
PHASE 3	12		0.00	11.00	0.15	792.00	13.20	3000.00	31.25	12
PHASE 3	13				0.00					13
PHASE 3	14				0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	846.00	14.10			16
PHASE 3	28.00		0.00		0.00			2900.00	30.21	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3	REACTOR 26		0.00					SO4	SO4	REACTOR 26
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	mg/L	mM	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM		0.00	
PHASE 3	0	930.40	10.34		0.00	98.00	1.63	4440.00	46.25	0
PHASE 3	1	879.00	9.77	tr	0.00	174.00	2.90	4200.00	43.75	1
PHASE 3	2	930.00	10.33	tr	0.00	196.00	3.27	4100.00	42.71	2
PHASE 3	3	908.00	10.09	tr	0.00	239.00	3.98	4300.00	44.79	3
PHASE 3	4									4

PHASE 3	5	841.00	9.34	6.00	0.08	301.00	5.02			5
PHASE 3	6									6
PHASE 3	7	732.00	8.13	9.00	0.12	347.00	5.78	4240.00	44.17	7
PHASE 3	8									8
PHASE 3	9	752.00	8.36	10.00	0.14	343.00	5.72			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12	603.00	6.70	14.00	0.19	442.00	7.37	3720.00	38.75	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	561.00	9.35			16
PHASE 3	28.00		0.00		0.00		0.00	2900.00	30.21	28.00
PHASE 3	REACTOR 27		0.00		0.00		0.00	SO4	SO4	REACTOR 27
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	mg/L	mM	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM		0.00	
PHASE 3	0	1000.70	11.12		0.00	69.00	1.15	4500.00	46.88	0
PHASE 3	1	929.50	10.33	tr	0.00	168.00	2.80	4600.00	47.92	1
PHASE 3	2	1015.00	11.28	tr	0.00	168.00	2.80	4500.00	46.88	2
PHASE 3	3	924.00	10.27	tr	0.00	199.00	3.32	4200.00	43.75	3
PHASE 3	4									4
PHASE 3	5	892.00	9.91	5.00	0.07	205.00	3.42			5
PHASE 3	6									6
PHASE 3	7	821.00	9.12	10.00	0.14	244.00	4.07	4480.00	46.67	7
PHASE 3	8									8
PHASE 3	9	824.00	9.16	13.00	0.18	267.00	4.45			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12	771.00	8.57	16.00	0.22	294.00	4.90	3280.00	34.17	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15
PHASE 3	16.00		0.00		0.00	325.00	5.42			16.00
PHASE 3	28.00		0.00		0.00			3280.00	34.17	28.00
PHASE 3	REACTOR 31		0.00		0.00		0.00	SO4	SO4	REACTOR 31
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	mg/L	mM	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM		0.00	
PHASE 3	0	870.30	9.67	0.00	0.00	379.00	6.32	3800.00	39.58	0
PHASE 3	1	368.10	4.09		0.00	672.00	11.20	3720.00	38.75	1
PHASE 3	2	17.00	0.19		0.00	724.00	12.07	2560.00	26.67	2
PHASE 3	3		0.00		0.00	659.00	10.98	2500.00	26.04	3
PHASE 3	4		0.00		0.00	599.00	9.98			4
PHASE 3	5		0.00		0.00	572.00	9.53			5
PHASE 3	6									6

PHASE 3	7		0.00		0.00	468.00	7.80	2560.00	26.67	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	342.00	5.70			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	237.00	3.95	2120.00	22.08	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	58.00	0.97			16
PHASE 3	28.00		0.00		0.00		0.00	1700.00	17.71	28.00
PHASE 3	REACTOR 32		0.00		0.00		0.00		0.00	REACTOR 32
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	931.10	10.35	0.00	0.00	421.00	7.02	4160.00	43.33	0
PHASE 3	1	333.00	3.70		0.00	708.00	11.80	3900.00	40.63	1
PHASE 3	2	13.00	0.14		0.00	835.00	13.92	2800.00	29.17	2
PHASE 3	3		0.00		0.00	825.00	13.75	2500.00	26.04	3
PHASE 3	4		0.00		0.00	708.00	11.80			4
PHASE 3	5		0.00		0.00	730.00	12.17			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	650.00	10.83	2640.00	27.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	566.00	9.43			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	433.00	7.22	1840.00	19.17	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	307.00	5.12			16
PHASE 3	28.00		0.00		0.00		0.00	1900.00	19.79	28.00
PHASE 3			0.00		0.00				0.00	
PHASE 3	REACTOR 33		0.00		0.00				0.00	REACTOR 33
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	909.90	10.11		0.00	406.00	6.77	4000.00	41.67	0
PHASE 3	1	297.90	3.31	tr	0.00	684.00	11.40	3600.00	37.50	1
PHASE 3	2	28.00	0.31		0.00	873.00	14.55	2900.00	30.21	2
PHASE 3	3		0.00		0.00	777.00	12.95	2600.00	27.08	3
PHASE 3	4		0.00		0.00	834.00	13.90			4
PHASE 3	5		0.00		0.00	733.00	12.22			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	737.00	12.28	2800.00	29.17	7

PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	674.00	11.23			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	587.00	9.78	2160.00	22.50	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	474.00	7.90			16
PHASE 3	28.00		0.00		0.00		0.00	1800.00	18.75	28.00
PHASE 3	REACTOR 34		0.00		0.00		0.00		0.00	REACTOR 34
PHASE 3	DAY	0.00	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	923.10	10.26		0.00	454.00	7.57	4000.00	41.67	0
PHASE 3	1	292.40	3.25	tr	0.00	640.00	10.67	3520.00	36.67	1
PHASE 3	2	32.00	0.36		0.00	949.00	15.82	2700.00	28.13	2
PHASE 3	3		0.00		0.00	871.00	14.52	2640.00	27.50	3
PHASE 3	4		0.00		0.00	822.00	13.70			4
PHASE 3	5		0.00		0.00	861.00	14.35			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	803.00	13.38	2840.00	29.58	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	849.00	14.15			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	818.00	13.63	2000.00	20.83	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00		0.00			15
PHASE 3	16.00		0.00		0.00	806.00	13.43			16.00
PHASE 3	28.00		0.00		0.00		0.00	1800.00	18.75	28.00
PHASE 3	REACTOR 35		0.00		0.00		0.00		0.00	REACTOR 35
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	965.80	10.73		0.00	450.00	7.50	4200.00	43.75	0
PHASE 3	1	315.30	3.50	tr	0.00	772.00	12.87	4040.00	42.08	1
PHASE 3	2	66.00	0.73		0.00	867.00	14.45	3100.00	32.29	2
PHASE 3	3	9.00	0.10		0.00	960.00	16.00	2480.00	25.83	3
PHASE 3	4		0.00		0.00	927.00	15.45			4
PHASE 3	5		0.00		0.00	932.00	15.53			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	957.00	15.95	2940.00	30.63	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	965.00	16.08			9

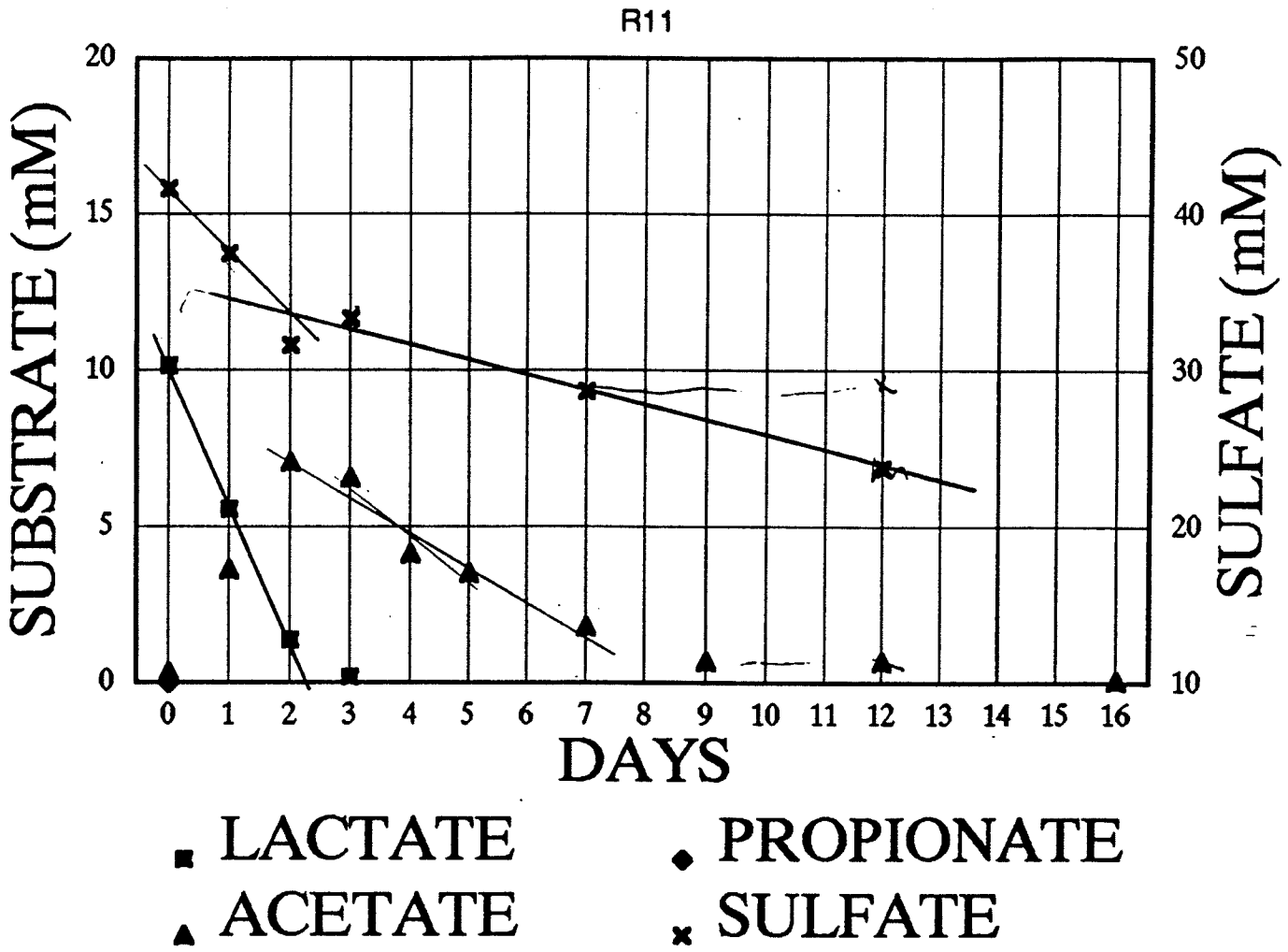
PHASE 3	10		0.00		0.00				10
PHASE 3	11		0.00		0.00				11
PHASE 3	12		0.00		0.00	965.00	16.08	2160.00	22.50	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	963.00	16.05			16
PHASE 3	28.00		0.00		0.00		0.00	2000.00	20.83	28.00
PHASE 3	REACTOR 36		0.00		0.00		0.00			REACTOR 36
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	871.20	9.68	0.00	0.00	369.00	6.15	3840.00	40.00	0
PHASE 3	1	340.60	3.78		0.00	760.00	12.67	3400.00	35.42	1
PHASE 3	2	71.00	0.79		0.00	875.00	14.58	3080.00	32.08	2
PHASE 3	3	2.00	0.02		0.00	963.00	16.05	3040.00	31.67	3
PHASE 3	4		0.00		0.00	931.00	15.52			4
PHASE 3	5		0.00		0.00	921.00	15.35			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	967.00	16.12	3120.00	32.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	1060.00	17.67			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	998.00	16.63	2440.00	25.42	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	1058.00	17.63			16
PHASE 3	28.00		0.00		0.00		0.00	2300.00	23.96	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3	REACTOR 37		0.00		0.00		0.00		0.00	REACTOR 37
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	924.60	10.27	0.00	0.00	428.00	7.13	4120.00	42.92	0
PHASE 3	1	352.60	3.92		0.00	726.00	12.10	3860.00	40.21	1
PHASE 3	2	83.00	0.92		0.00	895.00	14.92	3300.00	34.38	2
PHASE 3	3	5.00	0.06		0.00	995.00	16.58	3000.00	31.25	3
PHASE 3	4		0.00		0.00	950.00	15.83			4
PHASE 3	5		0.00		0.00	941.00	15.68			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	1062.00	17.70	3120.00	32.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	1033.00	17.22			9
PHASE 3	10		0.00		0.00					10

PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	1003.00	16.72	2800.00	29.17	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00		0.00			15
PHASE 3	16		0.00		0.00	1131.00	18.85			16
PHASE 3			0.00		0.00		0.00	2300.00	23.96	28.00
PHASE 3	REACTOR 41		0.00		0.00		0.00		0.00	REACTOR 41
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	855.20	9.50	0.00	0.00	89.00	1.48	3840.00	40.00	0
PHASE 3	1	58.50	0.65		0.00	557.00	9.28	3800.00	39.58	1
PHASE 3	2	5.00	0.06		0.00	574.00	9.57	3200.00	33.33	2
PHASE 3	3		0.00		0.00	544.00	9.07	3100.00	32.29	3
PHASE 3	4		0.00		0.00	431.00	7.18			4
PHASE 3	5		0.00		0.00	369.00	6.15			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	195.00	3.25	3200.00	33.33	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	124.00	2.07			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	88.00	1.47	2500.00	26.04	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16.00		0.00		0.00	140.00	2.33			16.00
PHASE 3	28.00		0.00		0.00		0.00	2400.00	25.00	28.00
PHASE 3	REACTOR 42		0.00		0.00		0.00		0.00	REACTOR 42
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	1012.60	11.25	0.00	0.00	54.00	0.90	4200.00	43.75	0
PHASE 3	1	76.10	0.85		0.00	673.00	11.22	3700.00	38.54	1
PHASE 3	2	5.00	0.06		0.00	679.00	11.32	3500.00	36.46	2
PHASE 3	3		0.00		0.00	640.00	10.67	3240.00	33.75	3
PHASE 3	4		0.00		0.00	589.00	9.82			4
PHASE 3	5		0.00		0.00	516.00	8.60			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	485.00	8.08	3200.00	33.33	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	388.00	6.47			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	324.00	5.40	2120.00	22.08	12

PHASE 3	13		0.00		0,00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	188.00	3.13			16
PHASE 3	28.00		0.00		0.00		0.00	2400.00	25.00	28.00
PHASE 3	REACTOR 43		0.00		0.00		0.00		0.00	REACTOR 43
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	1022.40	11.36	0.00	0.00	43.00	0.72	4600.00	47.92	0
PHASE 3	1	63.00	0.70		0.00	668.00	11.13	3760.00	39.17	1
PHASE 3	2	4.00	0.04		0.00	709.00	11.82	3360.00	35.00	2
PHASE 3	3		0.00		0.00	700.00	11.67	3500.00	36.46	3
PHASE 3	4		0.00		0.00	681.00	11.35			4
PHASE 3	5		0.00		0.00	706.00	11.77			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	715.00	11.92	3600.00	37.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	708.00	11.80			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	713.00	11.88	2200.00	22.92	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	682.00	11.37			16
PHASE 3	28.00		0.00		0.00		0.00	2400.00	25.00	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3	REACTOR 44		0.00		0.00		0.00		0.00	REACTOR 44
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	968.60	10.76	0.00	0.00	28.00	0.47	4280.00	44.58	0
PHASE 3	1	127.00	1.41		0.00	640.00	10.67	3760.00	39.17	1
PHASE 3	2	3.00	0.03		0.00	732.00	12.20	3600.00	37.50	2
PHASE 3	3		0.00	tr	0.00	762.00	12.70	3640.00	37.92	3
PHASE 3	4		0.00		0.00	749.00	12.48			4
PHASE 3	5		0.00		0.00	781.00	13.02			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	753.00	12.55	3600.00	37.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	724.00	12.07			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	767.00	12.78	2840.00	29.58	12
PHASE 3	13		0.00		0.00					13

PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00		0.00			15
PHASE 3	16		0.00		0.00	792.00	13.20			16
PHASE 3	28.00		0.00		0.00		0.00	2700.00	28.13	28.00
PHASE 3	REACTOR 45		0.00		0.00		0.00		0.00	REACTOR 45
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	1020.90	11.34		0.00	9.00	0.15	4380.00	45.63	0
PHASE 3	1	701.00	7.79		0.00	232.00	3.87	4000.00	41.67	1
PHASE 3	2	728.00	8.09		0.00	320.00	5.33	3900.00	40.63	2
PHASE 3	3	540.00	6.00	tr	0.00	374.00	6.23	3800.00	39.58	3
PHASE 3	4			tr	0.00	520.00	8.67	3600.00	37.50	4
PHASE 3	5	239.00	2.66	2.00	0.03	531.00	8.85			5
PHASE 3	6									6
PHASE 3	7	115.00	1.28	5.00	0.07	648.00	10.80	3600.00	37.50	7
PHASE 3	8									8
PHASE 3	9	3.00	0.03	5.00	0.07	720.00	12.00			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12		0.00	8.00	0.11	741.00	12.35	2680.00	27.92	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15
PHASE 3	16.00		0.00		0.00	794.00	13.23			16.00
PHASE 3	28.00		0.00		0.00		0.00	3000.00	31.25	28.00
PHASE 3	REACTOR 46		0.00		0.00		0.00		0.00	REACTOR 46
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	941.20	10.46		0.00	12.00	0.20	4300.00	44.79	0
PHASE 3	1	932.00	10.36		0.00	115.00	1.92	4300.00	44.79	1
PHASE 3	2	1008.00	11.20	tr	0.00	130.00	2.17	4160.00	43.33	2
PHASE 3	3	922.00	10.24	tr	0.00	177.00	2.95	3800.00	39.58	3
PHASE 3	4									4
PHASE 3	5	824.00	9.16	2.00	0.03	240.00	4.00			5
PHASE 3	6									6
PHASE 3	7	807.00	8.97	6.00	0.08	261.00	4.35	4100.00	42.71	7
PHASE 3	8									8
PHASE 3	9	753.00	8.37	7.00	0.09	302.00	5.03			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12	682.00	7.58	10.00	0.14	356.00	5.93	3500.00	36.46	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15

PHASE 3	16		0.00		0.00	438.00	7.30			16
PHASE 3	28.00		0.00		0.00		0.00	3200.00	33.33	28.00
PHASE 3	REACTOR 47		0.00		0.00		0.00		0.00	REACTOR 47
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	964.30	10.71	0.00	0.00	16.00	0.27	4600.00	47.92	0
PHASE 3	1	938.00	10.42	0.00	0.00	85.00	1.42	4400.00	45.83	1
PHASE 3	2	974.00	10.82	tr	0.00	97.00	1.62	4200.00	43.75	2
PHASE 3	3	966.00	10.73	tr	0.00	125.00	2.08	3600.00	37.50	3
PHASE 3	4									4
PHASE 3	5	941.00	10.46	3.00	0.04	147.00	2.45			5
PHASE 3	6									6
PHASE 3	7	855.00	9.50	7.00	0.09	188.00	3.13	4400.00	45.83	7
PHASE 3	8									8
PHASE 3	9	938.00	10.42	9.00	0.12	209.00	3.48			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12	811.00	9.01	12.00	0.16	234.00	3.90	3320.00	34.58	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	292.00	4.87			16
PHASE 3	28.00		0.00		0.00		0.00	3400.00	35.42	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3										



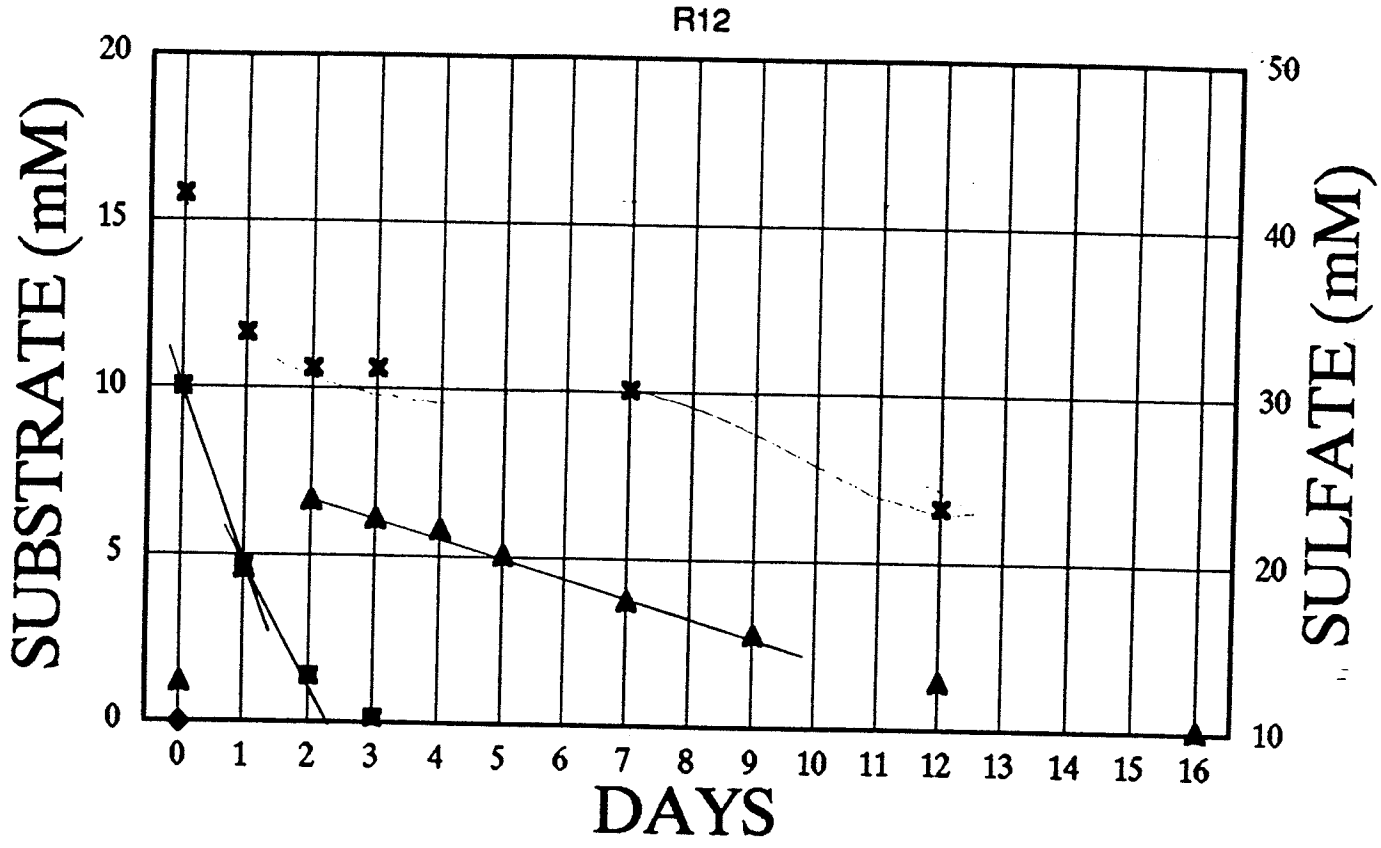
MIS = after HCl digested.

2590

48 mL CH_4

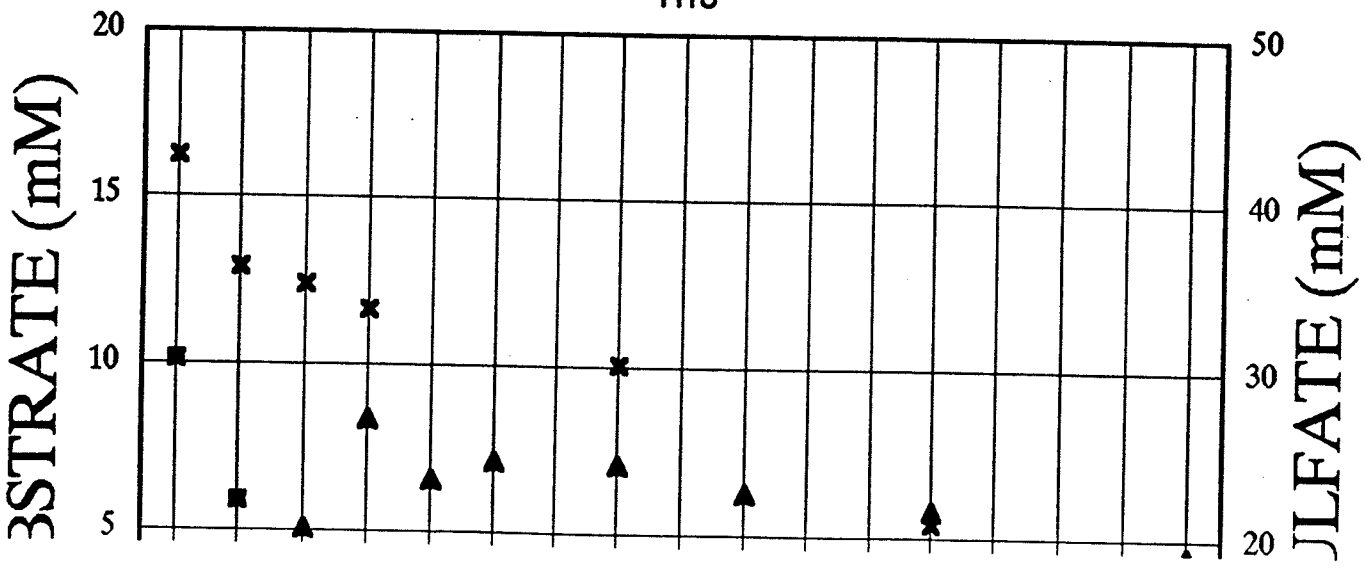
	ST	MIS
pH	7.0	7.4
TS	180	680
H_2S	78.9	161

$\frac{dS_t}{dt}$ =



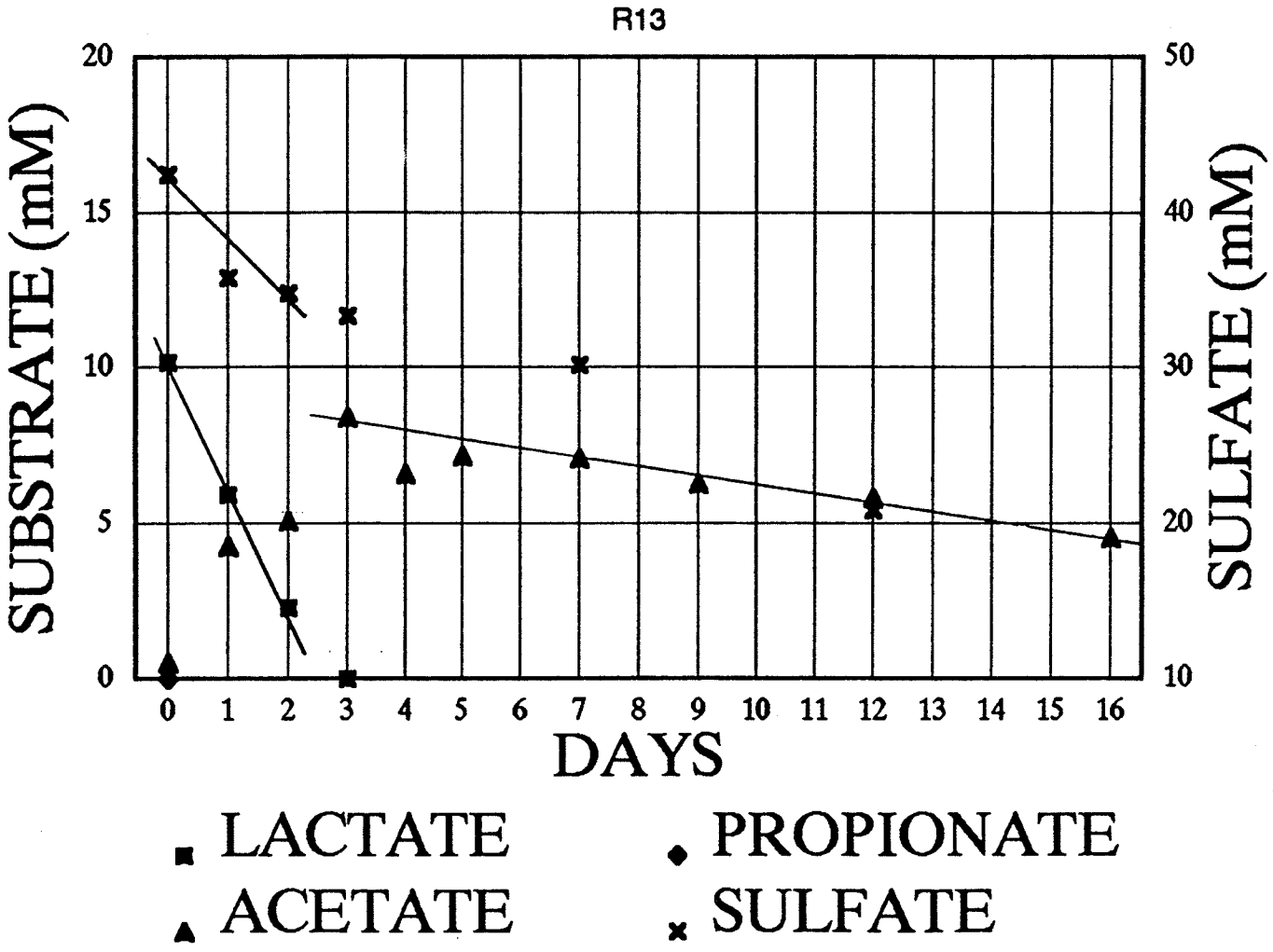
3.8 mL CH4

pH	7.0	7.4
TS	225	740
H2S	98.7	176



pH 7. 7.3
TS 275 760
H_2S 121 214

40 mL CH4

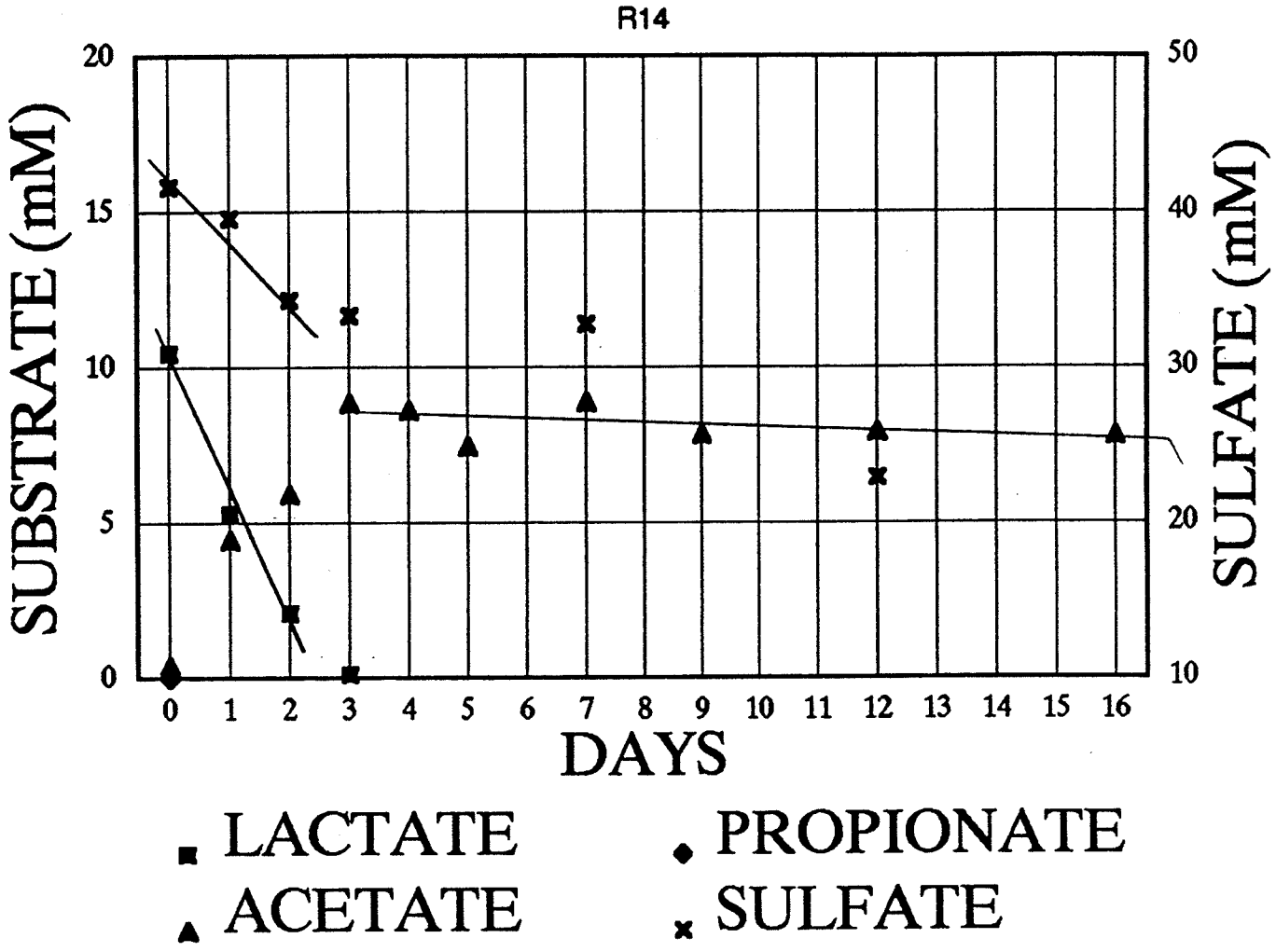


pH 7 7.4

B 330 770

H_2S 145 183

1.3 mL CH_4

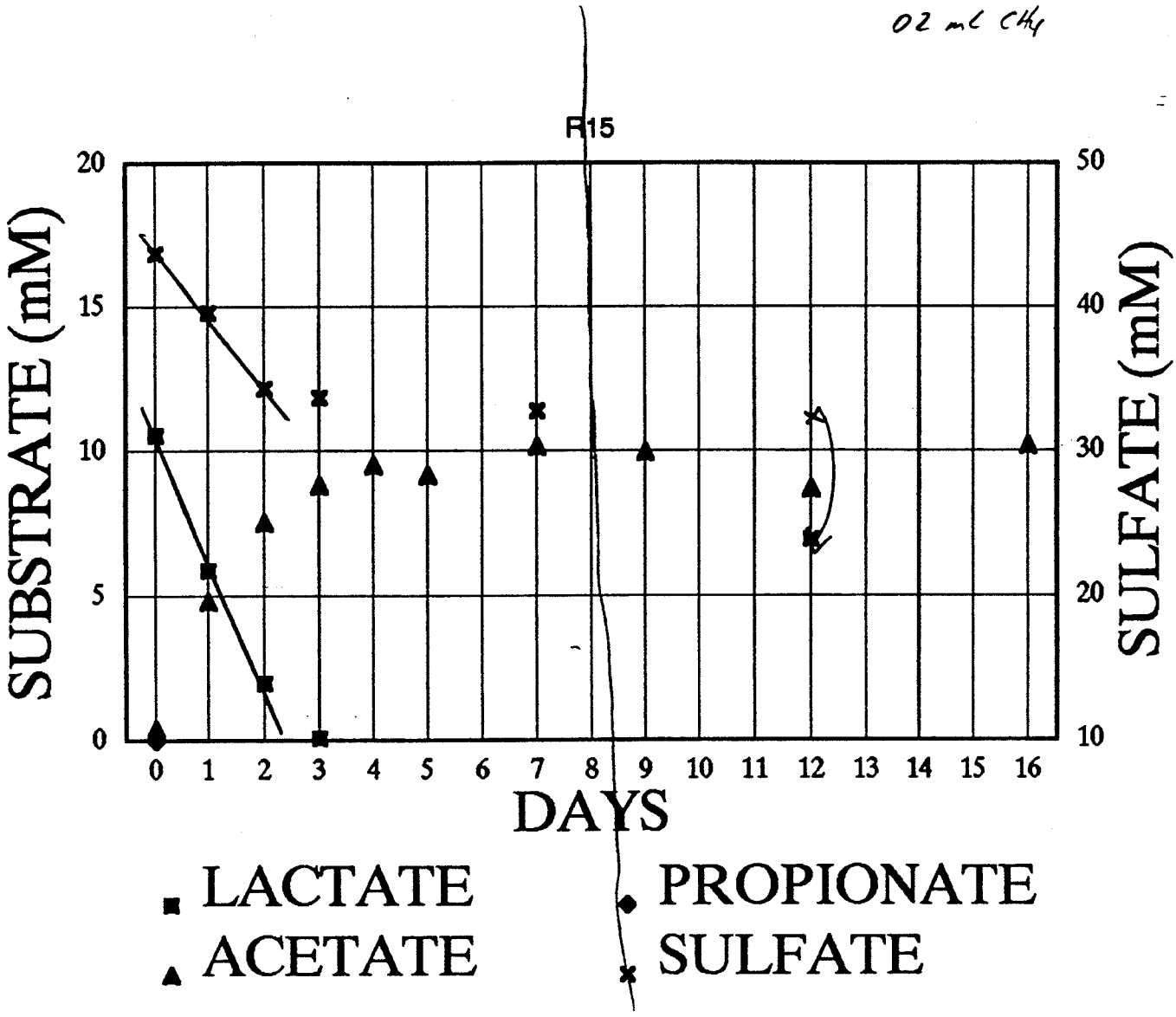


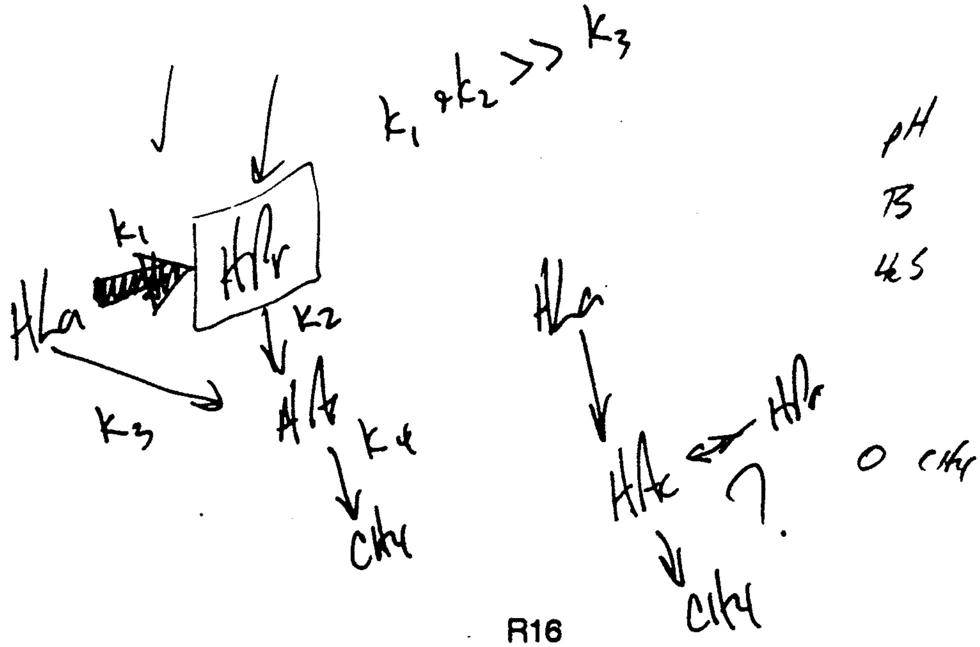
pH 7.0 7.3

TS 425 880

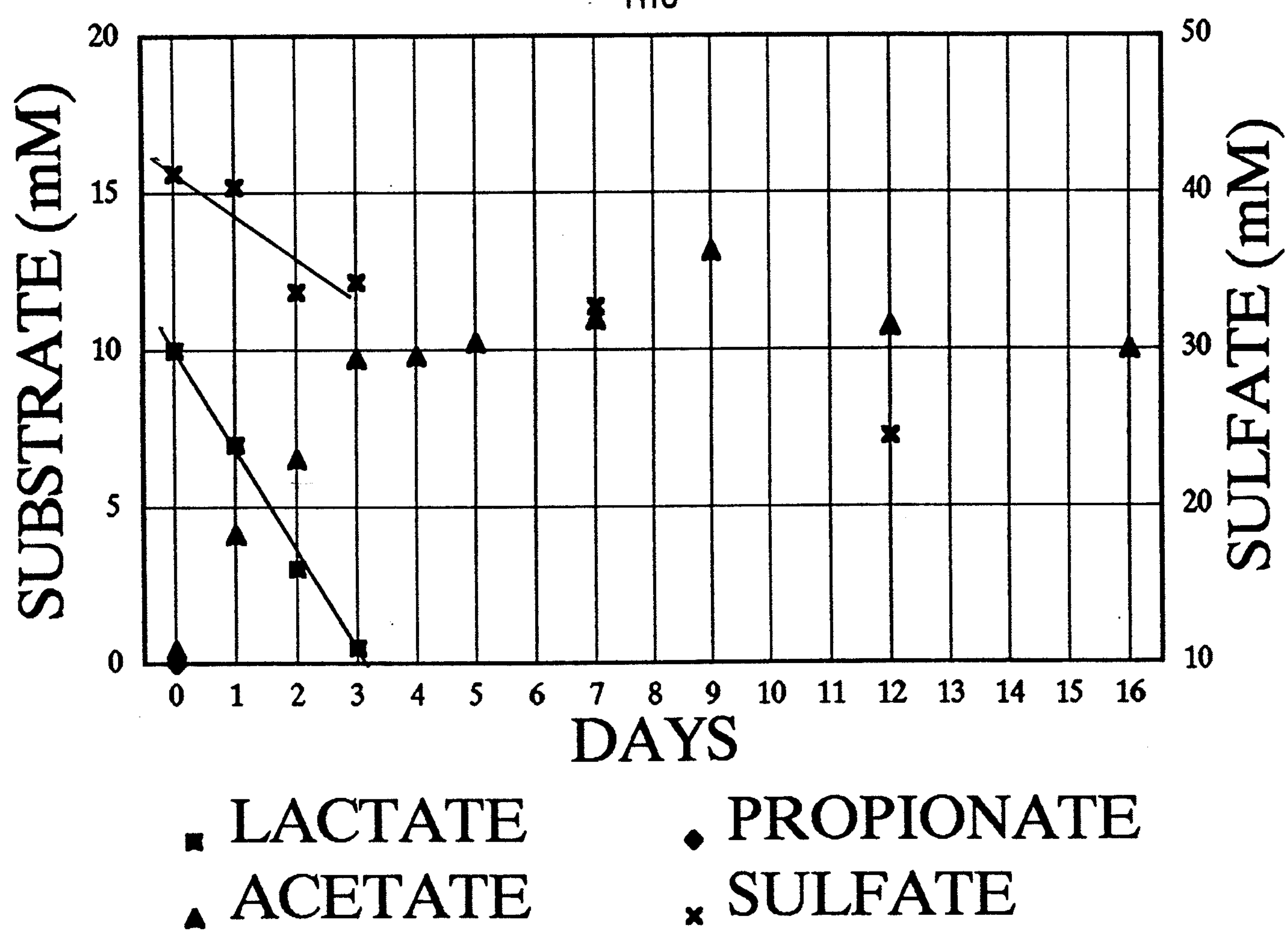
VS 186 248

0.2 mL CH_4





pH	7.0	7.4
B	520	1000
H_2S	228	237

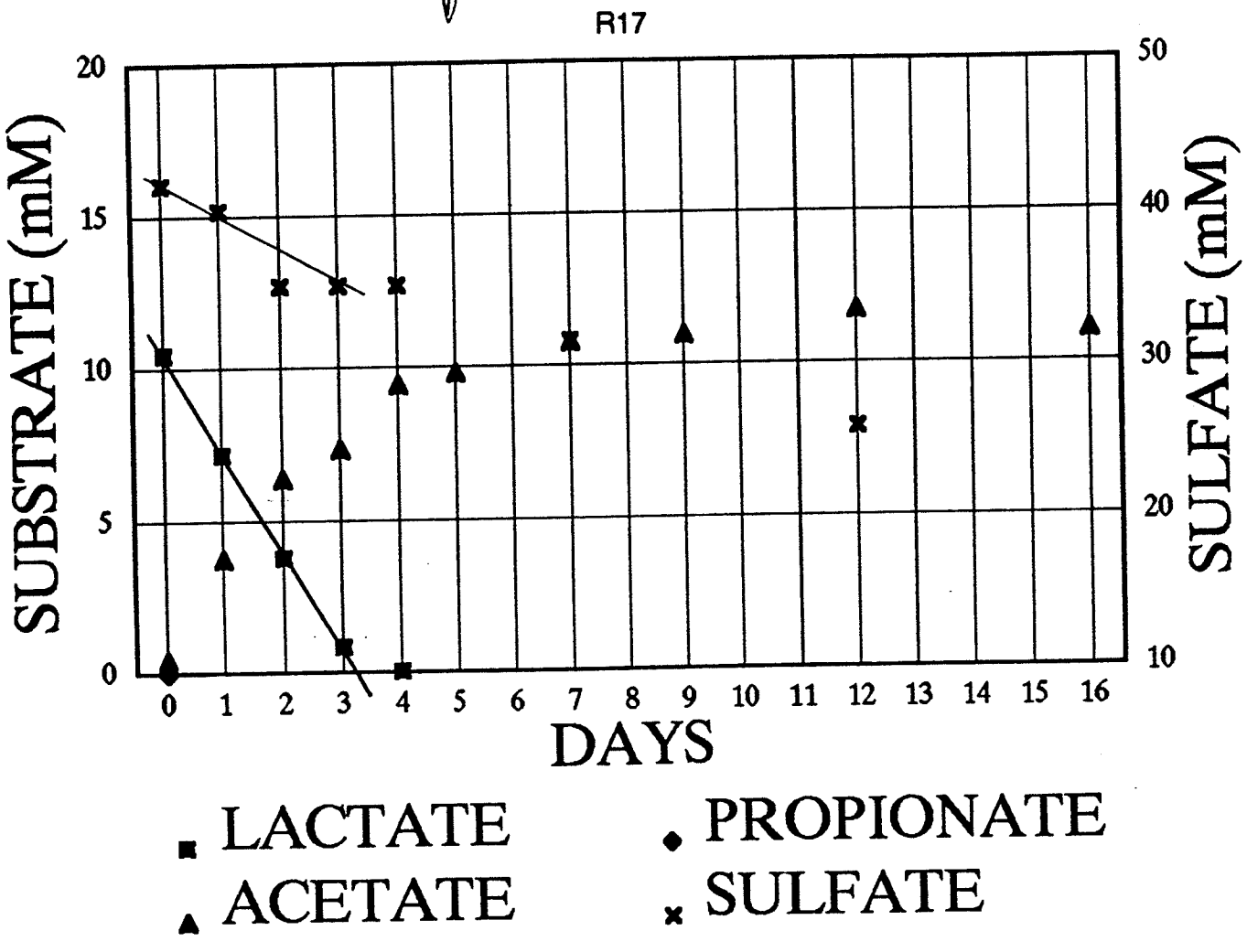


pH 7. 7.4

TS 600 1160

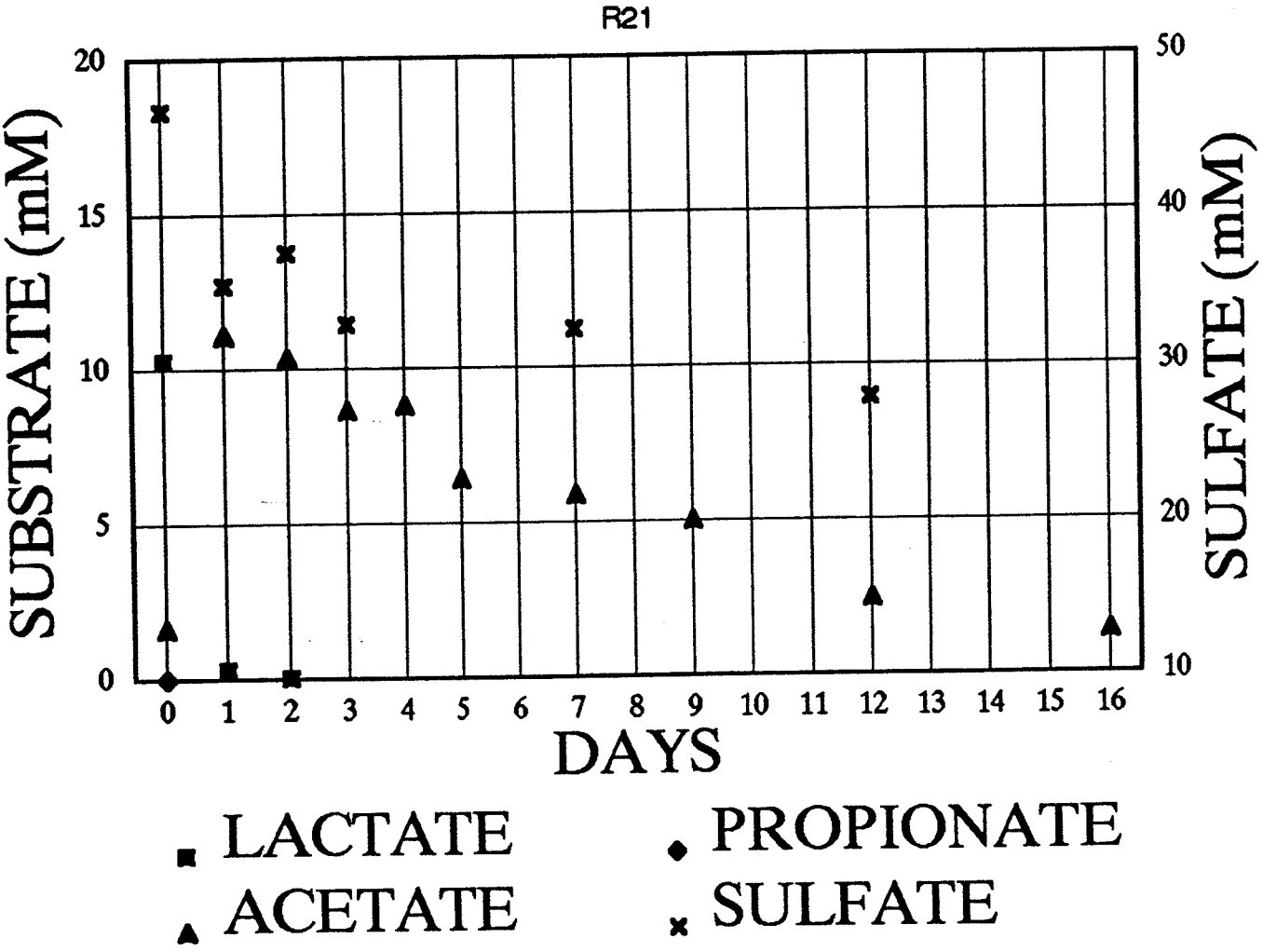
VS 263 261

0 CH4



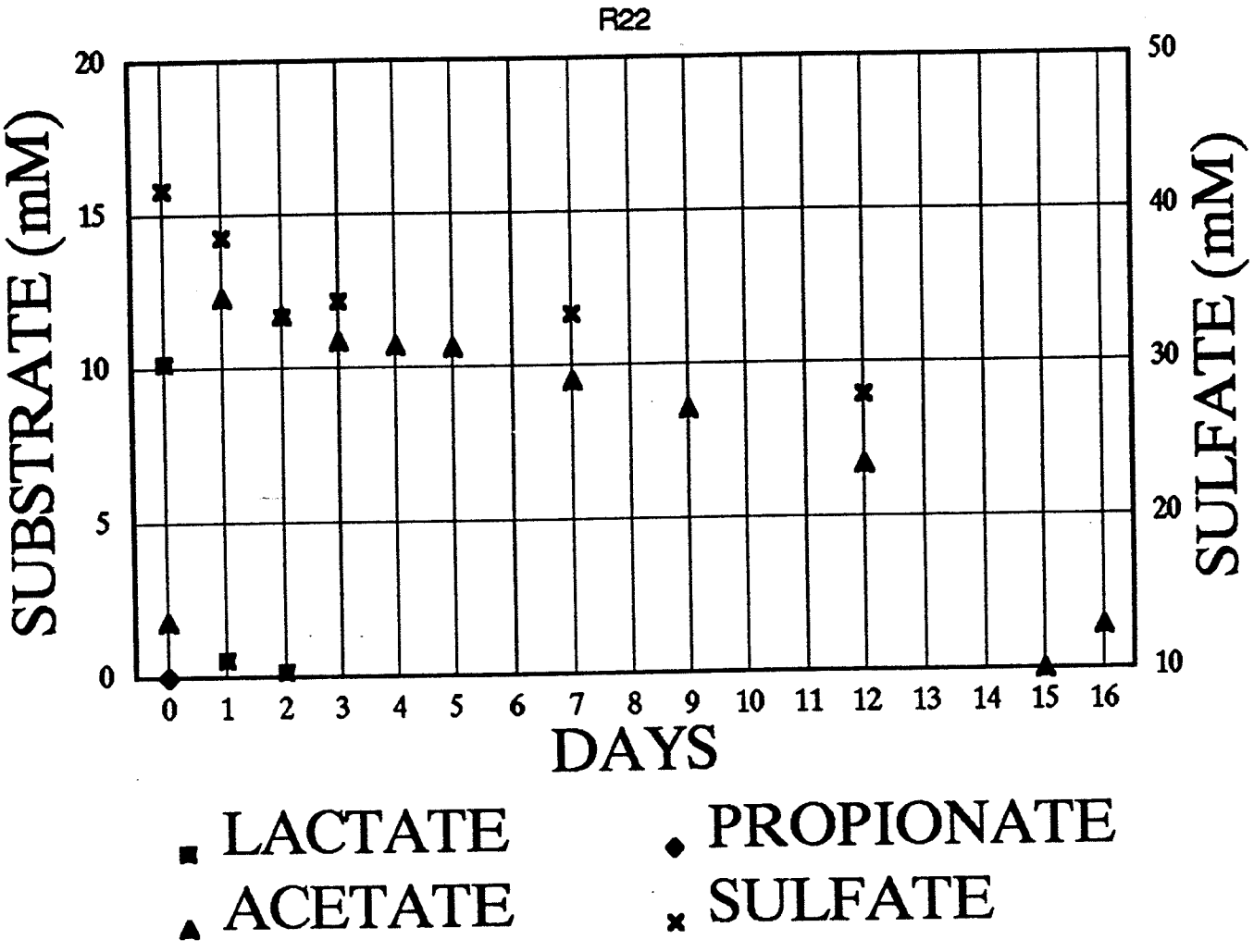
pH 8.0 8.0
TS 790 1350
H2S 57.2 97.8

5.9 mL CH4



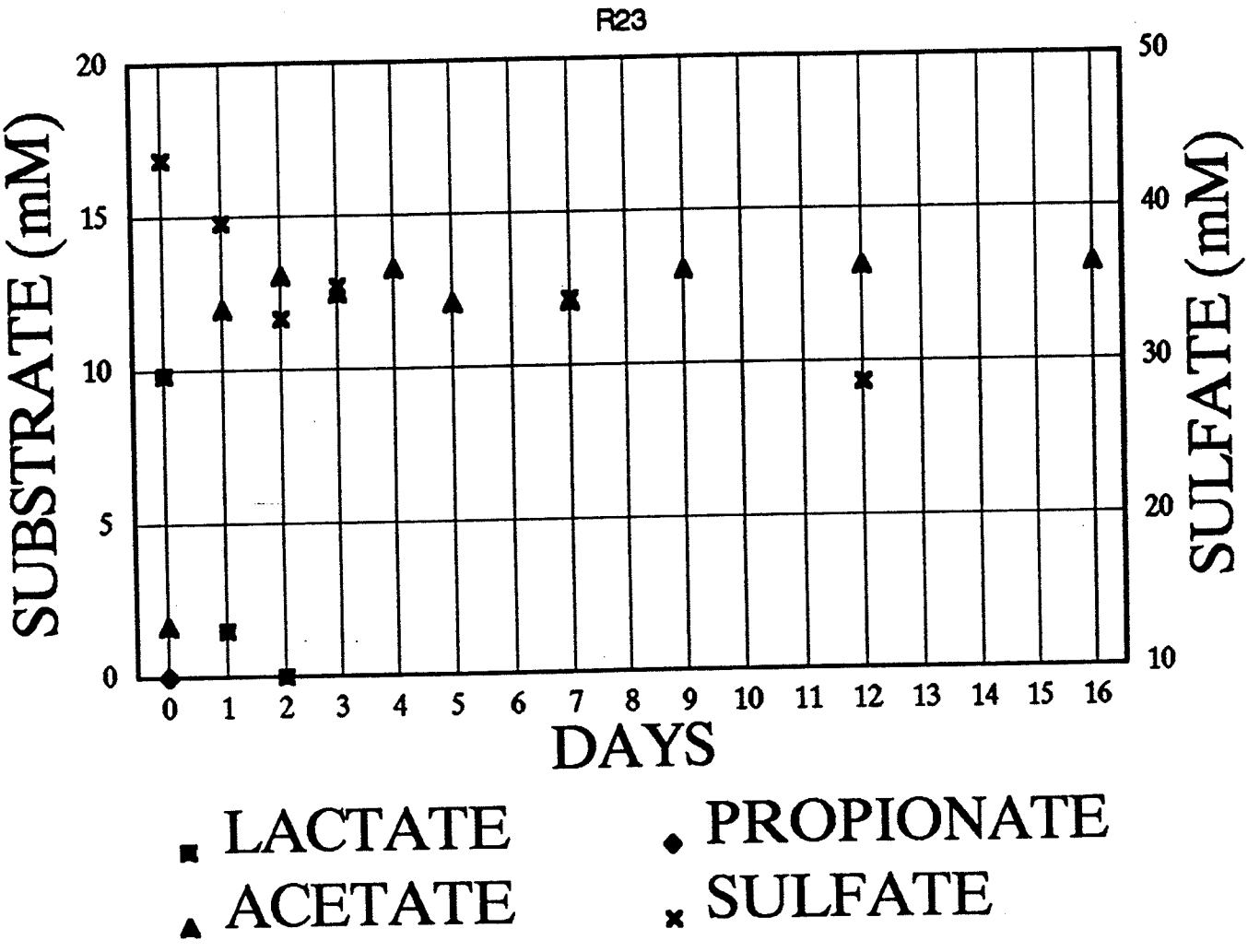
8. 81
1100 1630
79.7 95.2

4.0 mL CH4



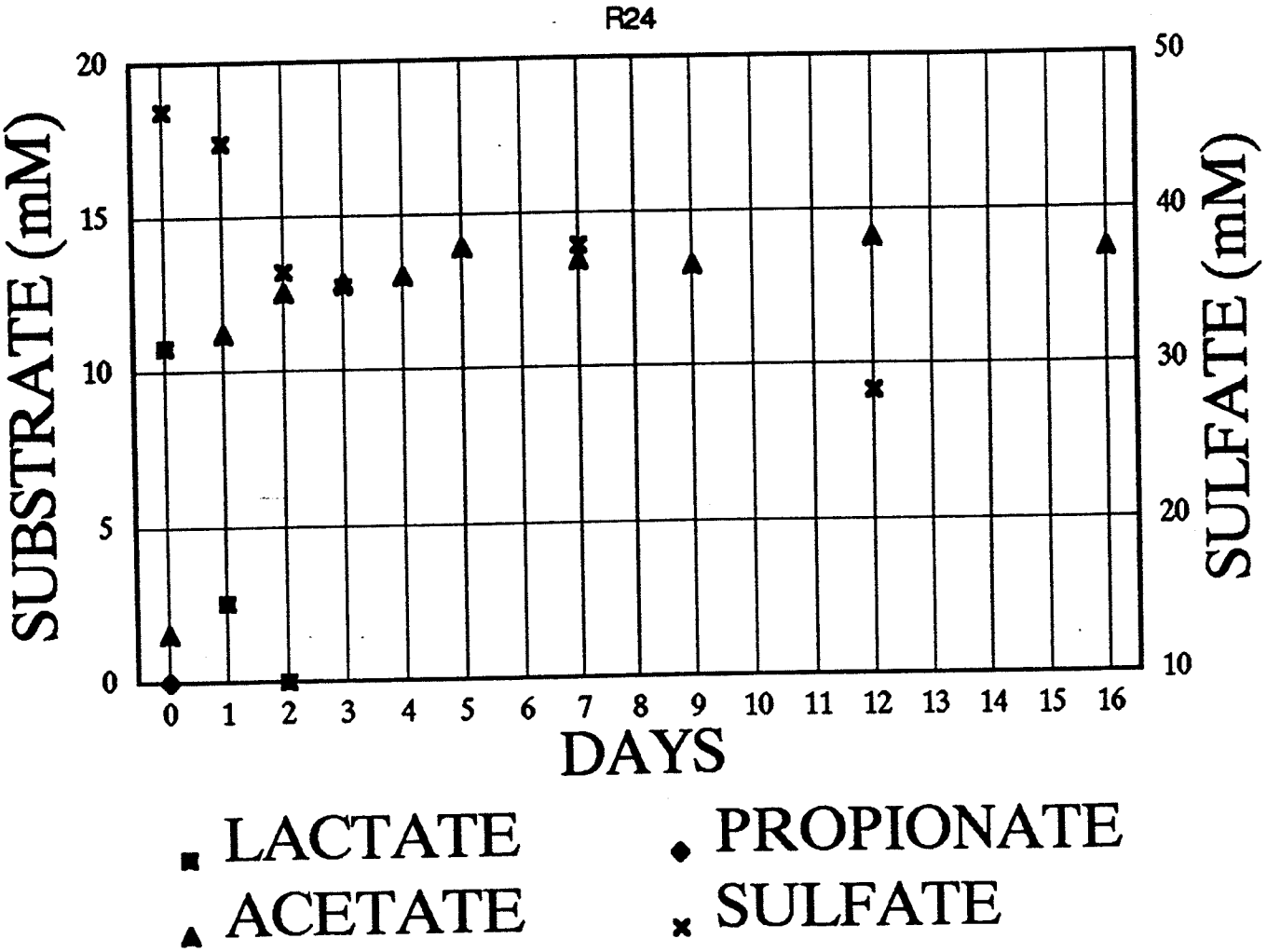
8 8
1370 1970
99.3 143

0.1 mL CH4



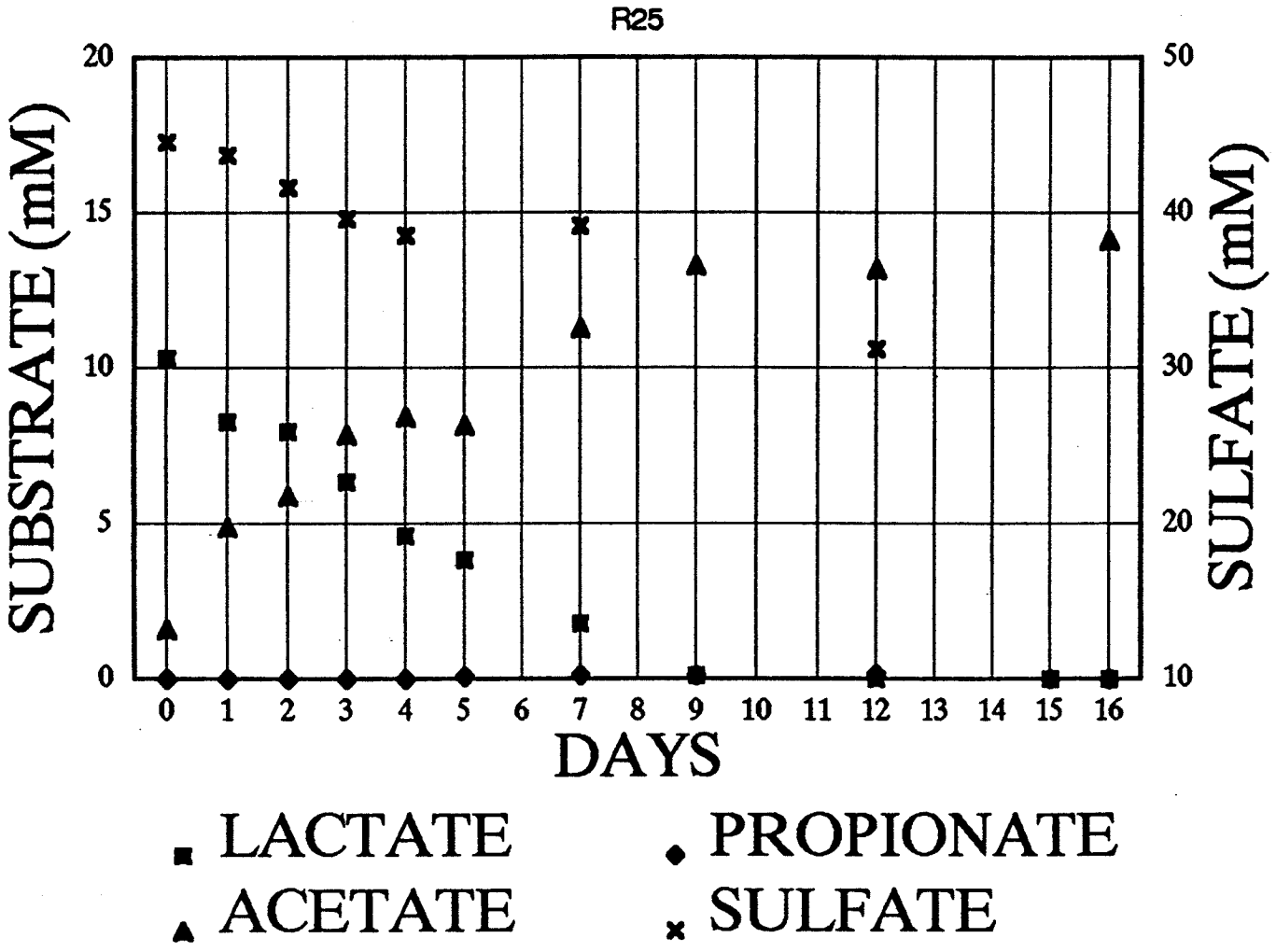
B B
1790 2420
130 175

$\bar{O}$ CH4



B B
2340 2880
170 209

0 CH4

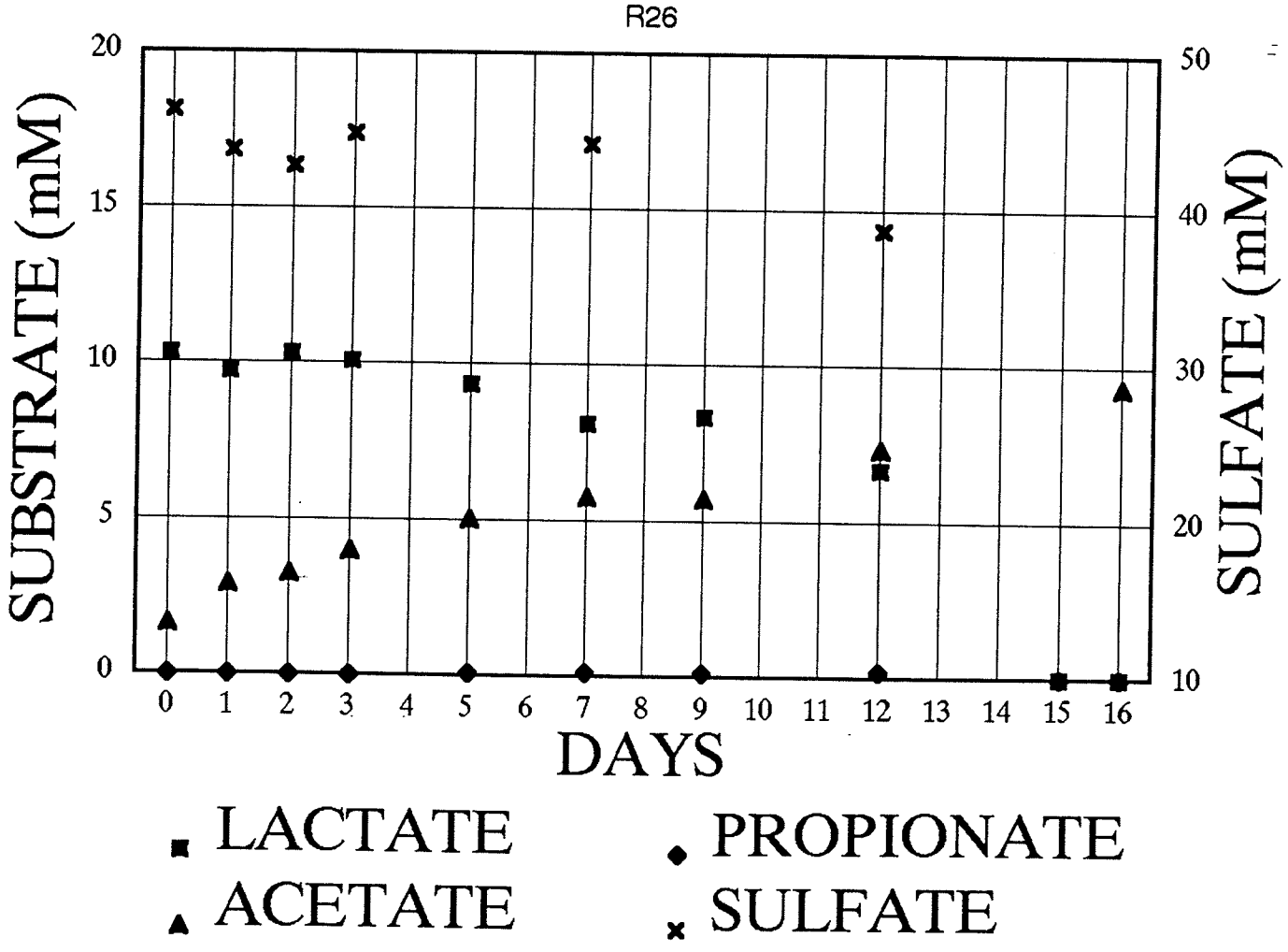


8 8

2900 2980

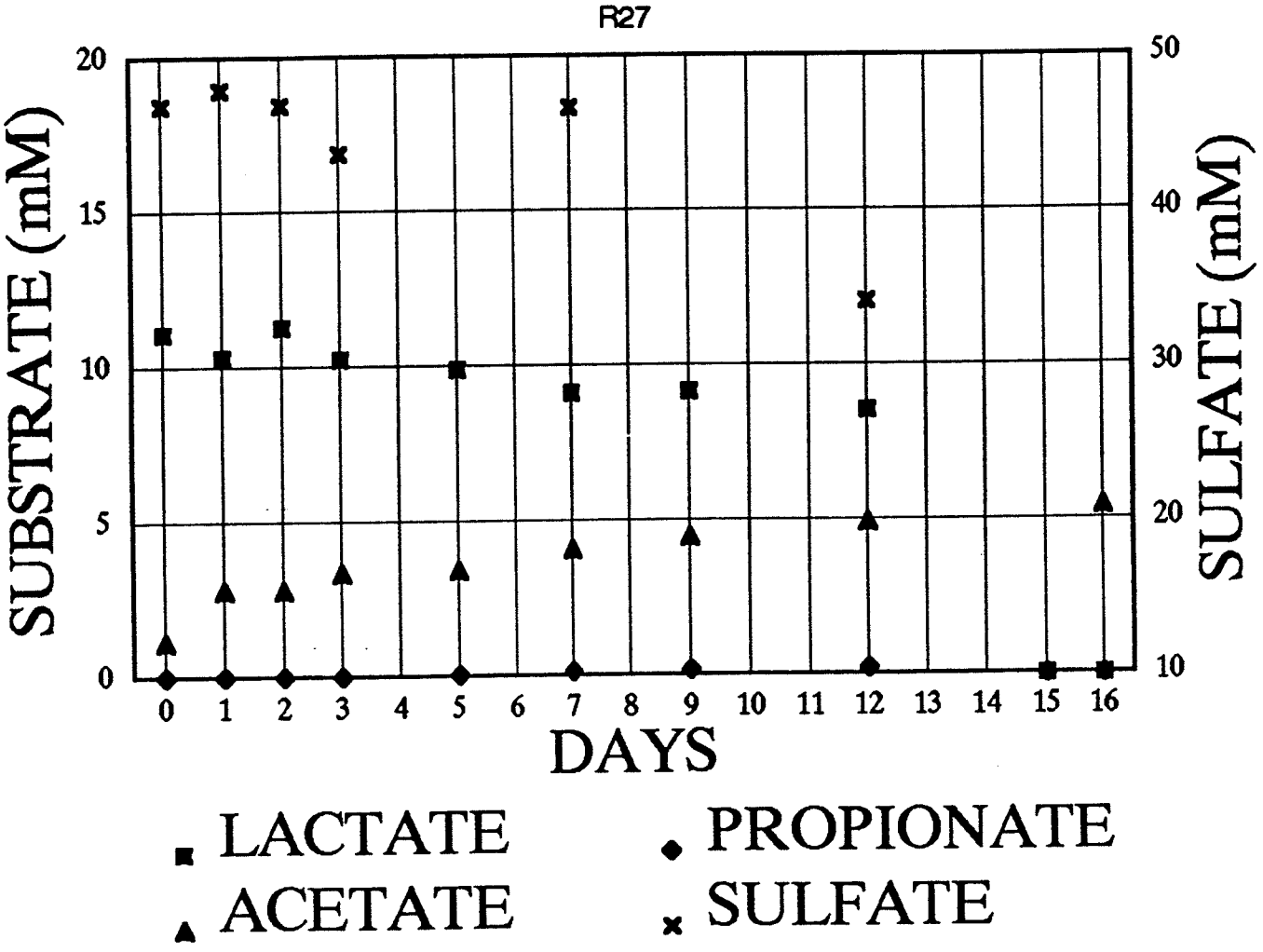
210 216

0 CH4



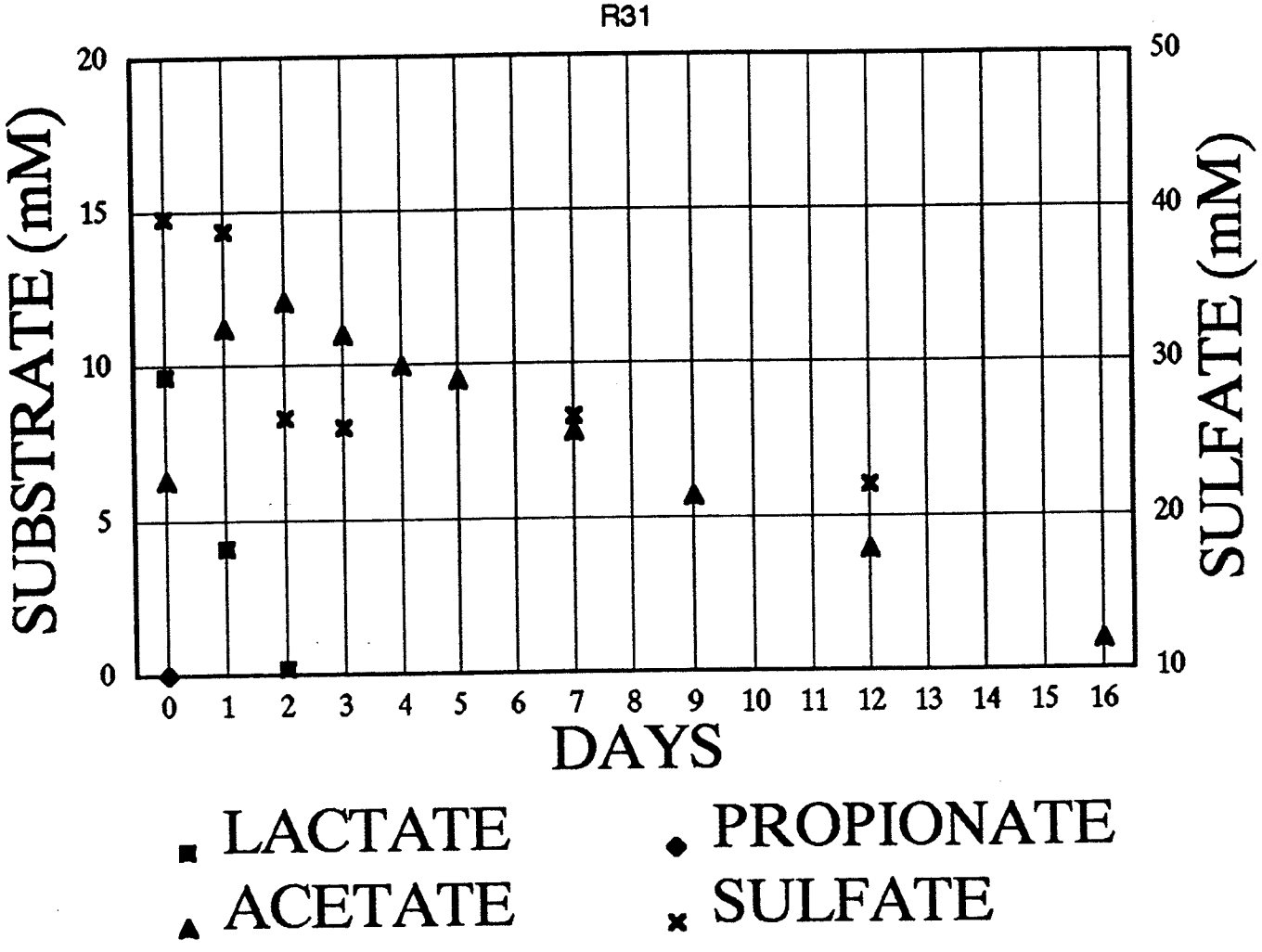
8 8.1
3450 5560
250 208

0 CH4



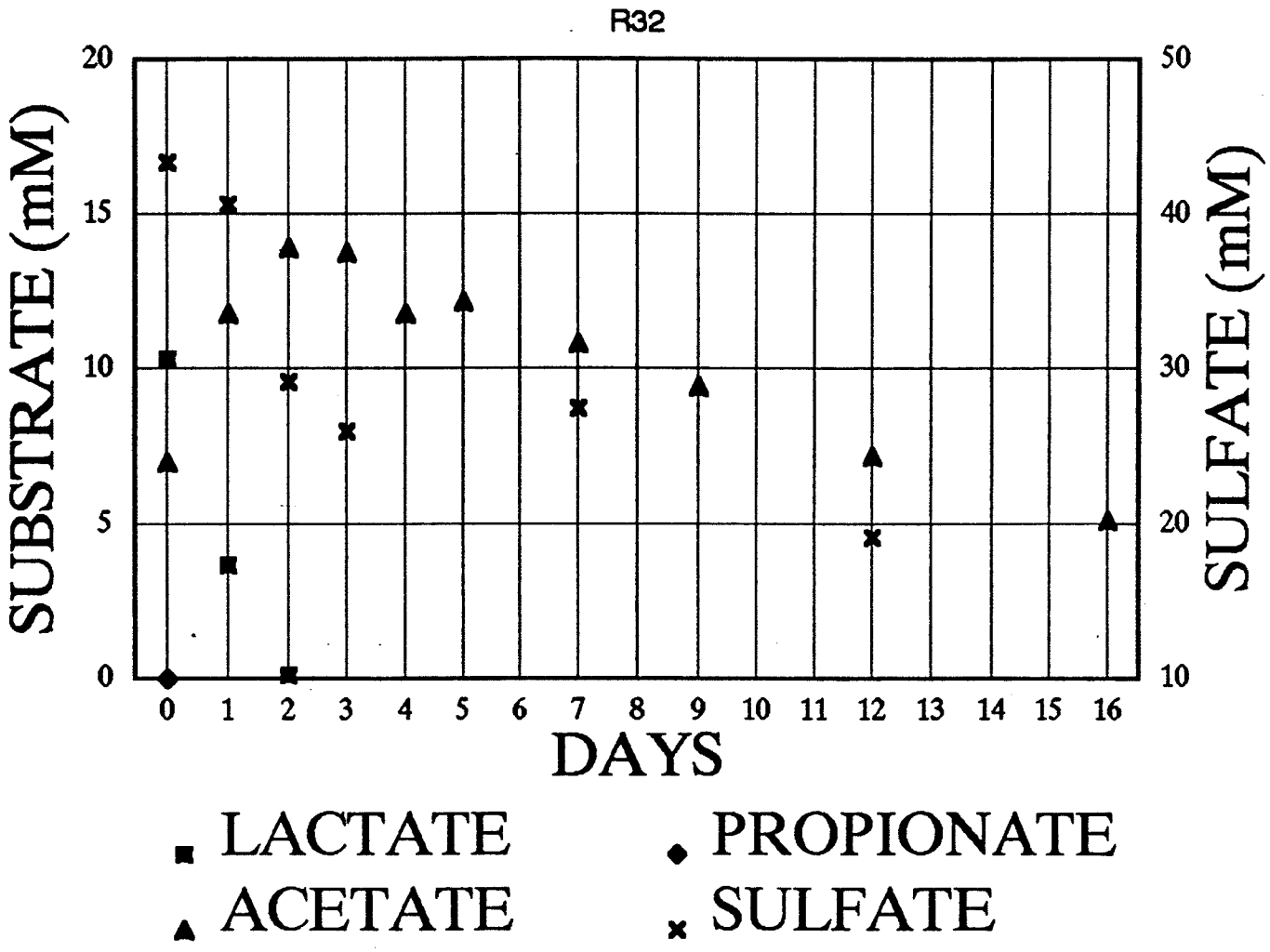
7.1 7.3
180 620
72.8 174

6.8 mL CH_4



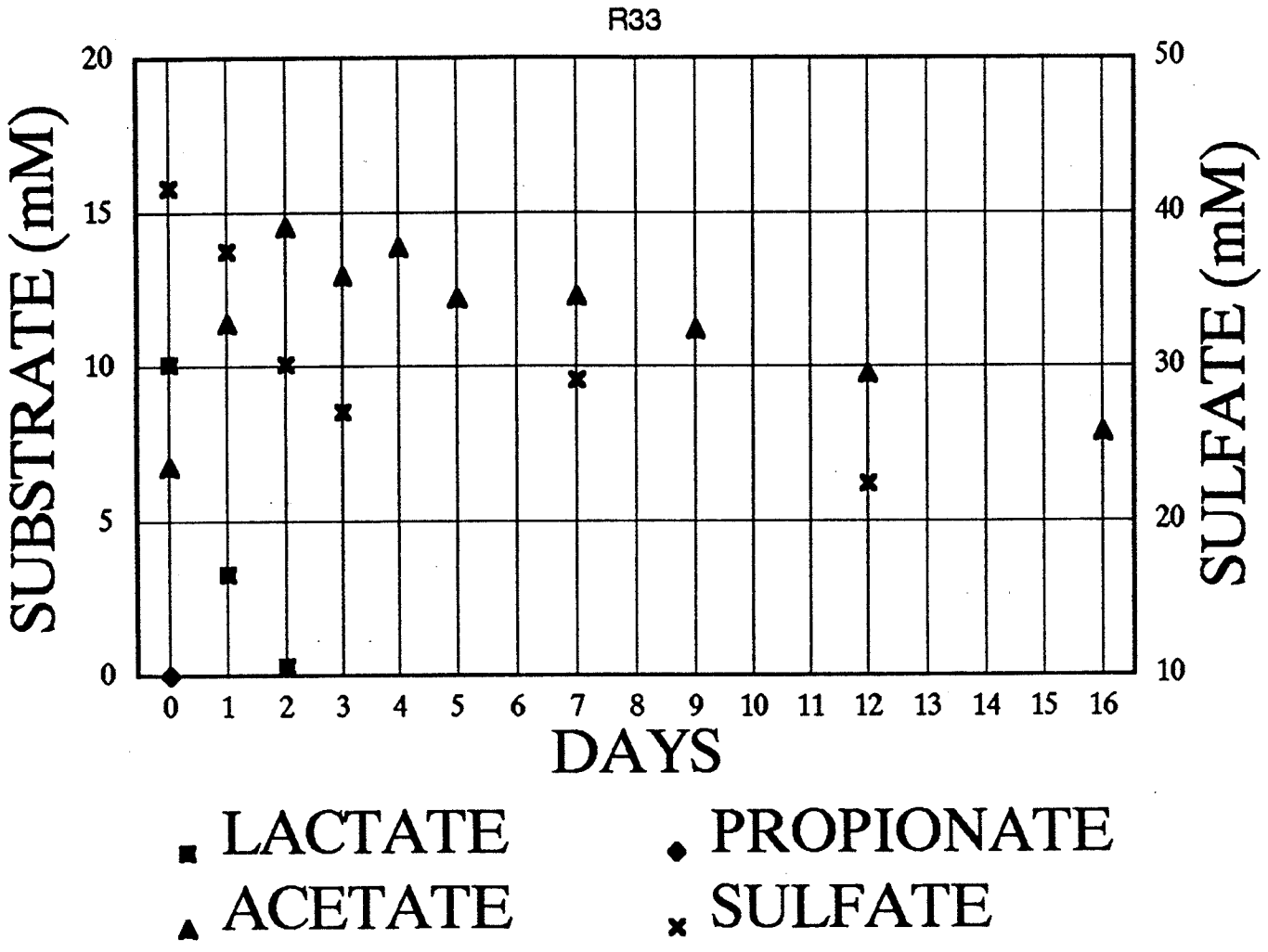
7· 7·5
21·5 72o
94 208

7·7 mL CH4



7. 75
265 750
116 211

74 mL CH_4

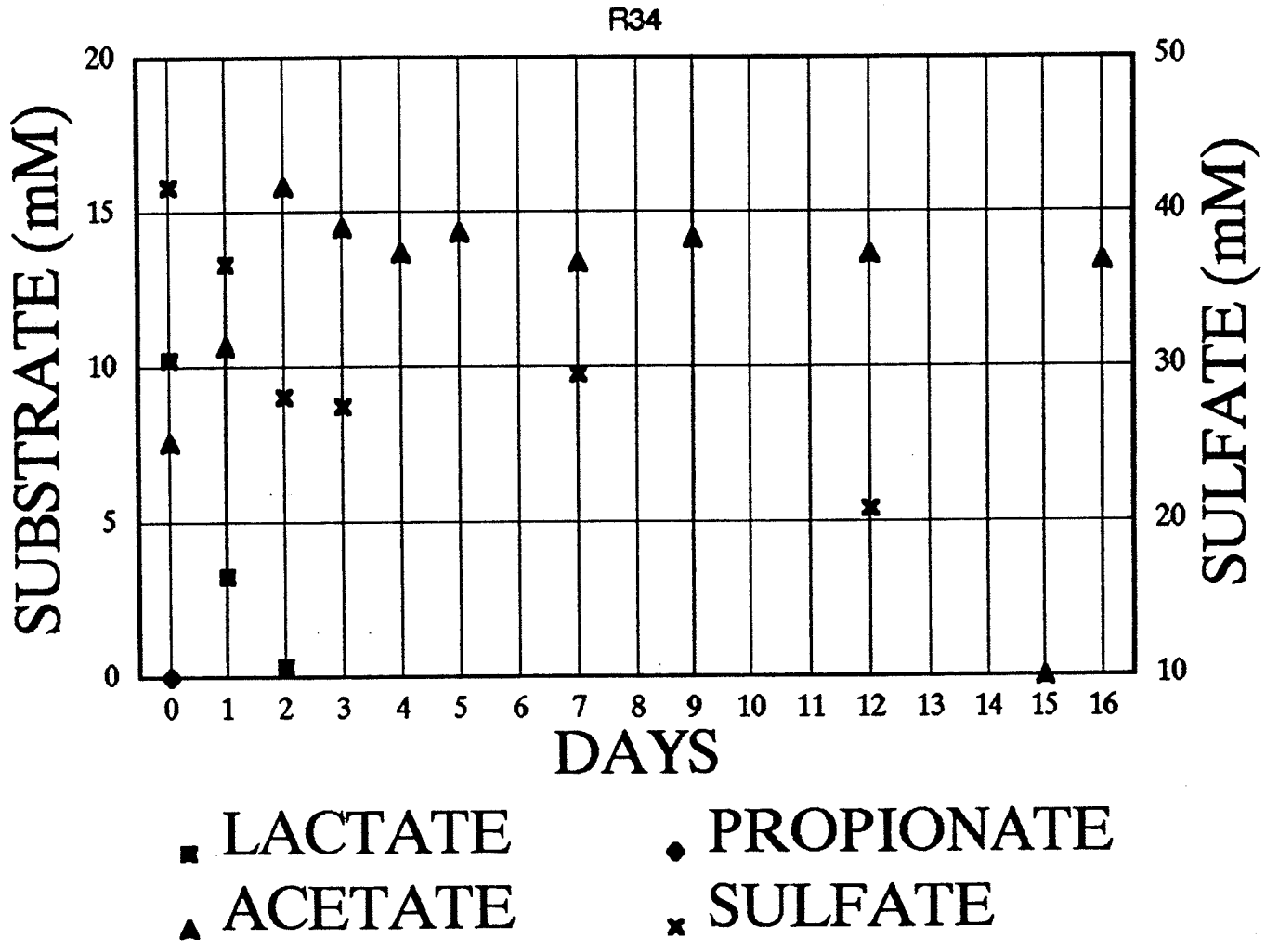


7.1 7.3

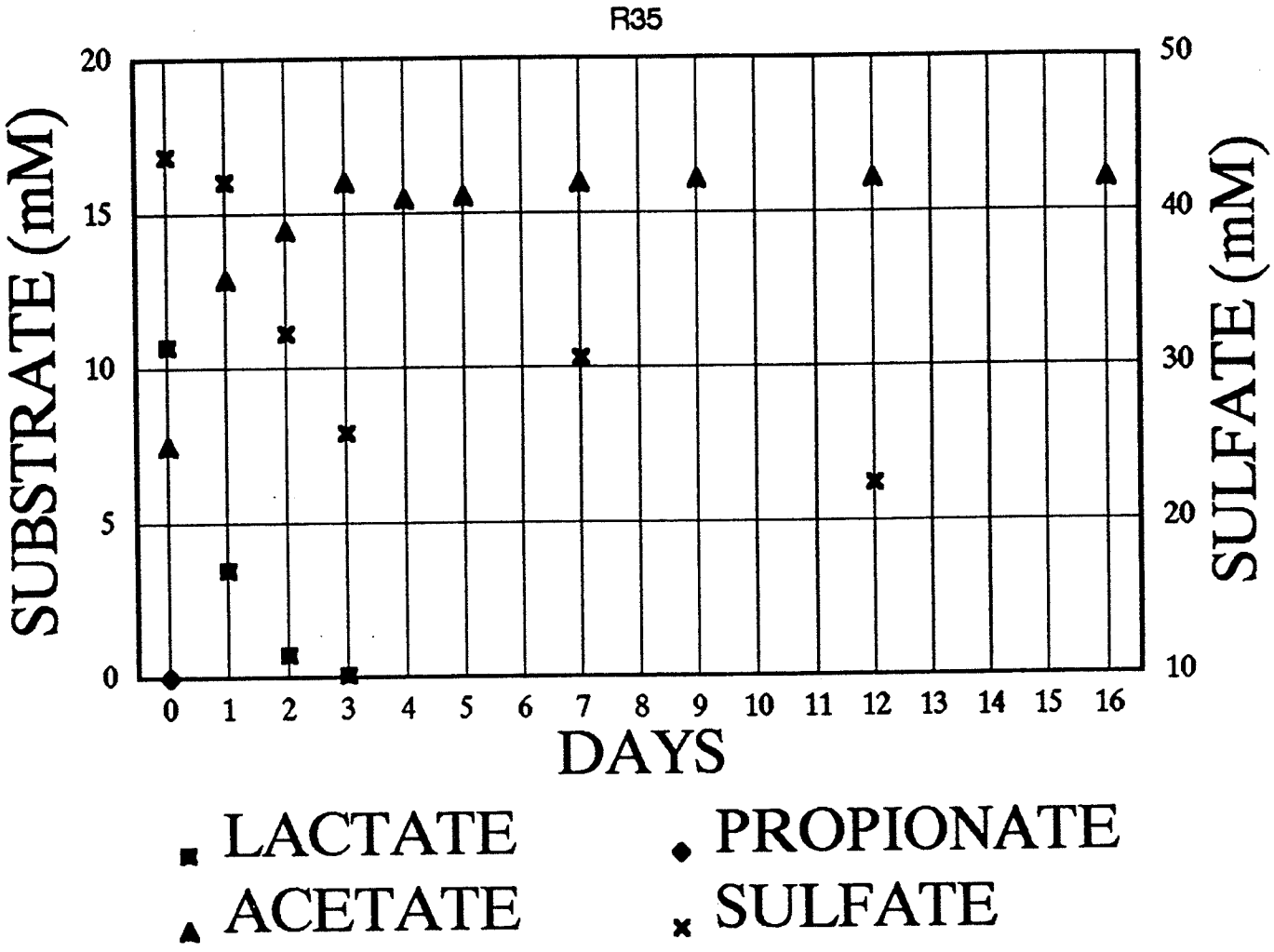
310 320

119 231

1.2 mL CH_4

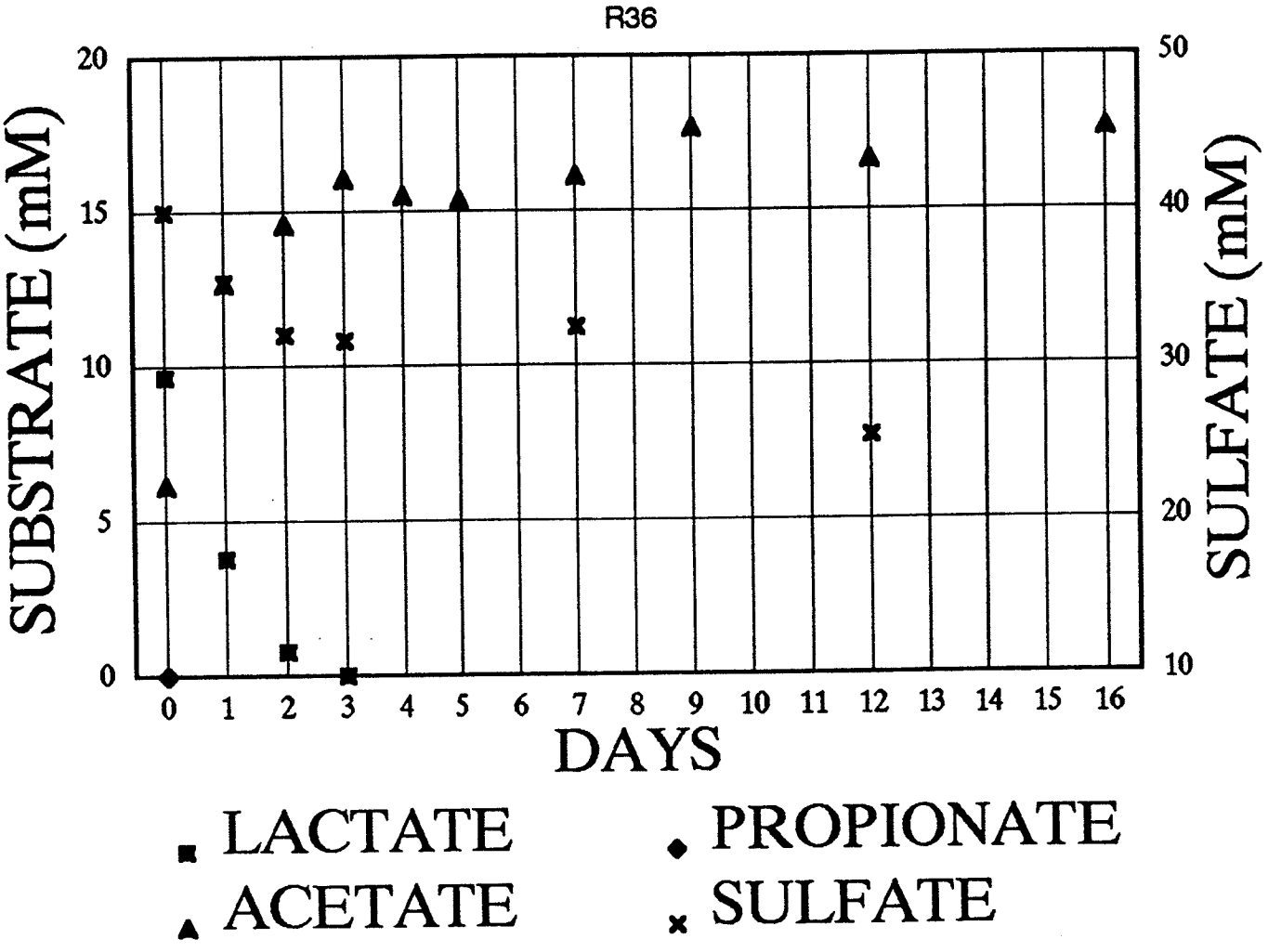


7.1 7.3
415 880
159 248
0 CH4



7.1 7.3
540 930
195 262

0 CH4

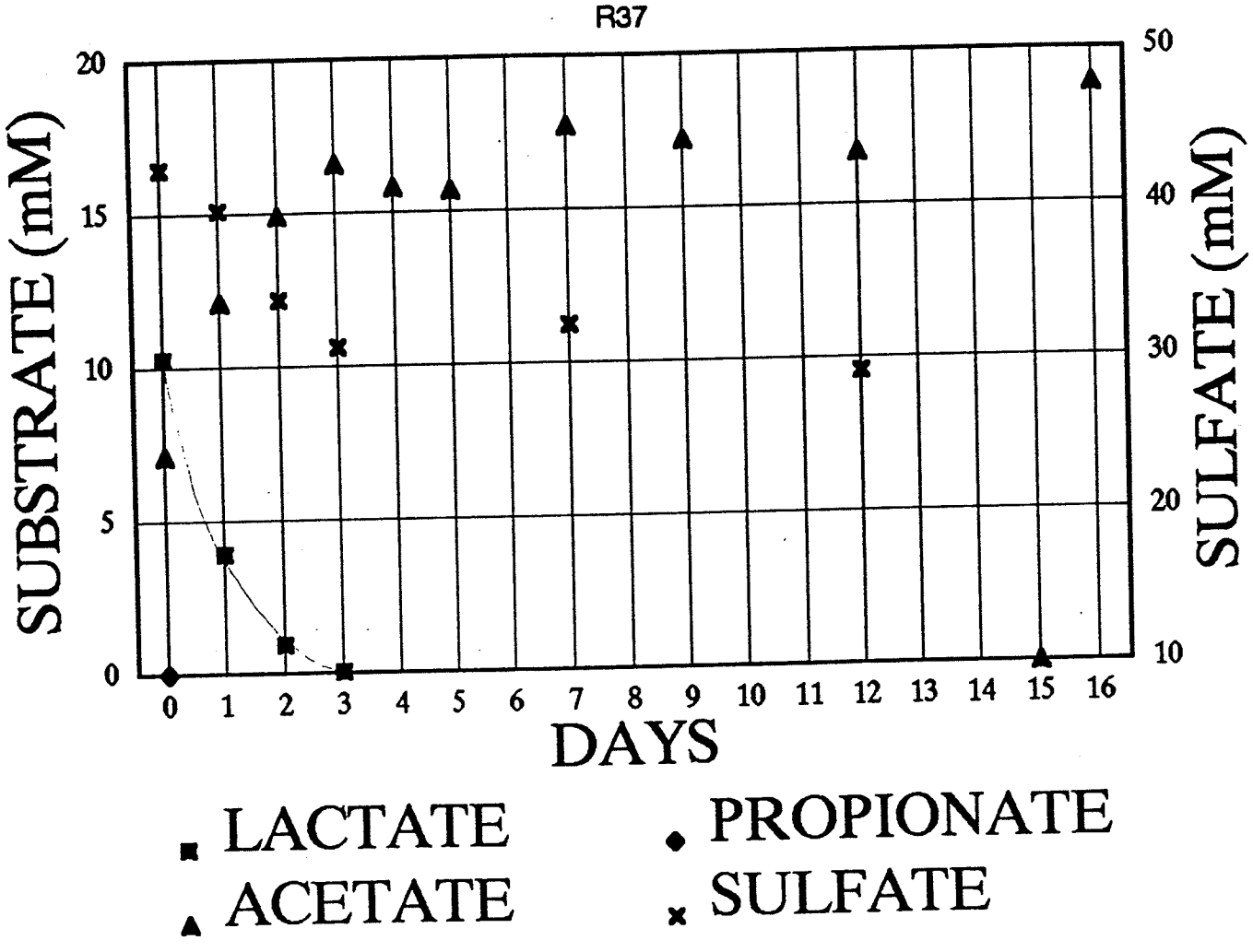


71 735

590 1080

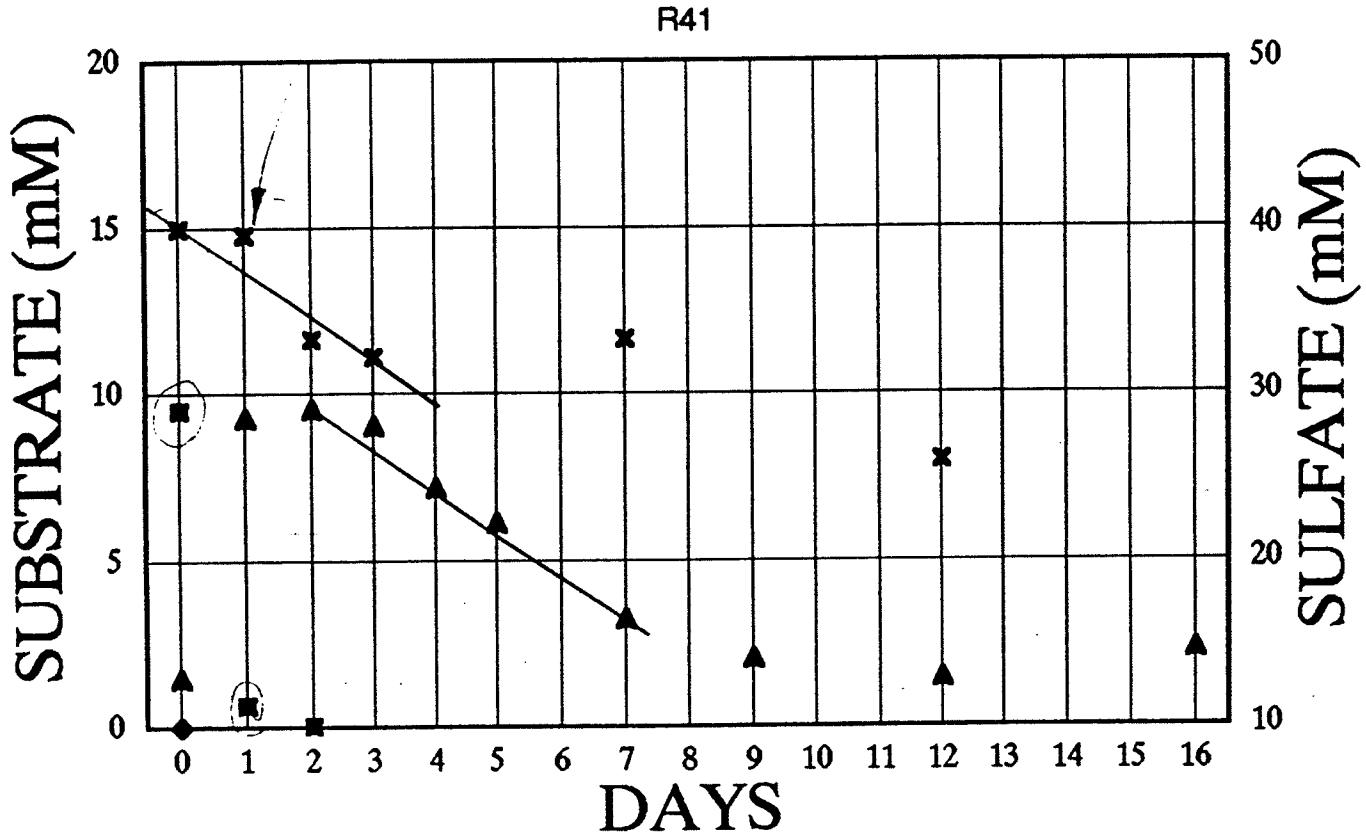
226 279

0 CH_4



B 8·1
800 1340
SB 783

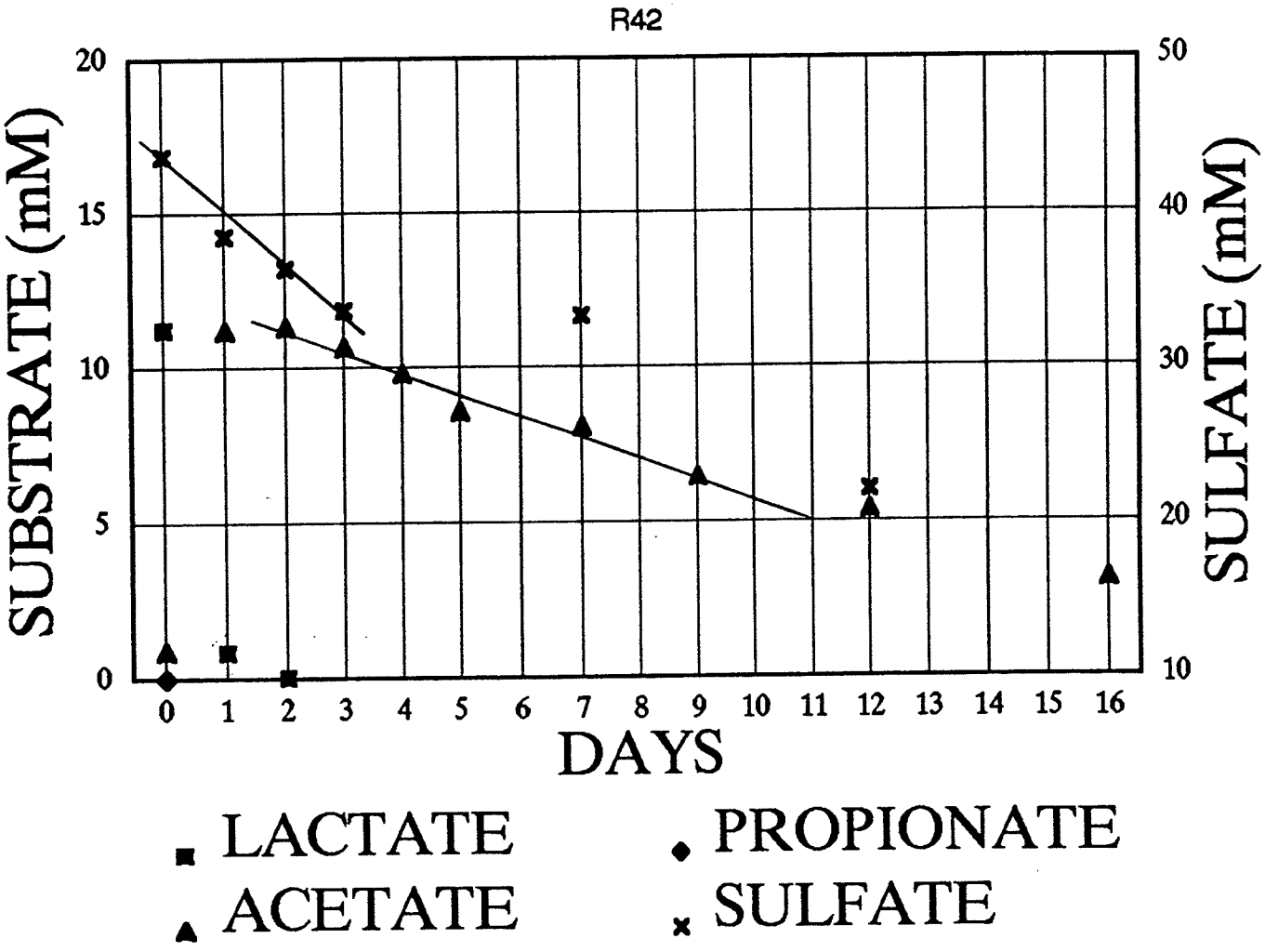
4.2 CH4



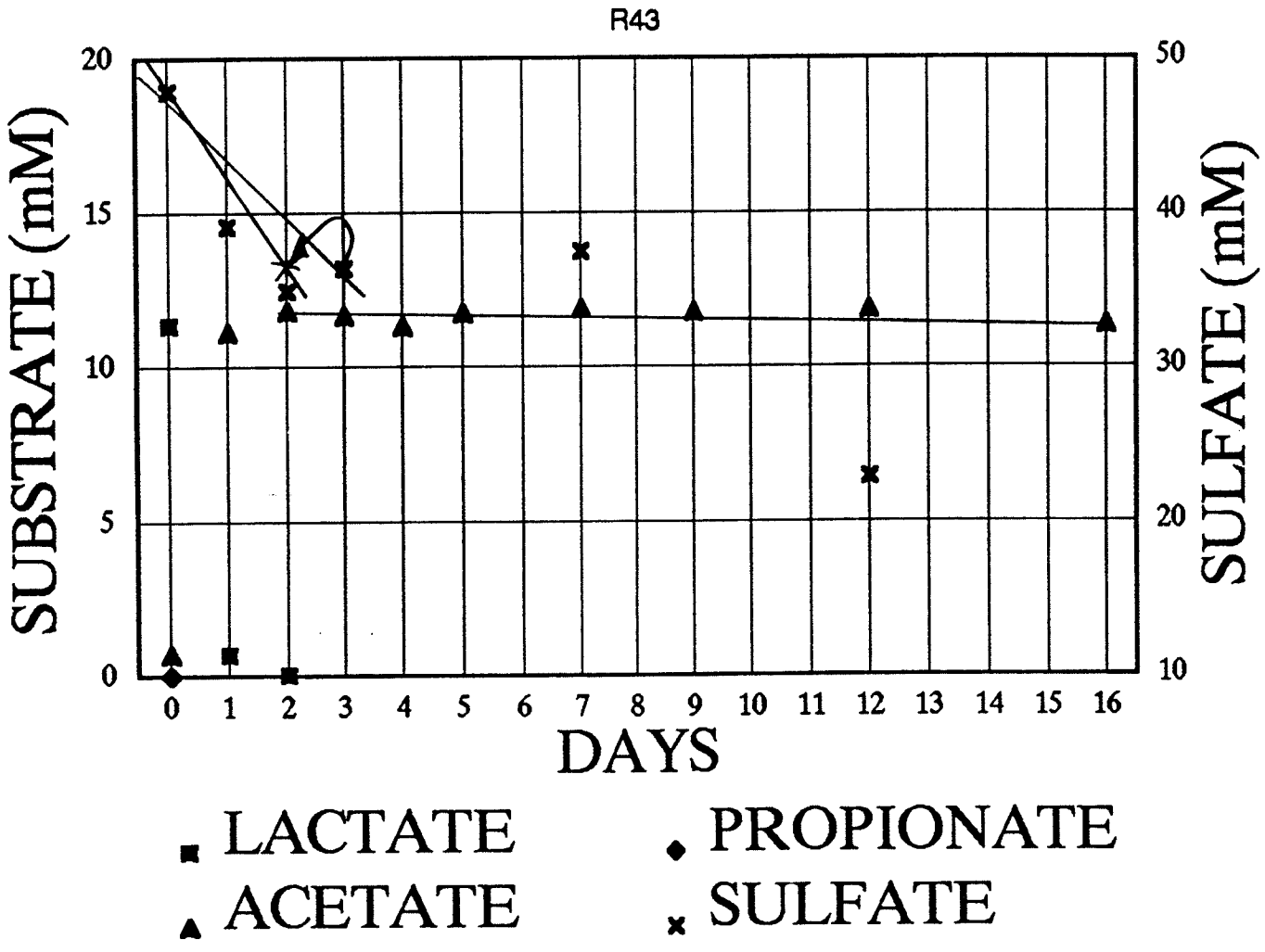
F4.17

7.9 8.2
1110 1700
99.4 79.9

4.3 mL CH4

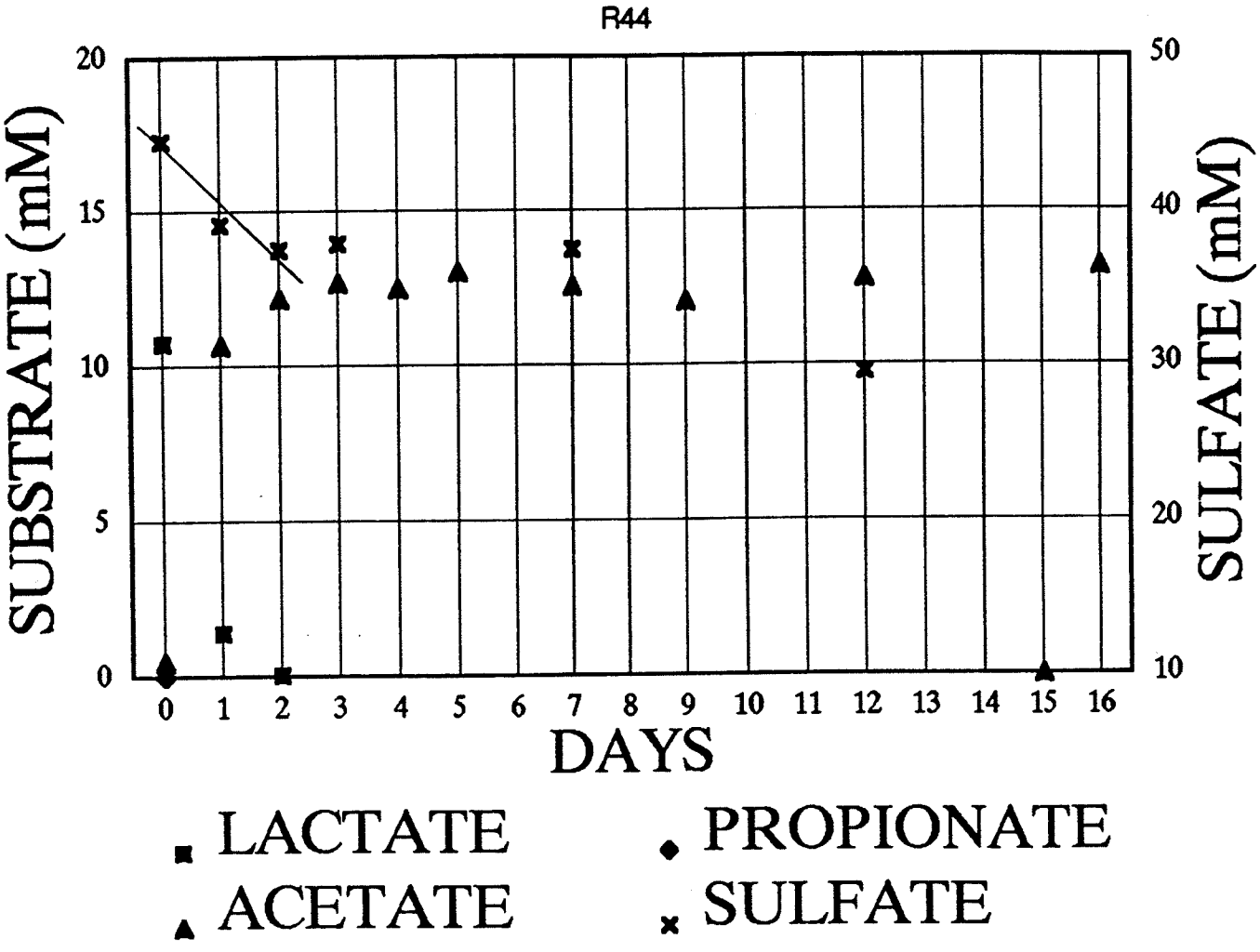


785 8.1
1380 1900
157 111
0.1 mL CH4



79 8.1
1800 2460
161 141

01 ~ CH4

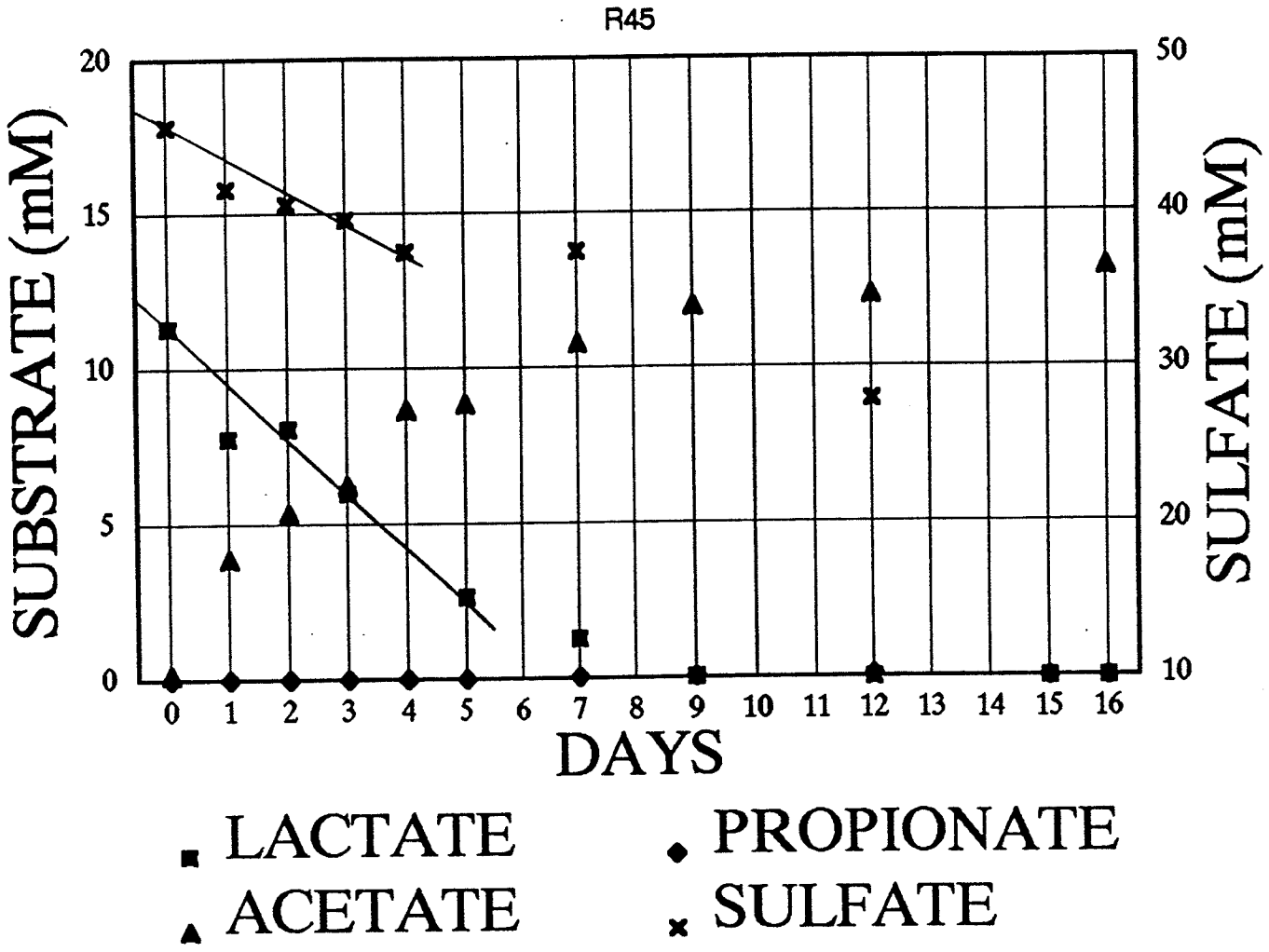


7.9 8 05

2350 2780

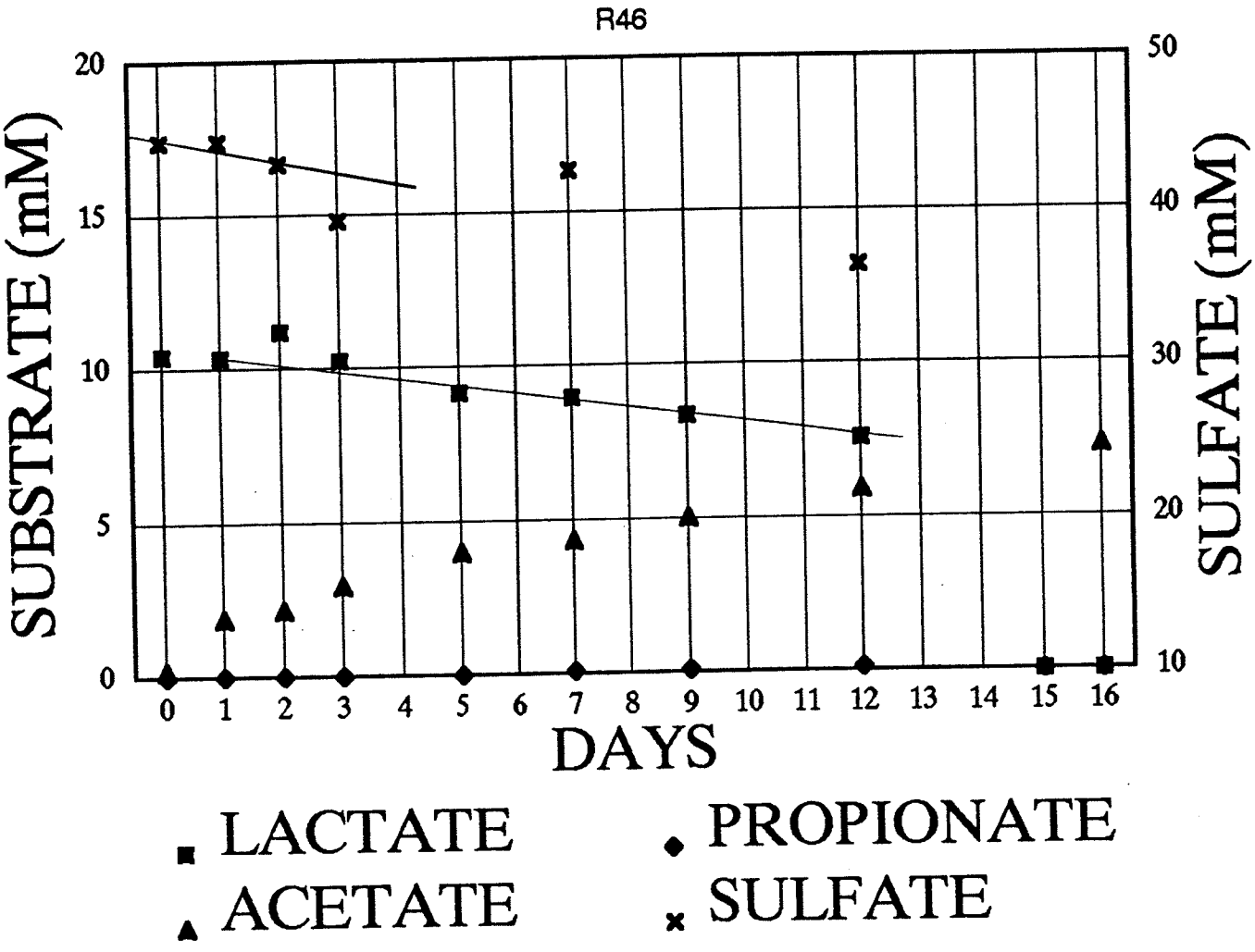
210 181

0 CH_4



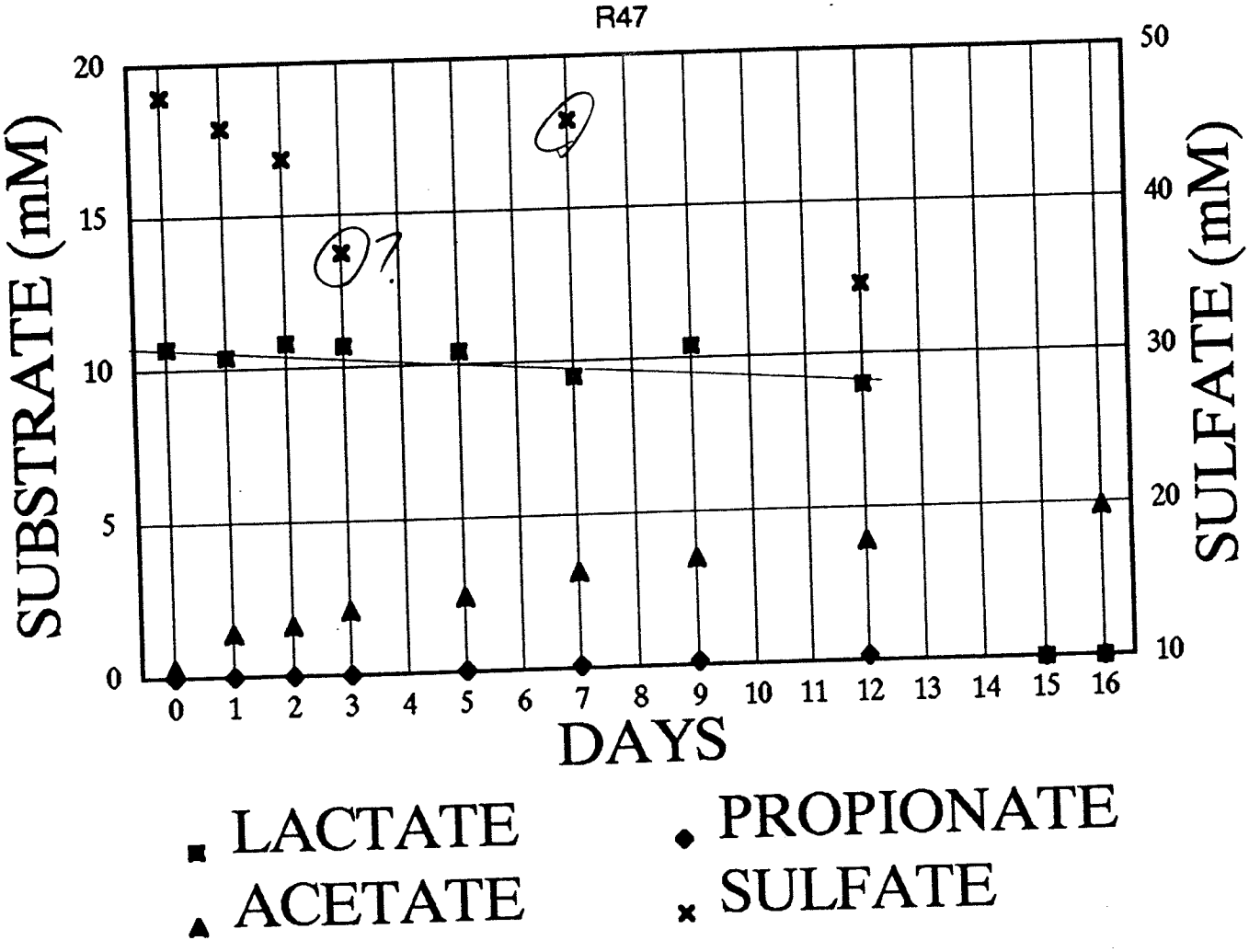
7.9 8.1
2910 3160
261 185

0 CH_4



795 B·1
3460 3560
279 208

0 CH4



App. B
Phase 3

PHASE 3											R11S
PHASE 3	REACTOR 11									REACTOR	R12S
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY	R13S
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM		R14S
PHASE 3	0	914.70	10.16	0.00	0.00	22.00	0.37	4000.00	41.67	0	R15
PHASE 3	1	500.90	5.57		0.00	219.00	3.65	3600.00	37.50	1	R16
PHASE 3	2	126.00	1.40		0.00	426.00	7.10	3040.00	31.67	2	R17
PHASE 3	3	20.00	0.22		0.00	395.00	6.58	3200.00	33.33	3	R21
PHASE 3	4		0.00		0.00	248.00	4.13			4	R22
PHASE 3	5		0.00		0.00	212.00	3.53			5	R23
PHASE 3	6									6	R24
PHASE 3	7		0.00		0.00	109.00	1.82	2760.00	28.75	7	R25
PHASE 3	8		0.00		0.00					8	R26
PHASE 3	9		0.00		0.00	43.00	0.72			9	R27
PHASE 3	10		0.00		0.00					10	R31
PHASE 3	11		0.00		0.00					11	R32
PHASE 3	12		0.00		0.00	43.00	0.72	2280.00	23.75	12	R33
PHASE 3	13		0.00		0.00					13	R34
PHASE 3	14		0.00		0.00					14	R35
PHASE 3	15		0.00		0.00					15	R36
PHASE 3	16		0.00		0.00	6.00	0.10			16	R37
PHASE 3	28.00		0.00		0.00			2100.00	21.88	28.00	R41
PHASE 3	REACTOR 12		0.00		0.00				0.00	REACTOR	R42
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY	R43
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM		R44
PHASE 3	0	906.90	10.08		0.00	72.00	1.20	4000.00	41.67	0	R45
PHASE 3	1	425.90	4.73		0.00	276.00	4.60	3200.00	33.33	1	R46
PHASE 3	2	126.00	1.40		0.00	399.00	6.65	3000.00	31.25	2	R47
PHASE 3	3	15.00	0.17		0.00	366.00	6.10	3000.00	31.25	3	R47S
PHASE 3	4		0.00		0.00	348.00	5.80			4	
PHASE 3	5		0.00		0.00	304.00	5.07			5	
PHASE 3	6									6	
PHASE 3	7		0.00		0.00	226.00	3.77	2900.00	30.21	7	
PHASE 3	8		0.00		0.00					8	
PHASE 3	9		0.00		0.00	167.00	2.78			9	
PHASE 3	10		0.00		0.00					10	
PHASE 3	11		0.00		0.00					11	
PHASE 3	12		0.00		0.00	86.00	1.43	2240.00	23.33	12	
PHASE 3	13		0.00		0.00					13	
PHASE 3	14		0.00		0.00					14	
PHASE 3	15		0.00		0.00					15	
PHASE 3	16.00		0.00		0.00	5.00	0.08				
PHASE 3	28.00		0.00		0.00			2160.00	22.50		
PHASE 3	REACTOR 13		0.00		0.00				0.00	REACTOR 13	

PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	914.30	10.16		0.00	30.00	0.50	4080.00	42.50	0
PHASE 3	1	532.70	5.92		0.00	255.00	4.25	3440.00	35.83	1
PHASE 3	2	206.00	2.29		0.00	304.00	5.07	3340.00	34.79	2
PHASE 3	3	2.00	0.02		0.00	503.00	8.38	3200.00	33.33	3
PHASE 3	4		0.00		0.00	395.00	6.58			4
PHASE 3	5		0.00		0.00	429.00	7.15			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	424.00	7.07	2900.00	30.21	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	377.00	6.28			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	349.00	5.82	2000.00	20.83	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	272.00	4.53			16
PHASE 3	28.00		0.00		0.00			2200.00	22.92	
PHASE 3 REACTOR 14			0.00		0.00				0.00	REACTOR 14
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	942.00	10.47	tr	0.00	24.00	0.40	4000.00	41.67	0
PHASE 3	1	476.70	5.30		0.00	268.00	4.47	3800.00	39.58	1
PHASE 3	2	189.00	2.10		0.00	355.00	5.92	3300.00	34.38	2
PHASE 3	3	13.00	0.14		0.00	531.00	8.85	3200.00	33.33	3
PHASE 3	4		0.00		0.00	518.00	8.63			4
PHASE 3	5		0.00		0.00	447.00	7.45			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	535.00	8.92	3150.00	32.81	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	472.00	7.87			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	475.00	7.92	2200.00	22.92	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	469.00	7.82			16
PHASE 3	28.00		0.00		0.00			2200.00	22.92	
PHASE 3			0.00		0.00				0.00	
PHASE 3 REACTOR 15			0.00		0.00				0.00	REACTOR 15
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY

PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	950.20	10.56		0.00	24.00	0.40	4200.00	43.75	0
PHASE 3	1	529.10	5.88		0.00	289.00	4.82	3800.00	39.58	1
PHASE 3	2	179.00	1.99		0.00	452.00	7.53	3300.00	34.38	2
PHASE 3	3	9.00	0.10		0.00	530.00	8.83	3240.00	33.75	3
PHASE 3	4		0.00		0.00	571.00	9.52			4
PHASE 3	5		0.00		0.00	549.00	9.15			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	611.00	10.18	3150.00	32.81	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	599.00	9.98			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	522.00	8.70	2300.00	23.96	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	613.00	10.22			16
PHASE 3	28.00		0.00		0.00			2200.00	22.92	
PHASE 3	REACTOR 16		0.00		0.00				0.00	REACTOR 16
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	902.00	10.02	tr	0.00	26.00	0.43	3960.00	41.25	0
PHASE 3	1	632.10	7.02		0.00	247.00	4.12	3880.00	40.42	1
PHASE 3	2	274.00	3.04		0.00	392.00	6.53	3240.00	33.75	2
PHASE 3	3	46.00	0.51		0.00	585.00	9.75	3300.00	34.38	3
PHASE 3	4		0.00		0.00	590.00	9.83		0.00	4
PHASE 3	5		0.00		0.00	616.00	10.27		0.00	5
PHASE 3	6								0.00	6
PHASE 3	7		0.00		0.00	658.00	10.97	3150.00	32.81	7
PHASE 3	8		0.00		0.00				0.00	8
PHASE 3	9		0.00		0.00	790.00	13.17		0.00	9
PHASE 3	10		0.00		0.00				0.00	10
PHASE 3	11		0.00		0.00				0.00	11
PHASE 3	12		0.00		0.00	646.00	10.77	2360.00	24.58	12
PHASE 3	13		0.00		0.00				0.00	13
PHASE 3	14		0.00		0.00				0.00	14
PHASE 3	15		0.00		0.00				0.00	15
PHASE 3	16.00		0.00		0.00	601.00	10.02		0.00	16.00
PHASE 3	28.00		0.00		0.00			2400.00	25.00	28.00
PHASE 3	REACTOR 17		0.00		0.00				0.00	REACTOR 17
PHASE 3	DAY	HLa	HLa	HPr	0.00	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	HPr	mg/L	mM	mg/L	mM	
PHASE 3	0	944.90	10.50	tr	mM	26.00	0.43	4040.00	42.08	0

PHASE 3	1	647.70	7.20		0.00	226.00	3.77	3880.00	40.42	1
PHASE 3	2	342.00	3.80		0.00	383.00	6.38	3400.00	35.42	2
PHASE 3	3	78.00	0.87		0.00	441.00	7.35	3400.00	35.42	3
PHASE 3	4	6.00	0.07		0.00	566.00	9.43	3400.00	35.42	4
PHASE 3	5		0.00		0.00	590.00	9.83			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	648.00	10.80	3040.00	31.67	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	660.00	11.00			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	705.00	11.75	2480.00	25.83	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	664.00	11.07			16
PHASE 3	28.00		0.00		0.00			2500.00	26.04	28.00
PHASE 3	REACTOR 21		0.00		0.00				0.00	REACTOR 21
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	925.50	10.28		0.00	97.00	1.62	4480.00	46.67	0
PHASE 3	1	29.80	0.33		0.00	665.00	11.08	3400.00	35.42	1
PHASE 3	2	6.00	0.07		0.00	621.00	10.35	3600.00	37.50	2
PHASE 3	3		0.00		0.00	521.00	8.68	3160.00	32.92	3
PHASE 3	4		0.00		0.00	530.00	8.83			4
PHASE 3	5		0.00		0.00	385.00	6.42			5 *
PHASE 3	6									6
PHASE 3	7		0.00		0.00	353.00	5.88	3120.00	32.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	301.00	5.02			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	149.00	2.48	2680.00	27.92	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	84.00	1.40			16
PHASE 3	28.00		0.00		0.00			2300.00	23.96	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3	REACTOR 22		0.00		0.00				0.00	REACTOR 22
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	917.00	10.19		0.00	108.00	1.80	4000.00	41.67	0
PHASE 3	1	48.20	0.54		0.00	739.00	12.32	3700.00	38.54	1

PHASE 3	2	15.00	0.17		0.00	703.00	11.72	3200.00	33.33	2
PHASE 3	3		0.00		0.00	651.00	10.85	3300.00	34.38	3
PHASE 3	4		0.00		0.00	643.00	10.72			4
PHASE 3	5		0.00			638.00	10.63			5
PHASE 3	6				0.00					6
PHASE 3	7		0.00		0.00	572.00	9.53	3200.00	33.33	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	512.00	8.53			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	402.00	6.70	2680.00	27.92	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00		0.00			15
PHASE 3	16		0.00		0.00	84.00	1.40			16
PHASE 3	28.00		0.00		0.00		0.00	2400.00	25.00	28.00
PHASE 3	REACTOR 23		0.00				0.00		0.00	REACTOR 23
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	886.90	9.85		0.00	101.00	1.68	4200.00	43.75	0
PHASE 3	1	136.60	1.52		0.00	719.00	11.98	3800.00	39.58	1
PHASE 3	2	2.00	0.02		0.00	785.00	13.08	3200.00	33.33	2
PHASE 3	3		0.00		0.00	750.00	12.50	3400.00	35.42	3
PHASE 3	4		0.00		0.00	797.00	13.28			4
PHASE 3	5		0.00		0.00	726.00	12.10			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	726.00	12.10	3300.00	34.38	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	784.00	13.07			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	789.00	13.15	2760.00	28.75	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16.00		0.00		0.00	791.00	13.18			16.00
PHASE 3	28.00		0.00		0.00			2360.00	24.58	28.00
PHASE 3	REACTOR 24		0.00		0.00				0.00	REACTOR 24
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	972.60	10.81	tr	0.00	93.00	1.55	4500.00	46.88	0
PHASE 3	1	230.90	2.57		0.00	674.00	11.23	4300.00	44.79	1
PHASE 3	2	1.00	0.01		0.00	752.00	12.53	3500.00	36.46	2
PHASE 3	3		0.00		0.00	771.00	12.85	3400.00	35.42	3

PHASE 3	4		0.00		0.00	782.00	13.03		4
PHASE 3	5		0.00		0.00	836.00	13.93		5
PHASE 3	6								6
PHASE 3	7		0.00		0.00	807.00	13.45	3640.00	37.92	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	795.00	13.25			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	846.00	14.10	2720.00	28.33	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	824.00	13.73			16
PHASE 3	28.00		0.00		0.00			2560.00	26.67	28.00
PHASE 3	REACTOR 25		0.00		0.00				0.00	REACTOR 25
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	930.00	10.33	0.00	0.00	95.00	1.58	4280.00	44.58	0
PHASE 3	1	746.50	8.29	tr	0.00	292.00	4.87	4200.00	43.75	1
PHASE 3	2	717.00	7.97	tr	0.00	353.00	5.88	4000.00	41.67	2
PHASE 3	3	571.00	6.34	tr	0.00	471.00	7.85	3800.00	39.58	3
PHASE 3	4	415.00	4.61	2.00	0.03	506.00	8.43	3700.00	38.54	4
PHASE 3	5	346.00	3.84	7.00	0.09	491.00	8.18			5
PHASE 3	6									6
PHASE 3	7	161.00	1.79	9.00	0.12	677.00	11.28	3760.00	39.17	7
PHASE 3	8				0.00					8
PHASE 3	9	13.00	0.14	10.00	0.14	798.00	13.30			9
PHASE 3	10				0.00					10
PHASE 3	11				0.00					11
PHASE 3	12		0.00	11.00	0.15	792.00	13.20	3000.00	31.25	12
PHASE 3	13				0.00					13
PHASE 3	14				0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	846.00	14.10			16
PHASE 3	28.00		0.00		0.00			2900.00	30.21	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3	REACTOR 26		0.00					SO4	SO4	REACTOR 26
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	mg/L	mM	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM		0.00	
PHASE 3	0	930.40	10.34		0.00	98.00	1.63	4440.00	46.25	0
PHASE 3	1	879.00	9.77	tr	0.00	174.00	2.90	4200.00	43.75	1
PHASE 3	2	930.00	10.33	tr	0.00	196.00	3.27	4100.00	42.71	2
PHASE 3	3	908.00	10.09	tr	0.00	239.00	3.98	4300.00	44.79	3
PHASE 3	4									4

PHASE 3	5	841.00	9.34	6.00	0.08	301.00	5.02			5
PHASE 3	6									6
PHASE 3	7	732.00	8.13	9.00	0.12	347.00	5.78	4240.00	44.17	7
PHASE 3	8									8
PHASE 3	9	752.00	8.36	10.00	0.14	343.00	5.72			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12	603.00	6.70	14.00	0.19	442.00	7.37	3720.00	38.75	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	561.00	9.35			16
PHASE 3	28.00		0.00		0.00		0.00	2900.00	30.21	28.00
PHASE 3	REACTOR 27		0.00		0.00		0.00	SO4	SO4	REACTOR 27
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	mg/L	mM	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM		0.00	
PHASE 3	0	1000.70	11.12		0.00	69.00	1.15	4500.00	46.88	0
PHASE 3	1	929.50	10.33	tr	0.00	168.00	2.80	4600.00	47.92	1
PHASE 3	2	1015.00	11.28	tr	0.00	168.00	2.80	4500.00	46.88	2
PHASE 3	3	924.00	10.27	tr	0.00	199.00	3.32	4200.00	43.75	3
PHASE 3	4									4
PHASE 3	5	892.00	9.91	5.00	0.07	205.00	3.42			5
PHASE 3	6									6
PHASE 3	7	821.00	9.12	10.00	0.14	244.00	4.07	4480.00	46.67	7
PHASE 3	8									8
PHASE 3	9	824.00	9.16	13.00	0.18	267.00	4.45			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12	771.00	8.57	16.00	0.22	294.00	4.90	3280.00	34.17	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15
PHASE 3	16.00		0.00		0.00	325.00	5.42			16.00
PHASE 3	28.00		0.00		0.00			3280.00	34.17	28.00
PHASE 3	REACTOR 31		0.00		0.00		0.00	SO4	SO4	REACTOR 31
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	mg/L	mM	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM		0.00	
PHASE 3	0	870.30	9.67	0.00	0.00	379.00	6.32	3800.00	39.58	0
PHASE 3	1	368.10	4.09		0.00	672.00	11.20	3720.00	38.75	1
PHASE 3	2	17.00	0.19		0.00	724.00	12.07	2560.00	26.67	2
PHASE 3	3		0.00		0.00	659.00	10.98	2500.00	26.04	3
PHASE 3	4		0.00		0.00	599.00	9.98			4
PHASE 3	5		0.00		0.00	572.00	9.53			5
PHASE 3	6									6

PHASE 3	7	0.00		0.00	468.00	7.80	2560.00	26.67	7
PHASE 3	8	0.00		0.00					8
PHASE 3	9	0.00		0.00	342.00	5.70			9
PHASE 3	10	0.00		0.00					10
PHASE 3	11	0.00		0.00					11
PHASE 3	12	0.00		0.00	237.00	3.95	2120.00	22.08	12
PHASE 3	13	0.00		0.00					13
PHASE 3	14	0.00		0.00					14
PHASE 3	15	0.00		0.00					15
PHASE 3	16	0.00		0.00	58.00	0.97			16
PHASE 3	28.00	0.00		0.00		0.00	1700.00	17.71	28.00
PHASE 3	REACTOR 32	0.00		0.00		0.00		0.00	REACTOR 32
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	931.10	10.35	0.00	0.00	421.00	7.02	4160.00	43.33	0
PHASE 3	1	333.00	3.70		0.00	708.00	11.80	3900.00	40.63	1
PHASE 3	2	13.00	0.14		0.00	835.00	13.92	2800.00	29.17	2
PHASE 3	3		0.00		0.00	825.00	13.75	2500.00	26.04	3
PHASE 3	4		0.00		0.00	708.00	11.80			4
PHASE 3	5		0.00		0.00	730.00	12.17			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	650.00	10.83	2640.00	27.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	566.00	9.43			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	433.00	7.22	1840.00	19.17	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	307.00	5.12			16
PHASE 3	28.00		0.00		0.00		0.00	1900.00	19.79	28.00
PHASE 3			0.00		0.00				0.00	
PHASE 3	REACTOR 33		0.00		0.00				0.00	REACTOR 33
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	909.90	10.11		0.00	406.00	6.77	4000.00	41.67	0
PHASE 3	1	297.90	3.31	tr	0.00	684.00	11.40	3600.00	37.50	1
PHASE 3	2	28.00	0.31		0.00	873.00	14.55	2900.00	30.21	2
PHASE 3	3		0.00		0.00	777.00	12.95	2600.00	27.08	3
PHASE 3	4		0.00		0.00	834.00	13.90			4
PHASE 3	5		0.00		0.00	733.00	12.22			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	737.00	12.28	2800.00	29.17	7

PHASE 3	8		0.00		0.00				8
PHASE 3	9		0.00		0.00	674.00	11.23		9
PHASE 3	10		0.00		0.00				10
PHASE 3	11		0.00		0.00				11
PHASE 3	12		0.00		0.00	587.00	9.78	2160.00	22.50	12
PHASE 3	13		0.00		0.00				13
PHASE 3	14		0.00		0.00				14
PHASE 3	15		0.00		0.00				15
PHASE 3	16		0.00		0.00	474.00	7.90		16
PHASE 3	28.00		0.00		0.00		0.00	1800.00	18.75	28.00
PHASE 3	REACTOR 34		0.00		0.00		0.00		0.00	REACTOR 34
PHASE 3	DAY	0.00	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	923.10	10.26		0.00	454.00	7.57	4000.00	41.67	0
PHASE 3	1	292.40	3.25	tr	0.00	640.00	10.67	3520.00	36.67	1
PHASE 3	2	32.00	0.36		0.00	949.00	15.82	2700.00	28.13	2
PHASE 3	3		0.00		0.00	871.00	14.52	2640.00	27.50	3
PHASE 3	4		0.00		0.00	822.00	13.70			4
PHASE 3	5		0.00		0.00	861.00	14.35			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	803.00	13.38	2840.00	29.58	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	849.00	14.15			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	818.00	13.63	2000.00	20.83	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00		0.00			15
PHASE 3	16.00		0.00		0.00	806.00	13.43			16.00
PHASE 3	28.00		0.00		0.00		0.00	1800.00	18.75	28.00
PHASE 3	REACTOR 35		0.00		0.00		0.00		0.00	REACTOR 35
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	965.80	10.73		0.00	450.00	7.50	4200.00	43.75	0
PHASE 3	1	315.30	3.50	tr	0.00	772.00	12.87	4040.00	42.08	1
PHASE 3	2	66.00	0.73		0.00	867.00	14.45	3100.00	32.29	2
PHASE 3	3	9.00	0.10		0.00	960.00	16.00	2480.00	25.83	3
PHASE 3	4		0.00		0.00	927.00	15.45			4
PHASE 3	5		0.00		0.00	932.00	15.53			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	957.00	15.95	2940.00	30.63	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	965.00	16.08			9

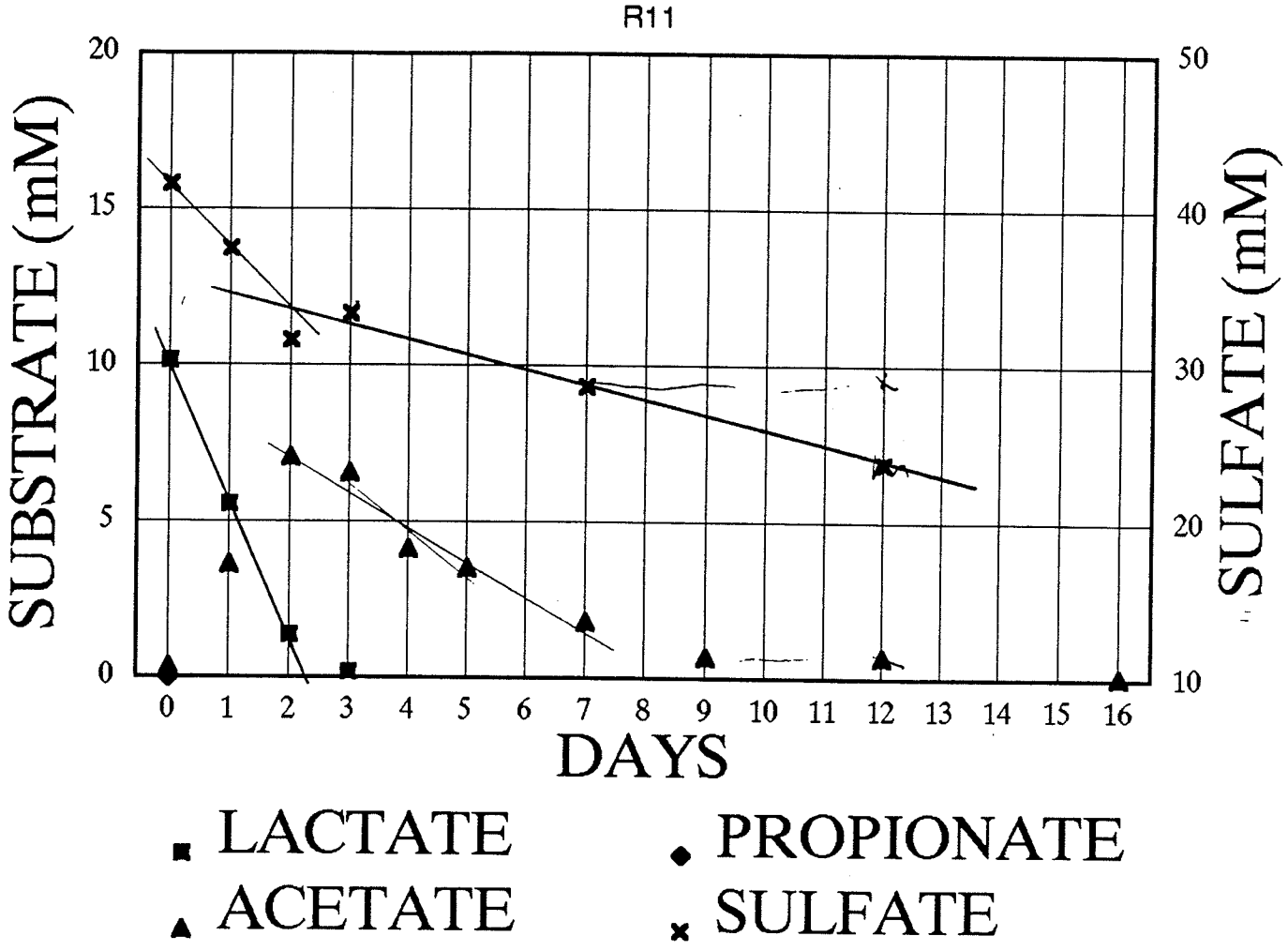
PHASE 3	10		0.00		0.00				10
PHASE 3	11		0.00		0.00				11
PHASE 3	12		0.00		0.00	965.00	16.08	2160.00	22.50	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	963.00	16.05			16
PHASE 3	28.00		0.00		0.00		0.00	2000.00	20.83	28.00
PHASE 3	REACTOR 36		0.00		0.00		0.00			REACTOR 36
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	871.20	9.68	0.00	0.00	369.00	6.15	3840.00	40.00	0
PHASE 3	1	340.60	3.78		0.00	760.00	12.67	3400.00	35.42	1
PHASE 3	2	71.00	0.79		0.00	875.00	14.58	3080.00	32.08	2
PHASE 3	3	2.00	0.02		0.00	963.00	16.05	3040.00	31.67	3
PHASE 3	4		0.00		0.00	931.00	15.52			4
PHASE 3	5		0.00		0.00	921.00	15.35			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	967.00	16.12	3120.00	32.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	1060.00	17.67			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	998.00	16.63	2440.00	25.42	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	1058.00	17.63			16
PHASE 3	28.00		0.00		0.00		0.00	2300.00	23.96	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3	REACTOR 37		0.00		0.00		0.00		0.00	REACTOR 37
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	924.60	10.27	0.00	0.00	428.00	7.13	4120.00	42.92	0
PHASE 3	1	352.60	3.92		0.00	726.00	12.10	3860.00	40.21	1
PHASE 3	2	83.00	0.92		0.00	895.00	14.92	3300.00	34.38	2
PHASE 3	3	5.00	0.06		0.00	995.00	16.58	3000.00	31.25	3
PHASE 3	4		0.00		0.00	950.00	15.83			4
PHASE 3	5		0.00		0.00	941.00	15.68			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	1062.00	17.70	3120.00	32.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	1033.00	17.22			9
PHASE 3	10		0.00		0.00					10

PHASE 3	11	0.00		0.00					11
PHASE 3	12	0.00		0.00	1003.00	16.72	2800.00	29.17	12
PHASE 3	13	0.00		0.00					13
PHASE 3	14	0.00		0.00					14
PHASE 3	15	0.00		0.00		0.00			15
PHASE 3	16	0.00		0.00	1131.00	18.85			16
PHASE 3		0.00		0.00		0.00	2300.00	23.96	28.00
PHASE 3	REACTOR 41	0.00		0.00		0.00		0.00	REACTOR 41
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	855.20	9.50	0.00	0.00	89.00	1.48	3840.00	40.00	0
PHASE 3	1	58.50	0.65		0.00	557.00	9.28	3800.00	39.58	1
PHASE 3	2	5.00	0.06		0.00	574.00	9.57	3200.00	33.33	2
PHASE 3	3		0.00		0.00	544.00	9.07	3100.00	32.29	3
PHASE 3	4		0.00		0.00	431.00	7.18			4
PHASE 3	5		0.00		0.00	369.00	6.15			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	195.00	3.25	3200.00	33.33	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	124.00	2.07			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	88.00	1.47	2500.00	26.04	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16.00		0.00		0.00	140.00	2.33			16.00
PHASE 3	28.00		0.00		0.00		0.00	2400.00	25.00	28.00
PHASE 3	REACTOR 42	0.00		0.00		0.00		0.00	REACTOR 42
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	1012.60	11.25	0.00	0.00	54.00	0.90	4200.00	43.75	0
PHASE 3	1	76.10	0.85		0.00	673.00	11.22	3700.00	38.54	1
PHASE 3	2	5.00	0.06		0.00	679.00	11.32	3500.00	36.46	2
PHASE 3	3		0.00		0.00	640.00	10.67	3240.00	33.75	3
PHASE 3	4		0.00		0.00	589.00	9.82			4
PHASE 3	5		0.00		0.00	516.00	8.60			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	485.00	8.08	3200.00	33.33	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	388.00	6.47			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	324.00	5.40	2120.00	22.08	12

PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	188.00	3.13			16
PHASE 3	28.00		0.00		0.00		0.00	2400.00	25.00	28.00
PHASE 3	REACTOR 43		0.00		0.00		0.00		0.00	REACTOR 43
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	1022.40	11.36	0.00	0.00	43.00	0.72	4600.00	47.92	0
PHASE 3	1	63.00	0.70		0.00	668.00	11.13	3760.00	39.17	1
PHASE 3	2	4.00	0.04		0.00	709.00	11.82	3360.00	35.00	2
PHASE 3	3		0.00		0.00	700.00	11.67	3500.00	36.46	3
PHASE 3	4		0.00		0.00	681.00	11.35			4
PHASE 3	5		0.00		0.00	706.00	11.77			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	715.00	11.92	3600.00	37.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	708.00	11.80			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	713.00	11.88	2200.00	22.92	12
PHASE 3	13		0.00		0.00					13
PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	682.00	11.37			16
PHASE 3	28.00		0.00		0.00		0.00	2400.00	25.00	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3	REACTOR 44		0.00		0.00		0.00		0.00	REACTOR 44
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	968.60	10.76	0.00	0.00	28.00	0.47	4280.00	44.58	0
PHASE 3	1	127.00	1.41		0.00	640.00	10.67	3760.00	39.17	1
PHASE 3	2	3.00	0.03		0.00	732.00	12.20	3600.00	37.50	2
PHASE 3	3		0.00	tr	0.00	762.00	12.70	3640.00	37.92	3
PHASE 3	4		0.00		0.00	749.00	12.48			4
PHASE 3	5		0.00		0.00	781.00	13.02			5
PHASE 3	6									6
PHASE 3	7		0.00		0.00	753.00	12.55	3600.00	37.50	7
PHASE 3	8		0.00		0.00					8
PHASE 3	9		0.00		0.00	724.00	12.07			9
PHASE 3	10		0.00		0.00					10
PHASE 3	11		0.00		0.00					11
PHASE 3	12		0.00		0.00	767.00	12.78	2840.00	29.58	12
PHASE 3	13		0.00		0.00					13

PHASE 3	14		0.00		0.00					14
PHASE 3	15		0.00		0.00		0.00			15
PHASE 3	16		0.00		0.00	792.00	13.20			16
PHASE 3	28.00		0.00		0.00		0.00	2700.00	28.13	28.00
PHASE 3	REACTOR 45		0.00		0.00		0.00		0.00	REACTOR 45
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	1020.90	11.34		0.00	9.00	0.15	4380.00	45.63	0
PHASE 3	1	701.00	7.79		0.00	232.00	3.87	4000.00	41.67	1
PHASE 3	2	728.00	8.09		0.00	320.00	5.33	3900.00	40.63	2
PHASE 3	3	540.00	6.00	tr	0.00	374.00	6.23	3800.00	39.58	3
PHASE 3	4			tr	0.00	520.00	8.67	3600.00	37.50	4
PHASE 3	5	239.00	2.66	2.00	0.03	531.00	8.85			5
PHASE 3	6									6
PHASE 3	7	115.00	1.28	5.00	0.07	648.00	10.80	3600.00	37.50	7
PHASE 3	8									8
PHASE 3	9	3.00	0.03	5.00	0.07	720.00	12.00			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12		0.00	8.00	0.11	741.00	12.35	2680.00	27.92	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15
PHASE 3	16.00		0.00		0.00	794.00	13.23			16.00
PHASE 3	28.00		0.00		0.00		0.00	3000.00	31.25	28.00
PHASE 3	REACTOR 46		0.00		0.00		0.00		0.00	REACTOR 46
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	941.20	10.46		0.00	12.00	0.20	4300.00	44.79	0
PHASE 3	1	932.00	10.36		0.00	115.00	1.92	4300.00	44.79	1
PHASE 3	2	1008.00	11.20	tr	0.00	130.00	2.17	4160.00	43.33	2
PHASE 3	3	922.00	10.24	tr	0.00	177.00	2.95	3800.00	39.58	3
PHASE 3	4									4
PHASE 3	5	824.00	9.16	2.00	0.03	240.00	4.00			5
PHASE 3	6									6
PHASE 3	7	807.00	8.97	6.00	0.08	261.00	4.35	4100.00	42.71	7
PHASE 3	8									8
PHASE 3	9	753.00	8.37	7.00	0.09	302.00	5.03			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12	682.00	7.58	10.00	0.14	356.00	5.93	3500.00	36.46	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15

PHASE 3	16		0.00		0.00	438.00	7.30			16
PHASE 3	28.00		0.00		0.00		0.00	3200.00	33.33	28.00
PHASE 3	REACTOR 47		0.00		0.00		0.00		0.00	REACTOR 47
PHASE 3	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	DAY
PHASE 3		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	
PHASE 3	0	964.30	10.71	0.00	0.00	16.00	0.27	4600.00	47.92	0
PHASE 3	1	938.00	10.42	0.00	0.00	85.00	1.42	4400.00	45.83	1
PHASE 3	2	974.00	10.82	tr	0.00	97.00	1.62	4200.00	43.75	2
PHASE 3	3	966.00	10.73	tr	0.00	125.00	2.08	3600.00	37.50	3
PHASE 3	4									4
PHASE 3	5	941.00	10.46	3.00	0.04	147.00	2.45			5
PHASE 3	6									6
PHASE 3	7	855.00	9.50	7.00	0.09	188.00	3.13	4400.00	45.83	7
PHASE 3	8									8
PHASE 3	9	938.00	10.42	9.00	0.12	209.00	3.48			9
PHASE 3	10									10
PHASE 3	11									11
PHASE 3	12	811.00	9.01	12.00	0.16	234.00	3.90	3320.00	34.58	12
PHASE 3	13									13
PHASE 3	14									14
PHASE 3	15		0.00		0.00					15
PHASE 3	16		0.00		0.00	292.00	4.87			16
PHASE 3	28.00		0.00		0.00		0.00	3400.00	35.42	28.00
PHASE 3			0.00		0.00		0.00		0.00	
PHASE 3										



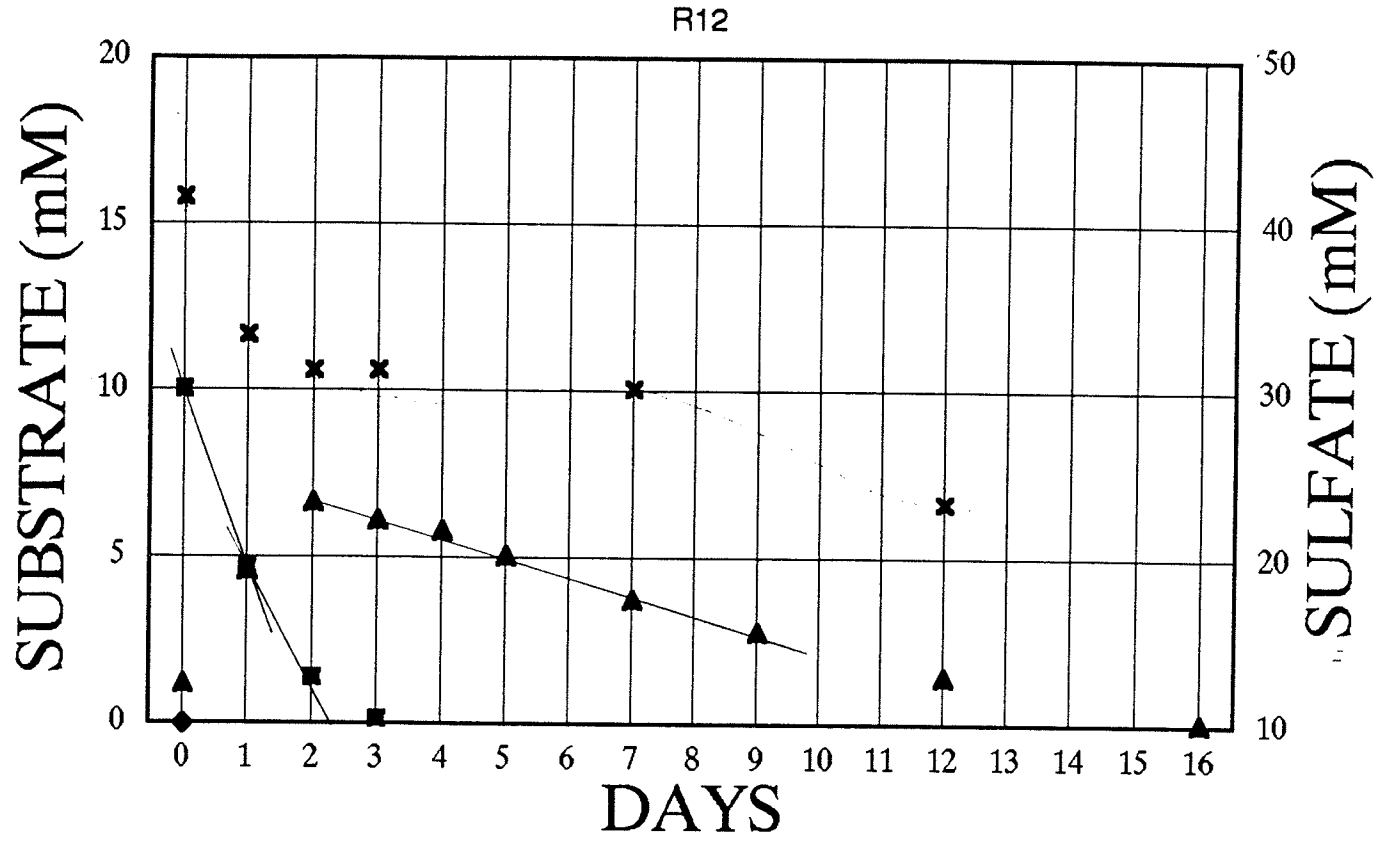
MIS = after the degraded

2590

48 mL CH_4

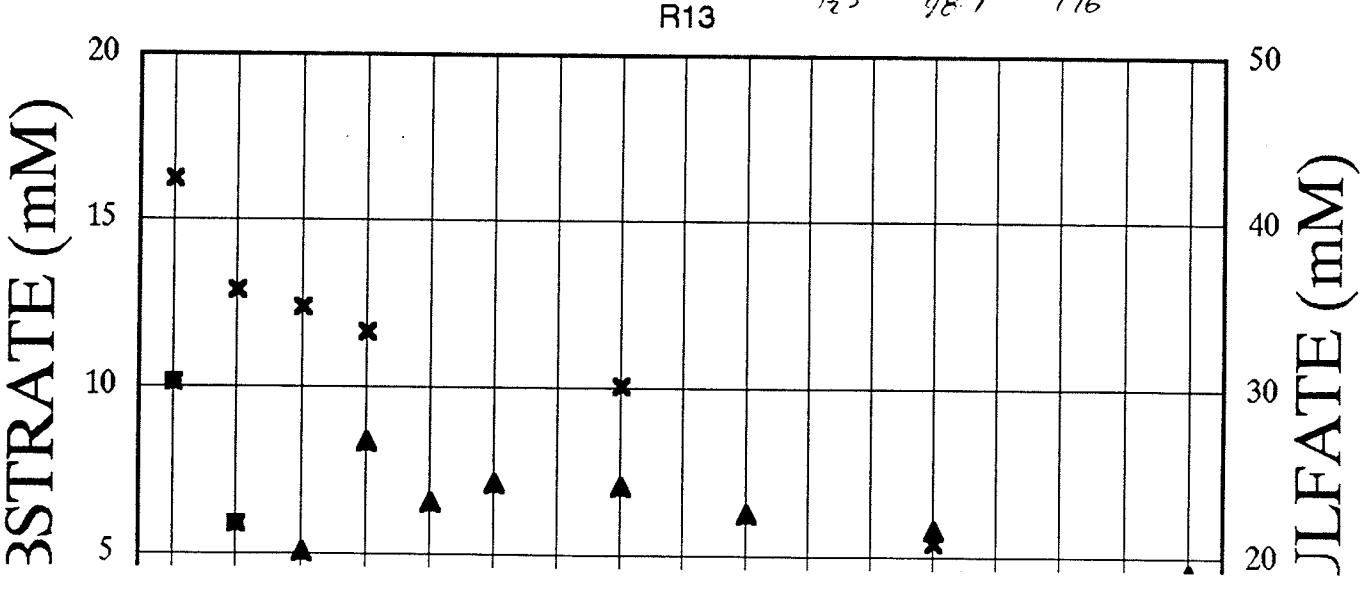
	T	MS
pH	7.0	7.4
TS	180	680
H_2S	18.9	161

$\frac{dS_x}{dt}$ =



3.8 mL CH4

pH	7.0	7.4
TS	225	740
H2S	98.7	176

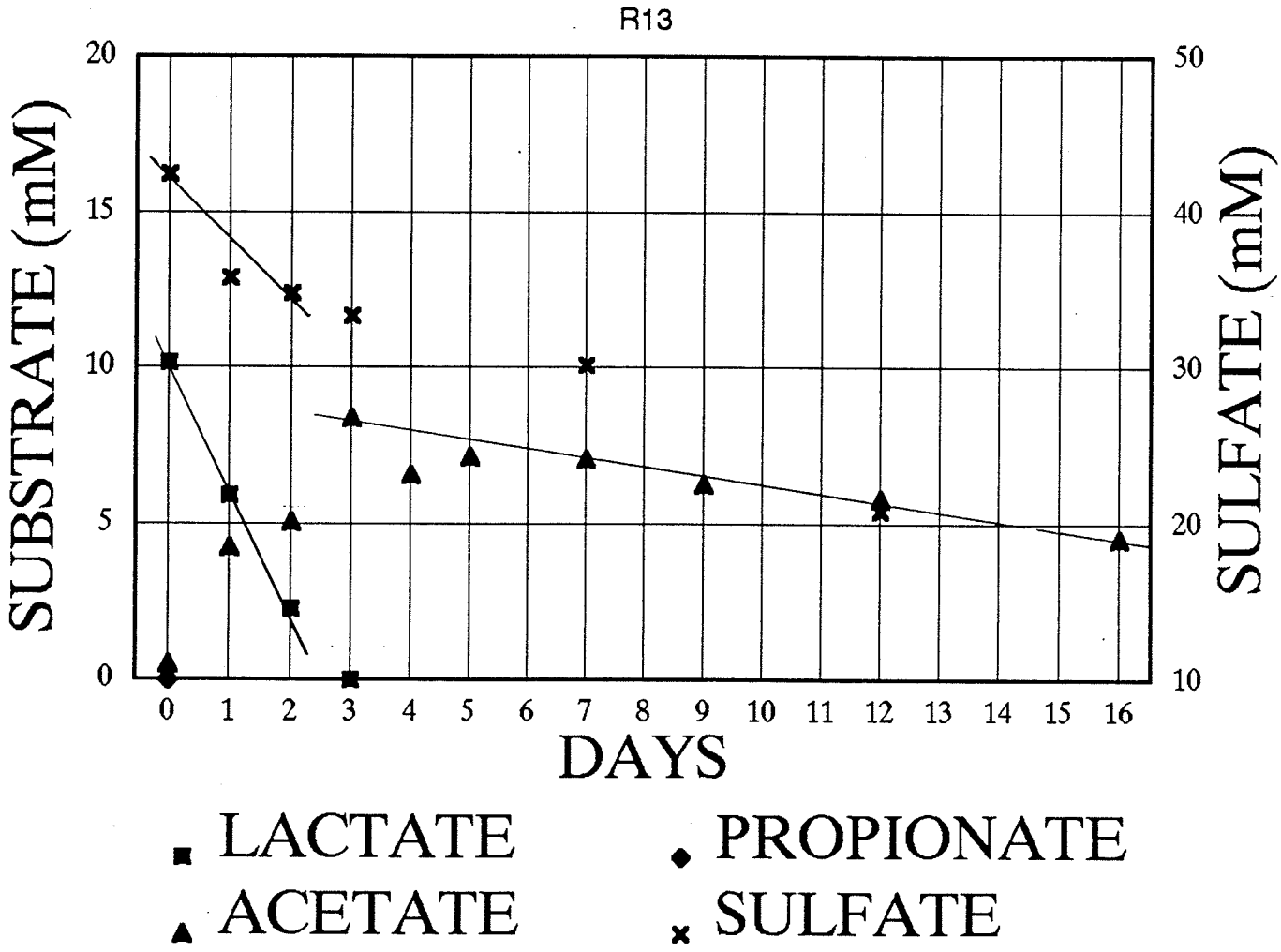


pH 7 7.3

TS 275 760

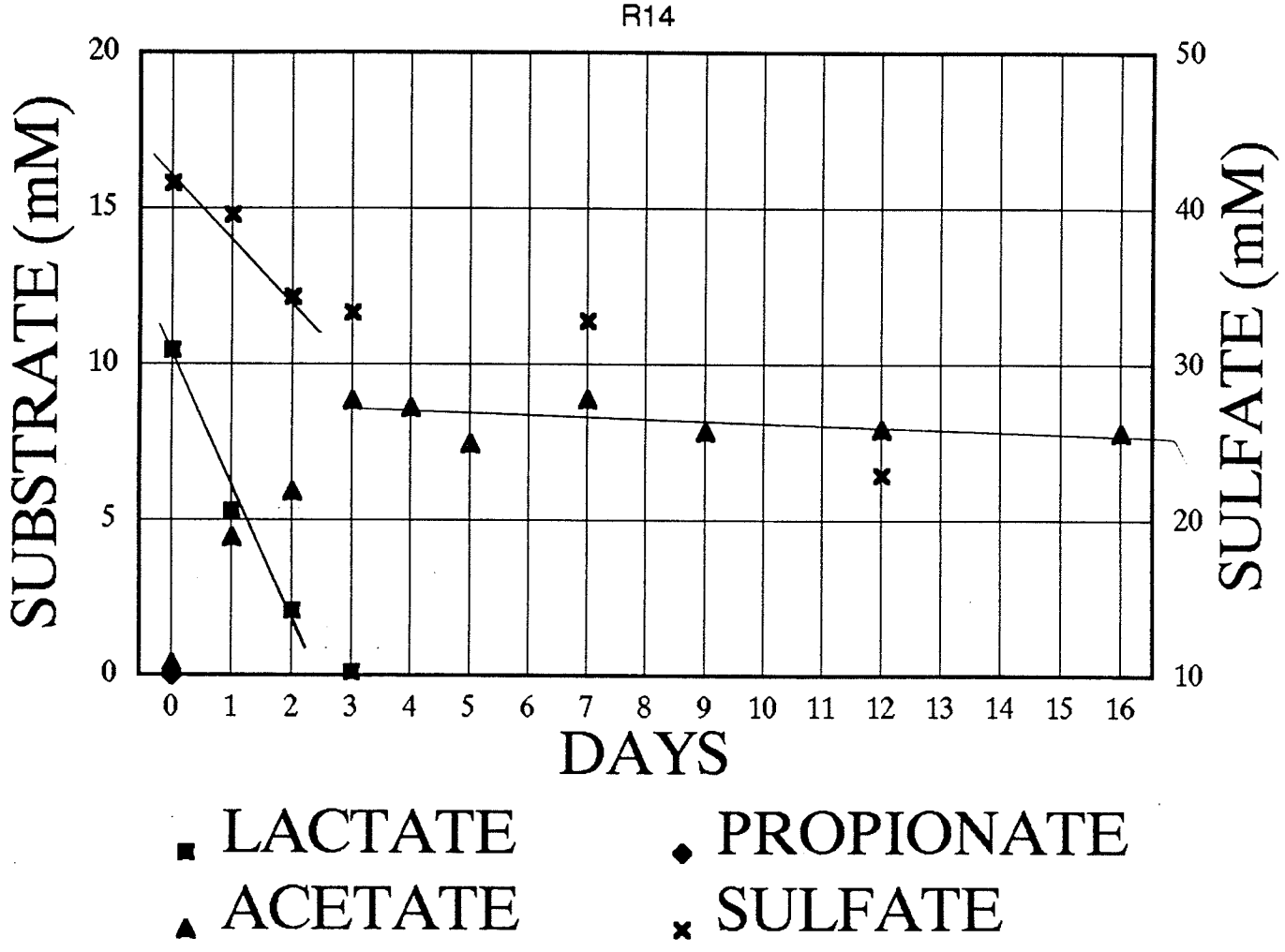
H_2S 121 214

40 mL CH4



pH	7	7.4
H_2	330	770
H_2S	145	183

1.3 mL CH_4

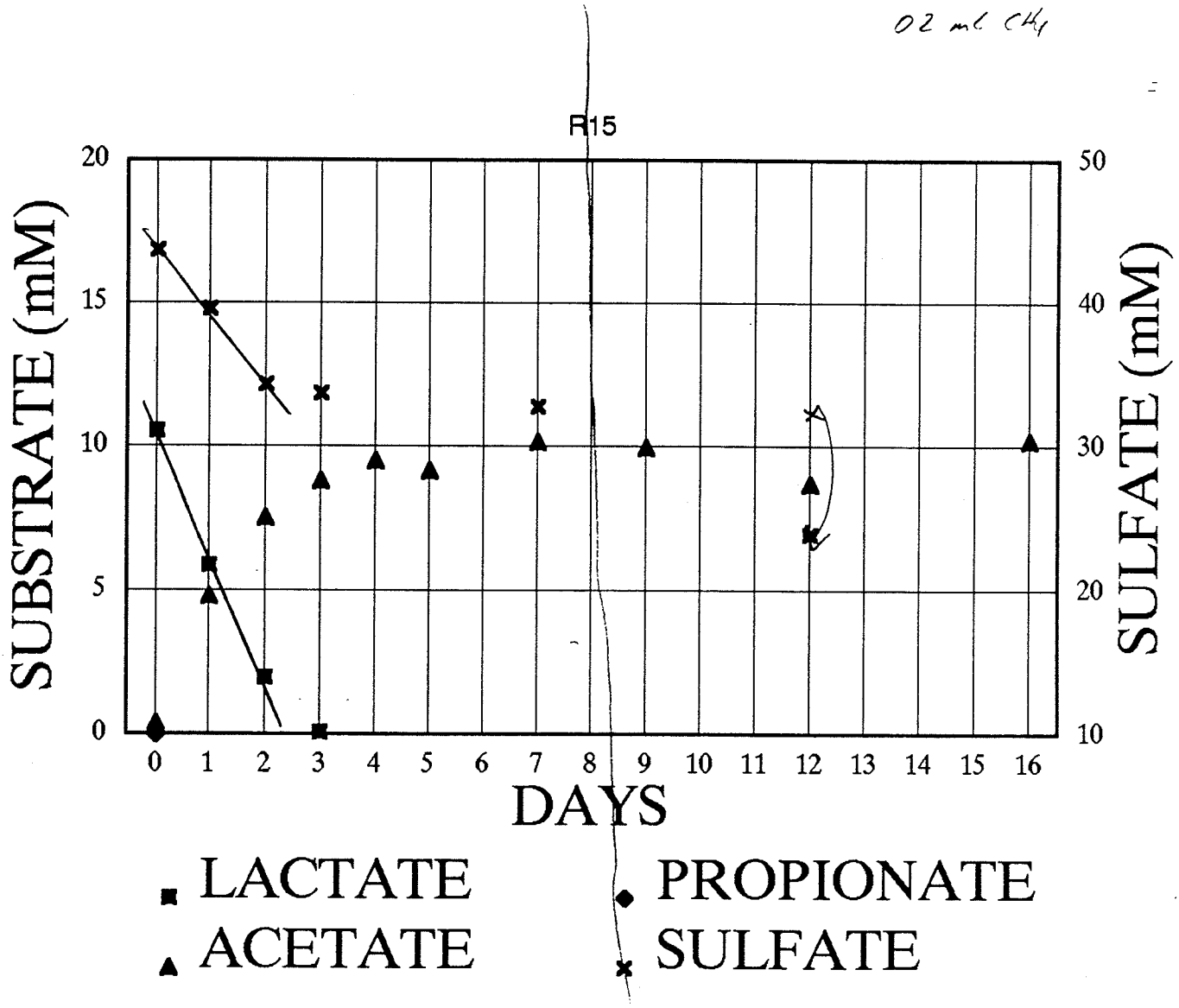


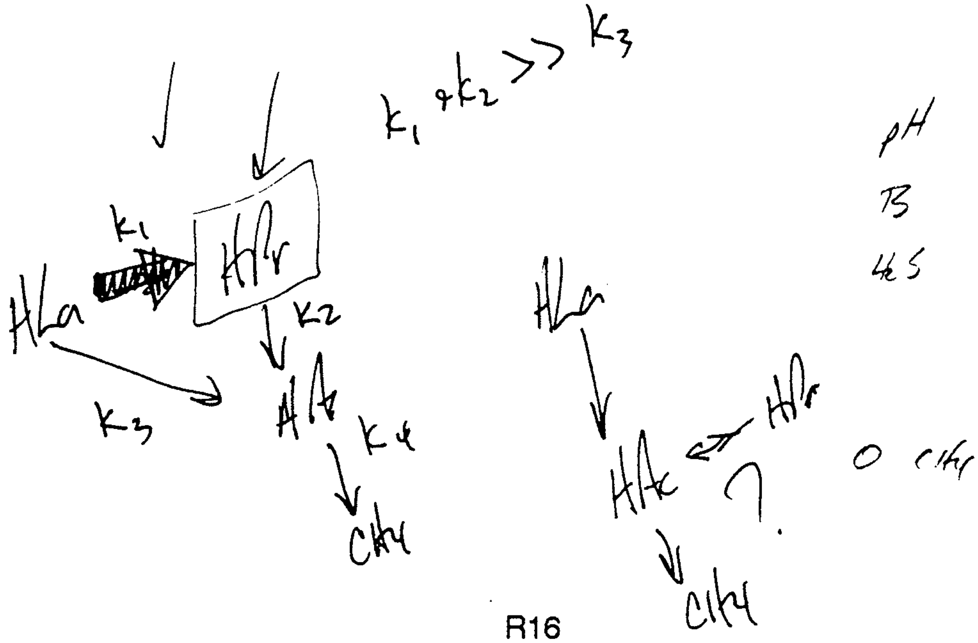
pH 7.0 7.3

TB 425 [illegible]

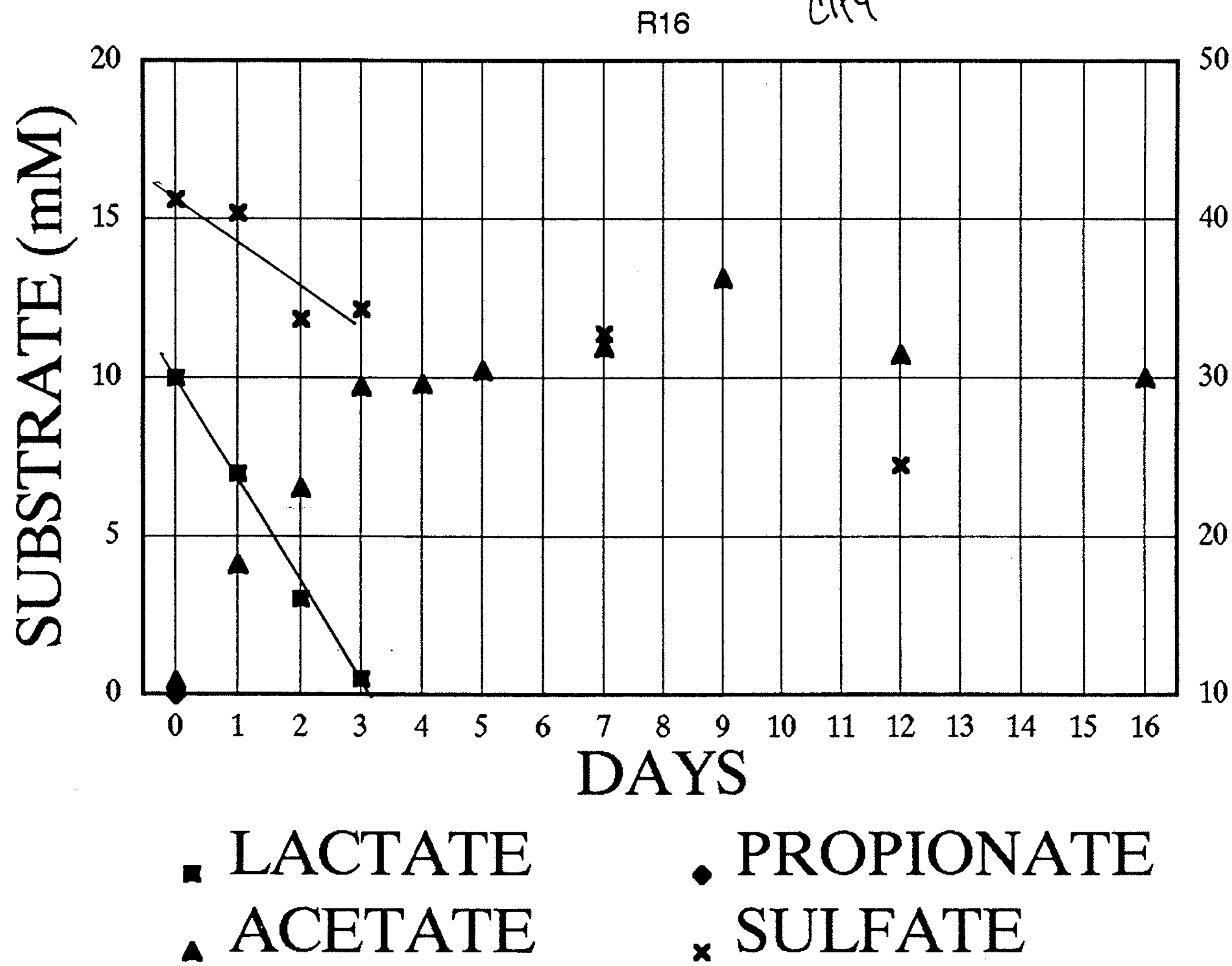
H2S 186 248

0.2 mL CH4





pH	70	74
B	560	[illegible]
H_2S	228	237

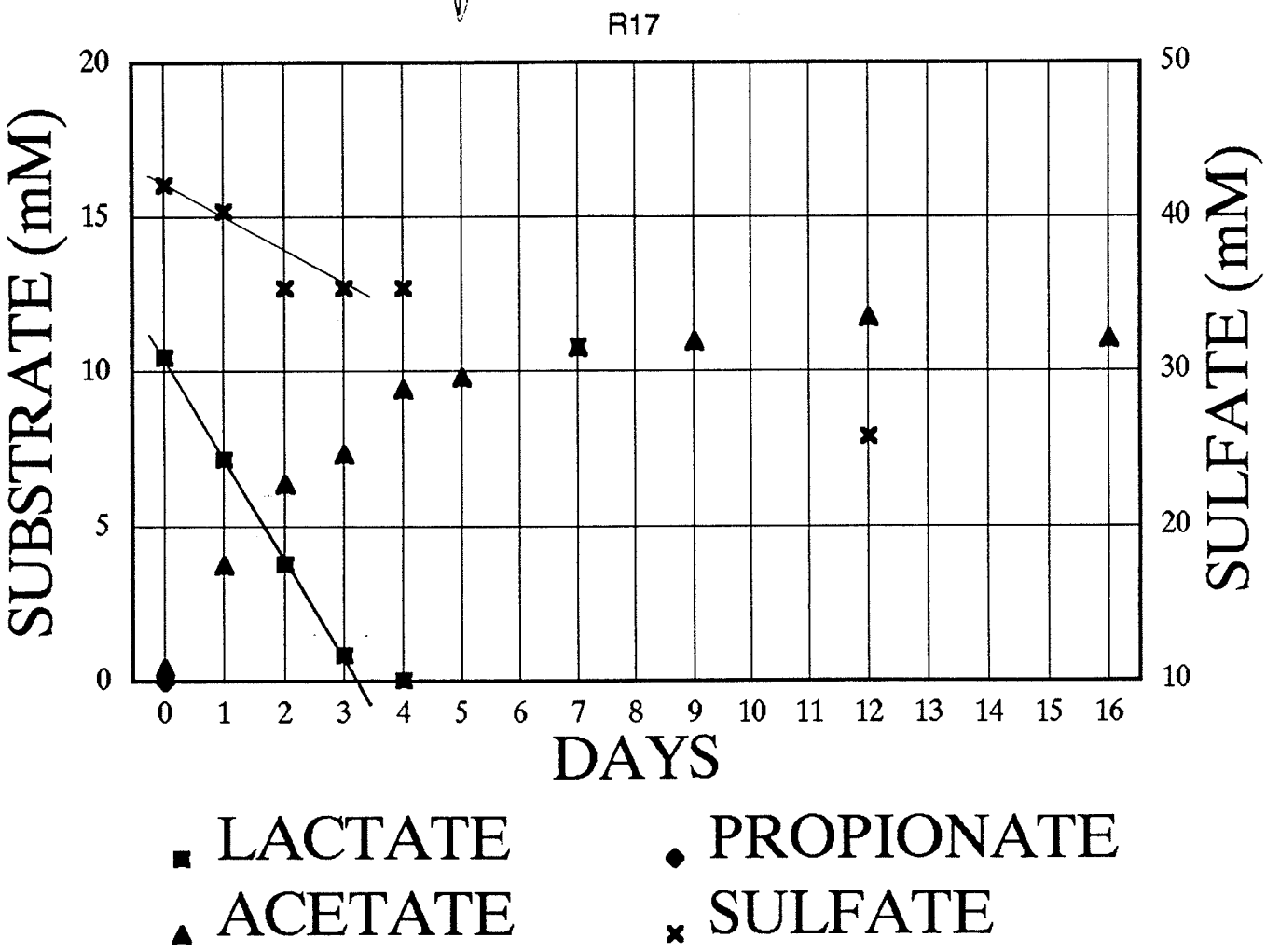


pH 7 7.4

TS 600 1100

VS 263 261

OCHA

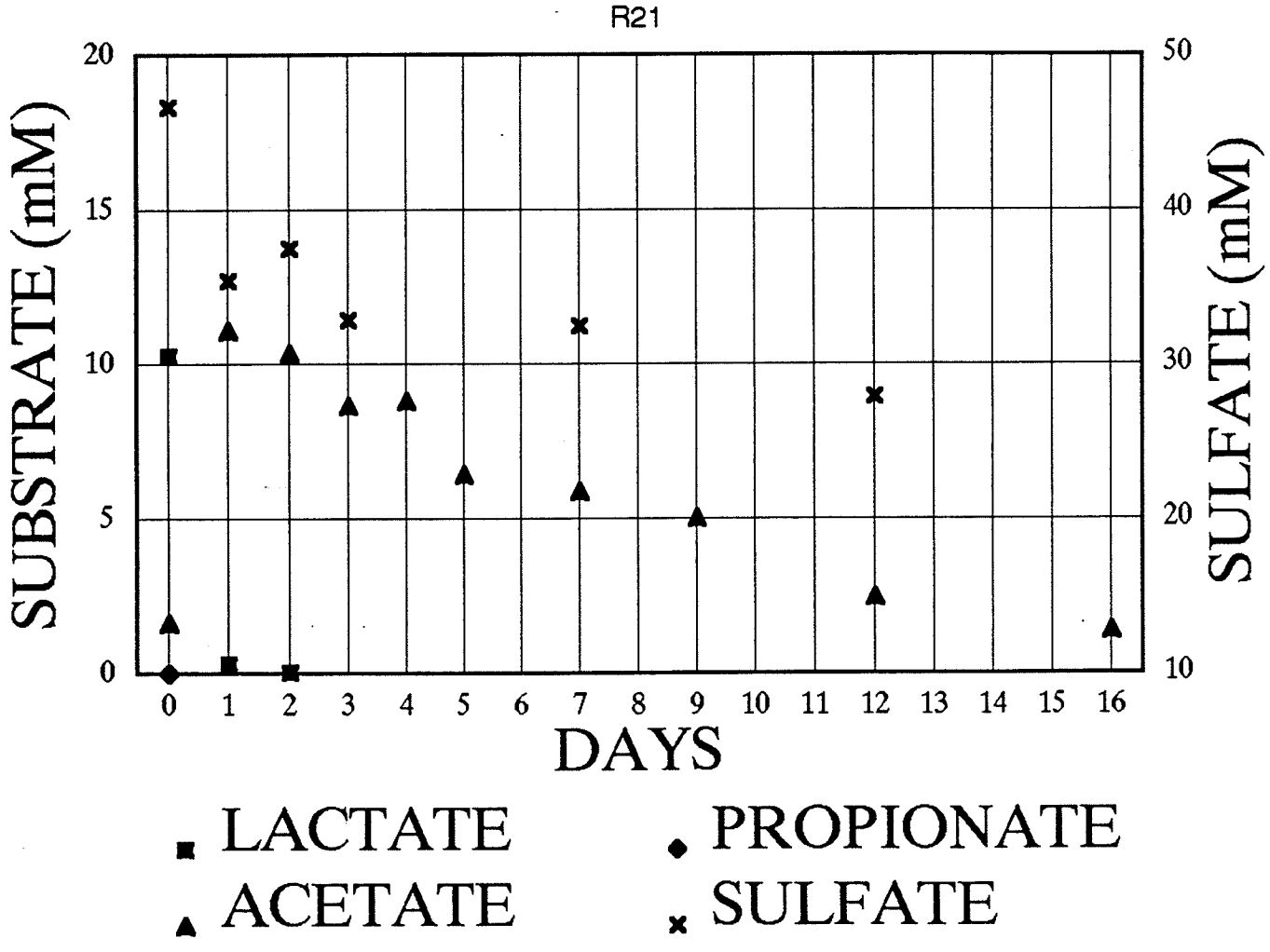


pH 8.0 8.0

TS 790 1350

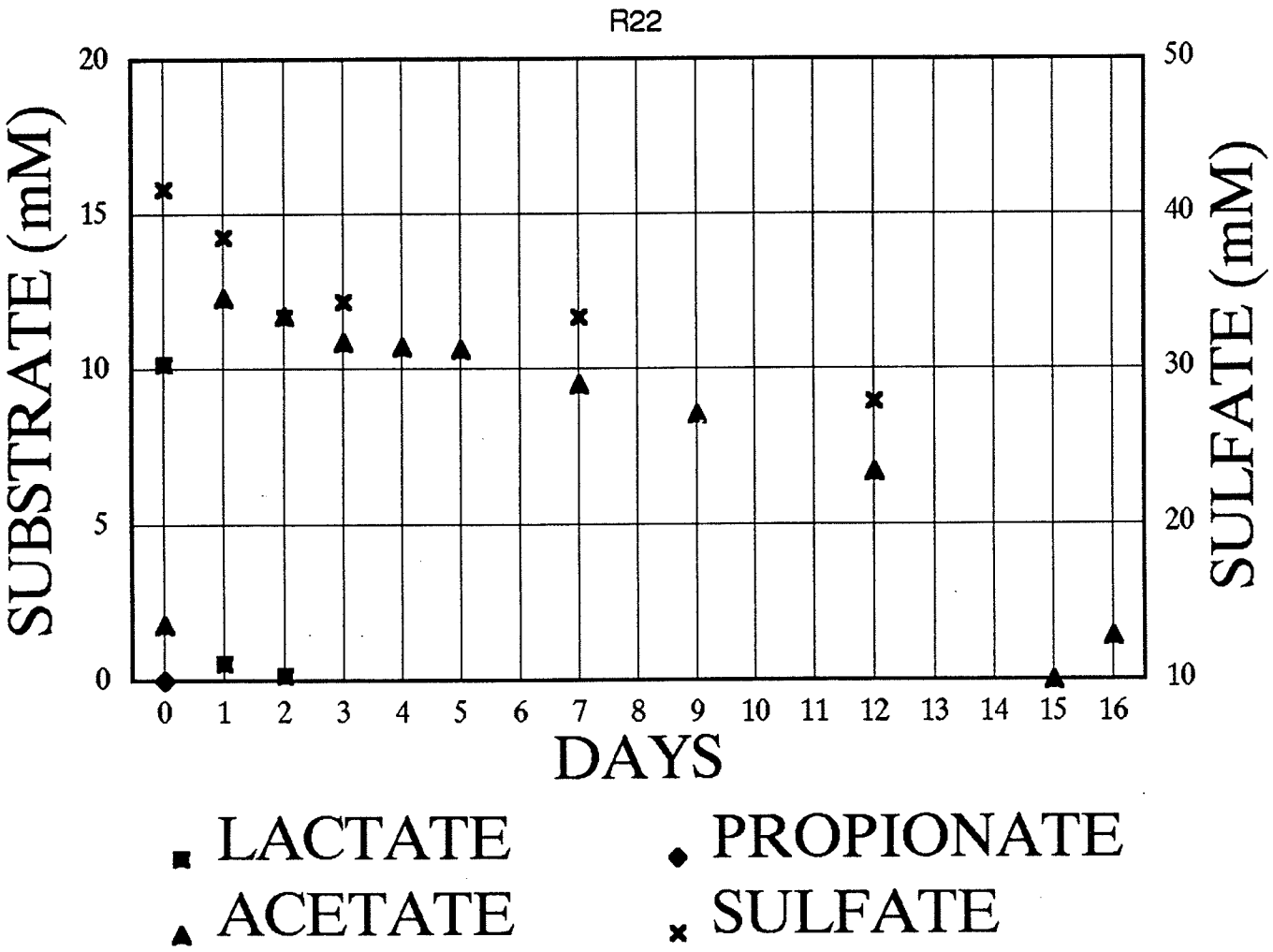
H_2S 57.2 97.8

5.9 mL CH_4



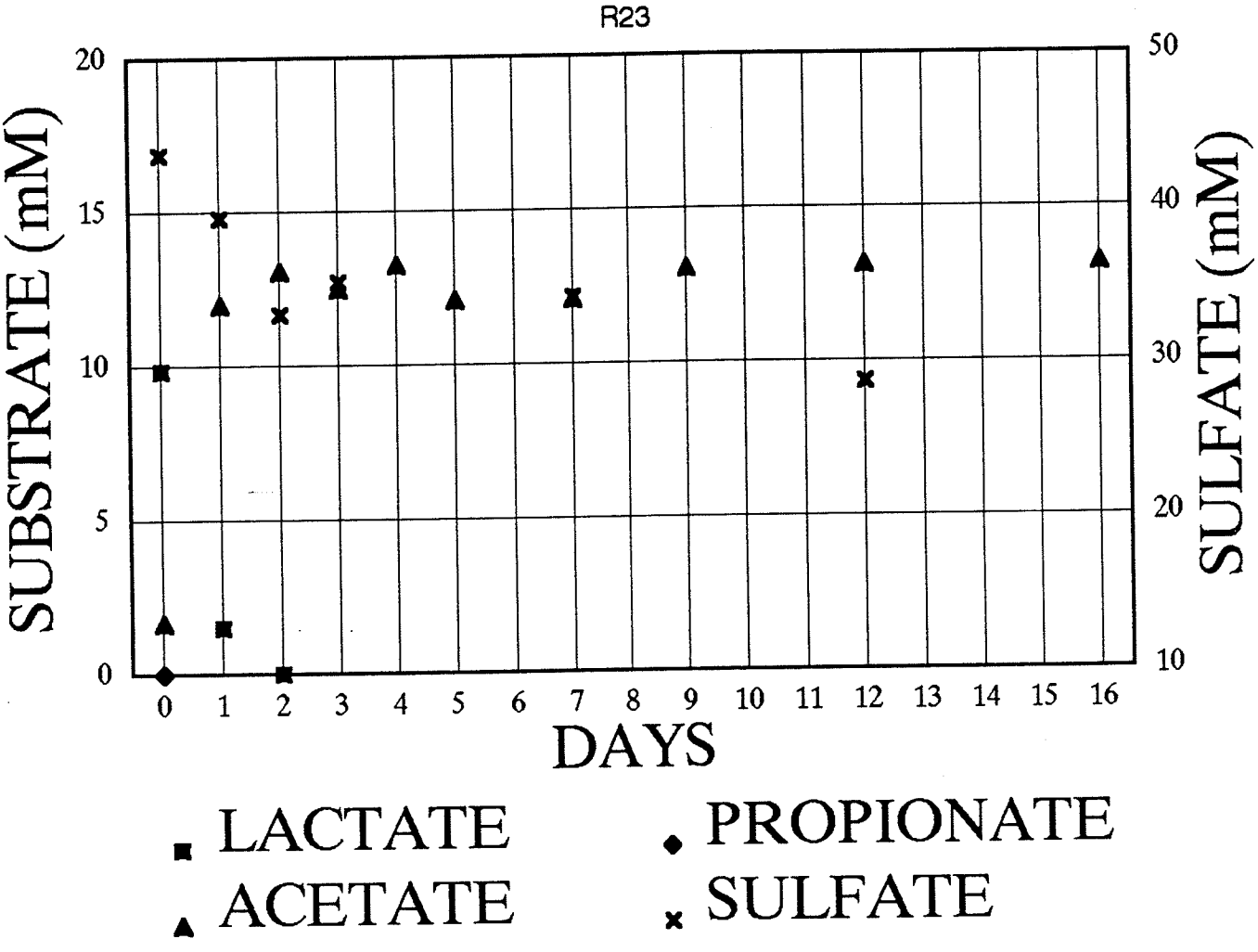
8. 81
1100 1630
79.7 95.2

4.0 mL CH4



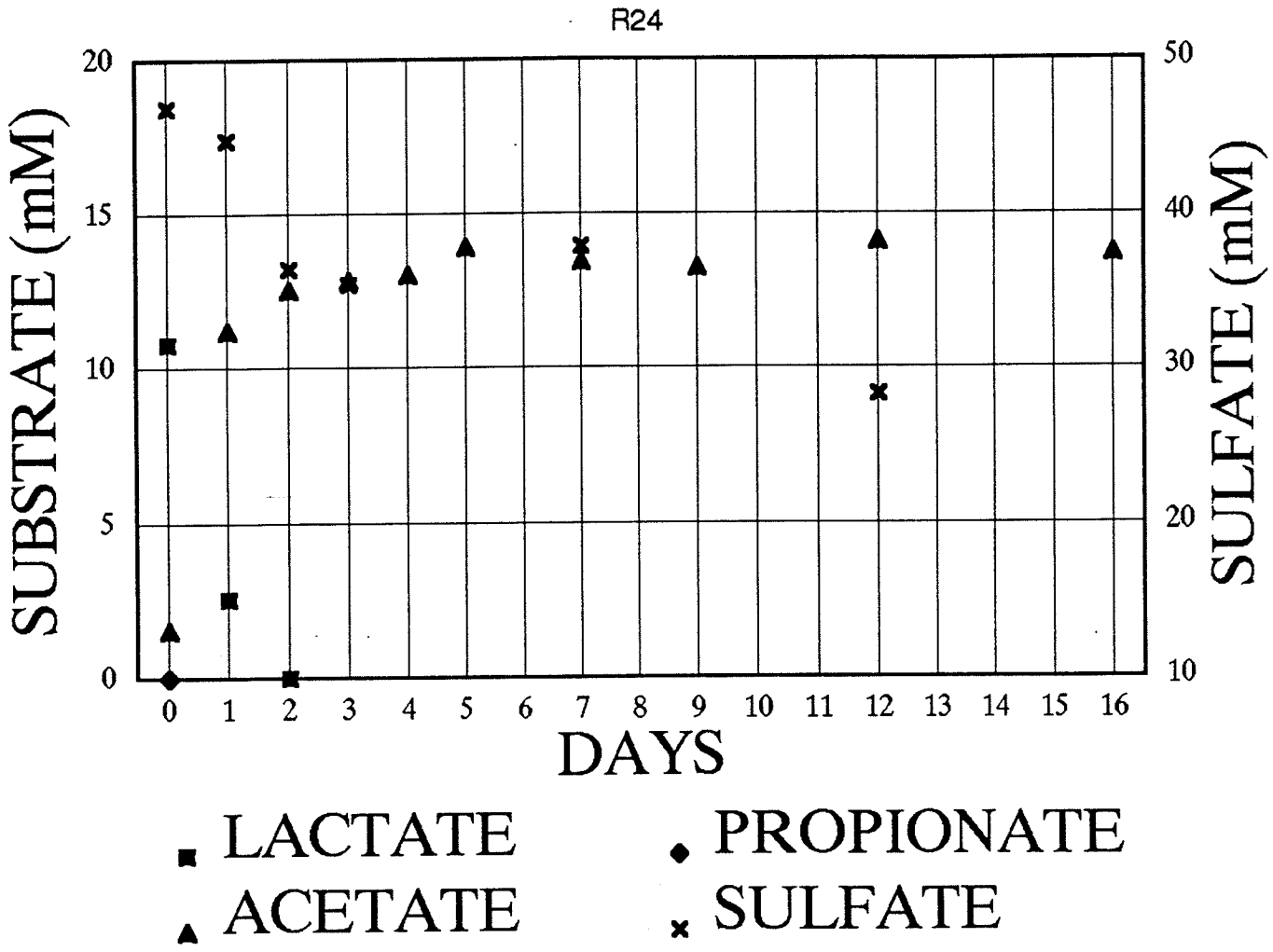
8 8
1370 1970
99.3 143

0.1 mL ClH



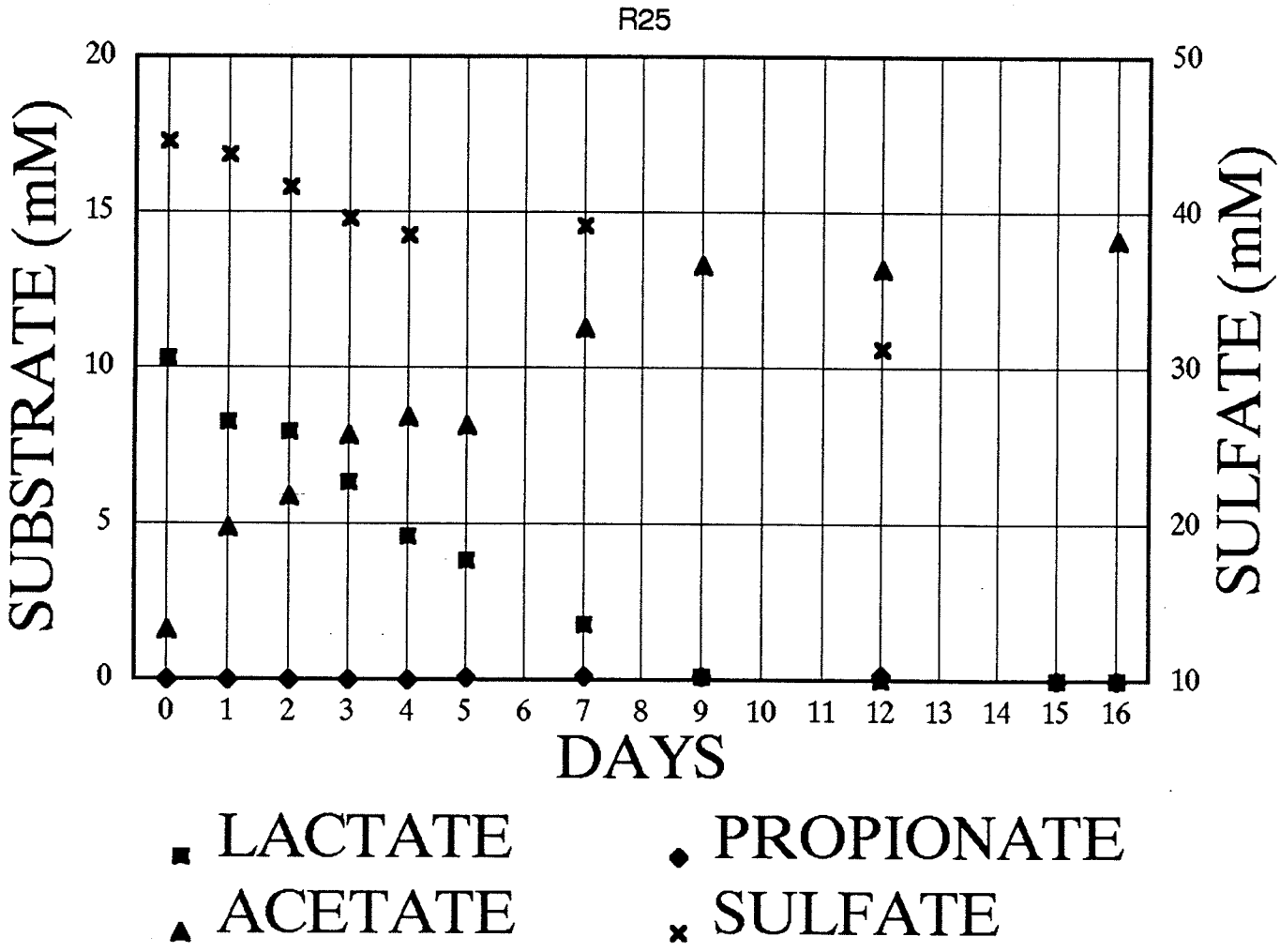
B B
179m 2420
130 175

0 CH4



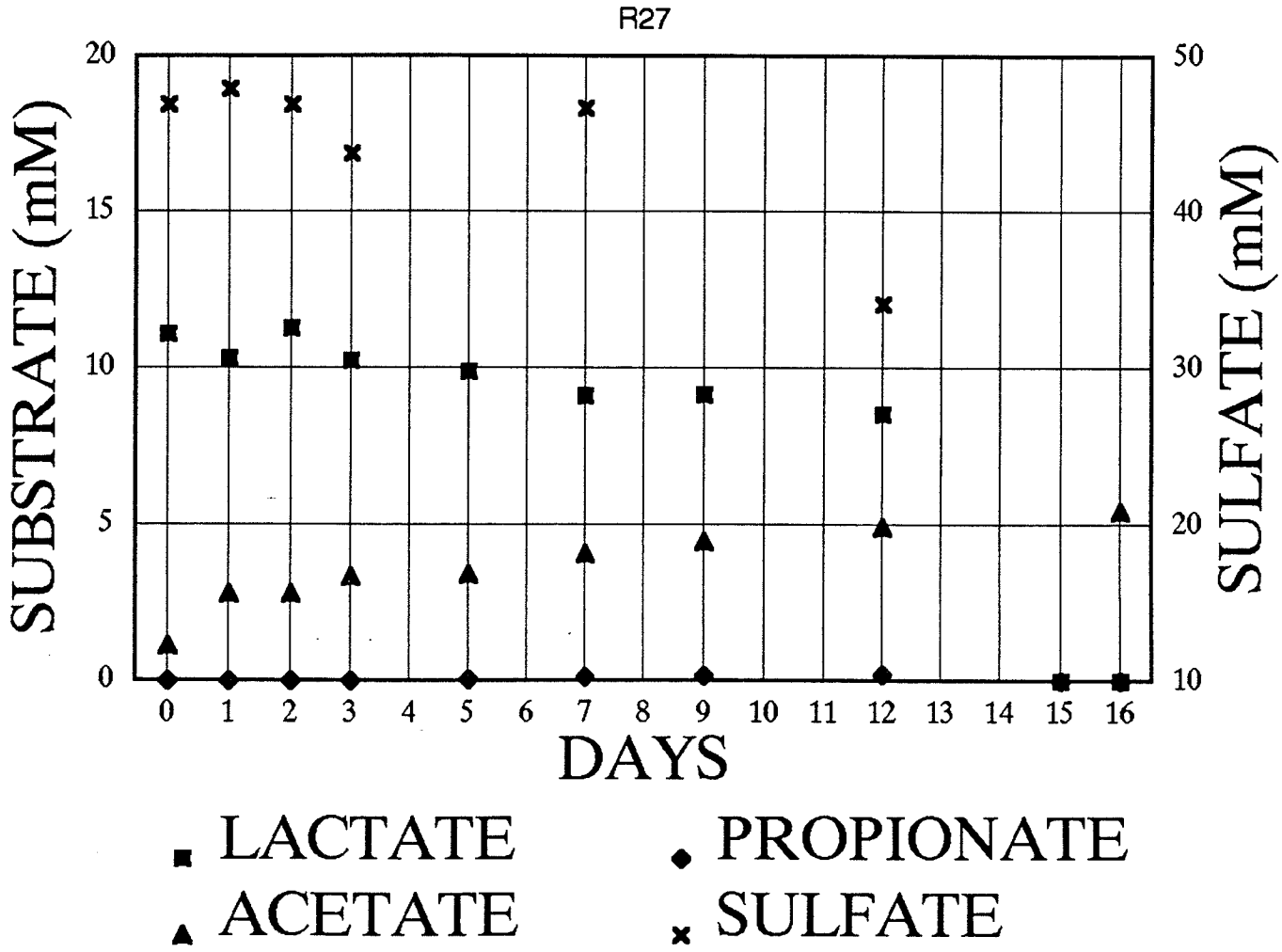
8 8
2345 2880
170 209

0 CH4



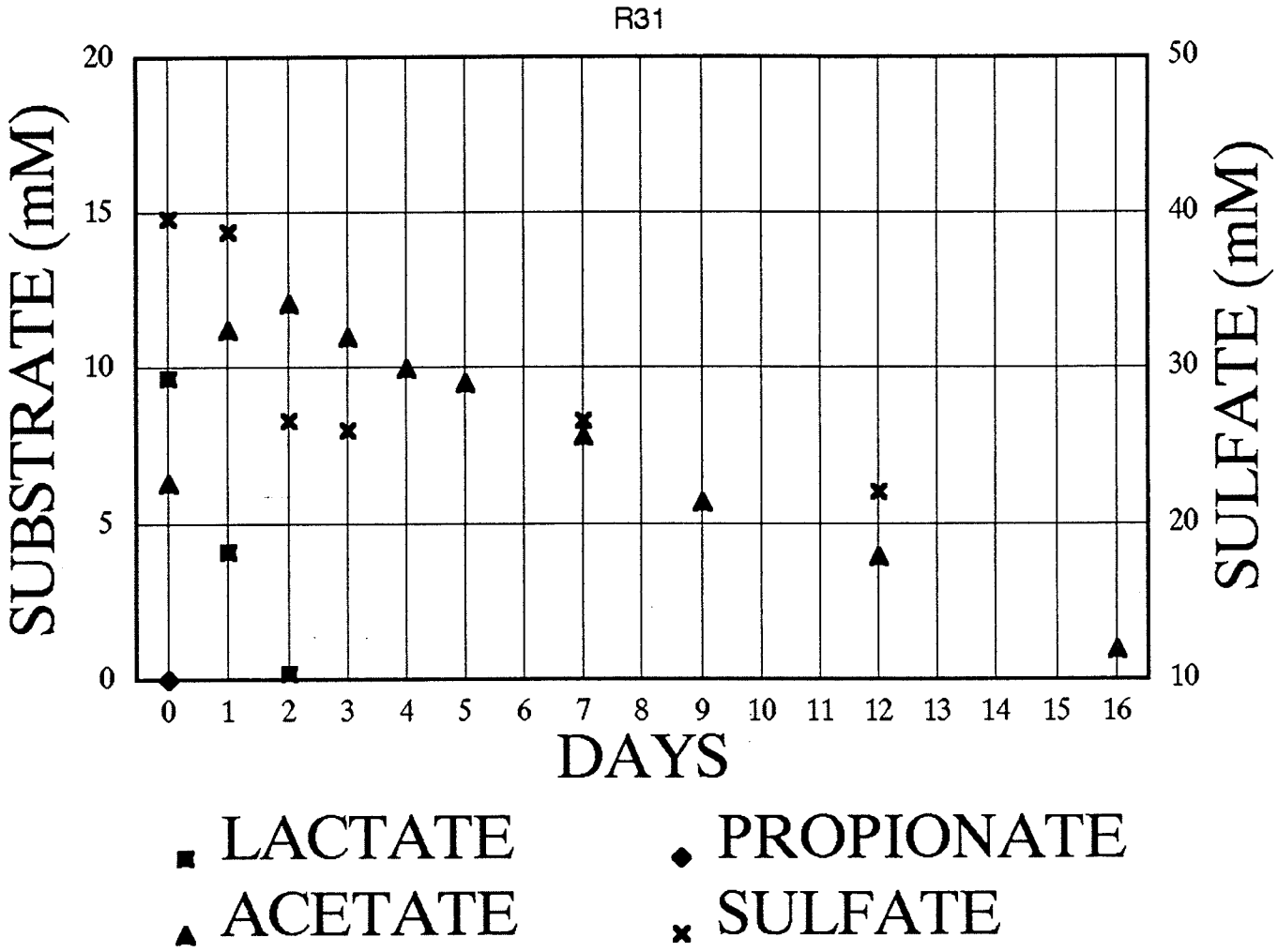
8 8·1
3450 3460
250 208

0 CH4



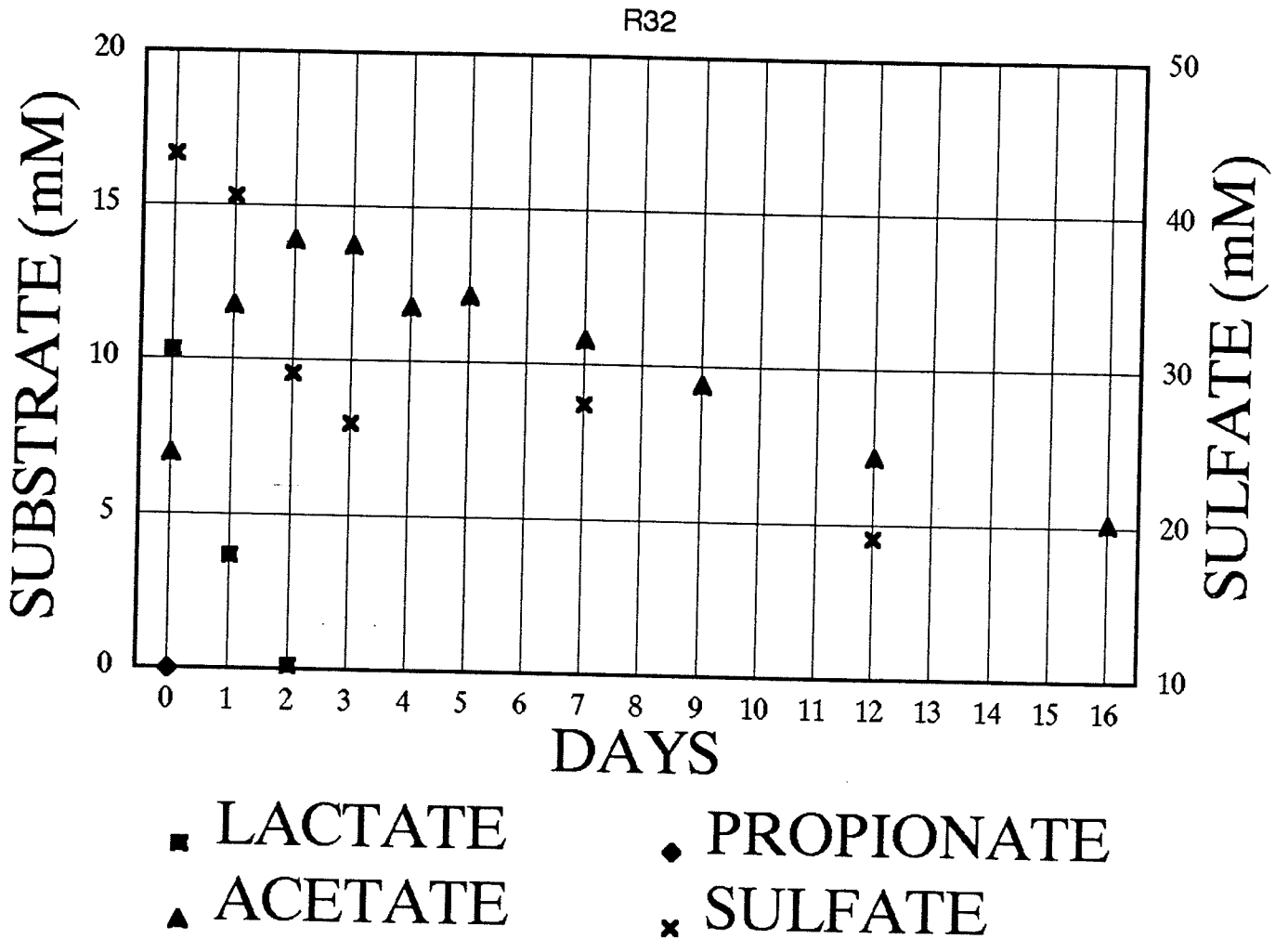
71 7.3
120 620
72.8 174

6.8 mL CH_4



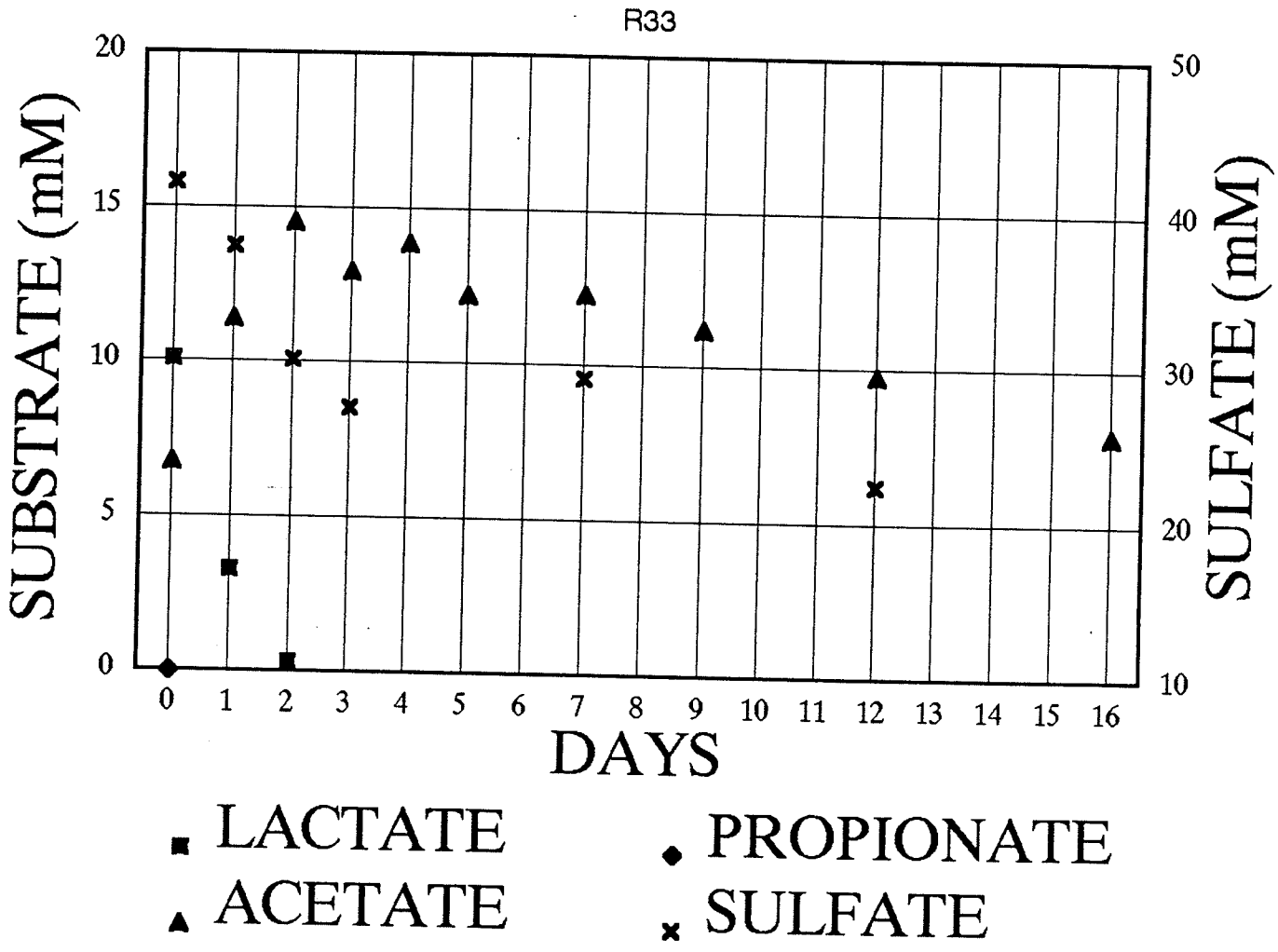
7. 7.5
21.5 72.0
94 208

7.7 mL CH4



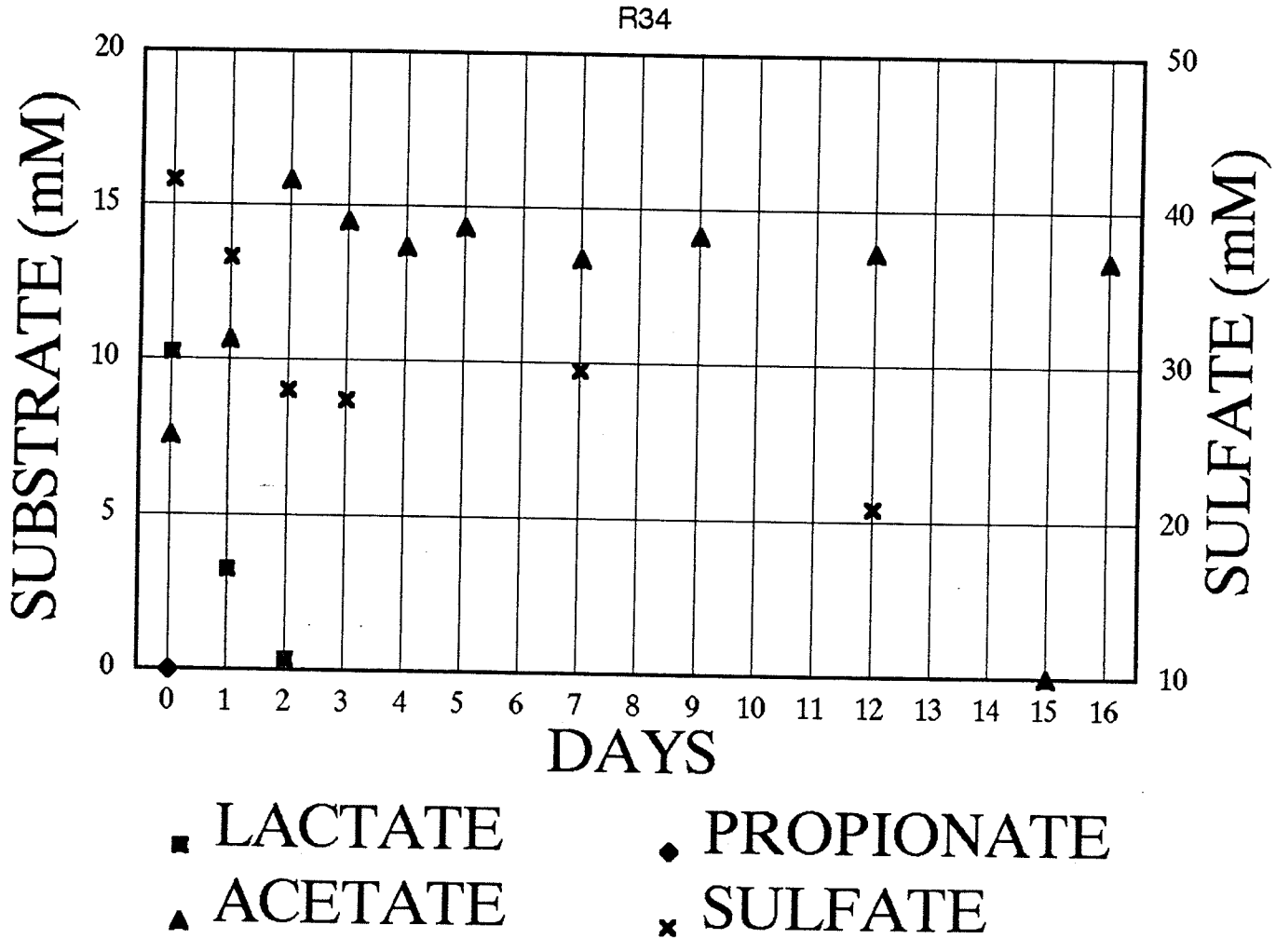
7. 75
265 750
116 211

74 mL Eq

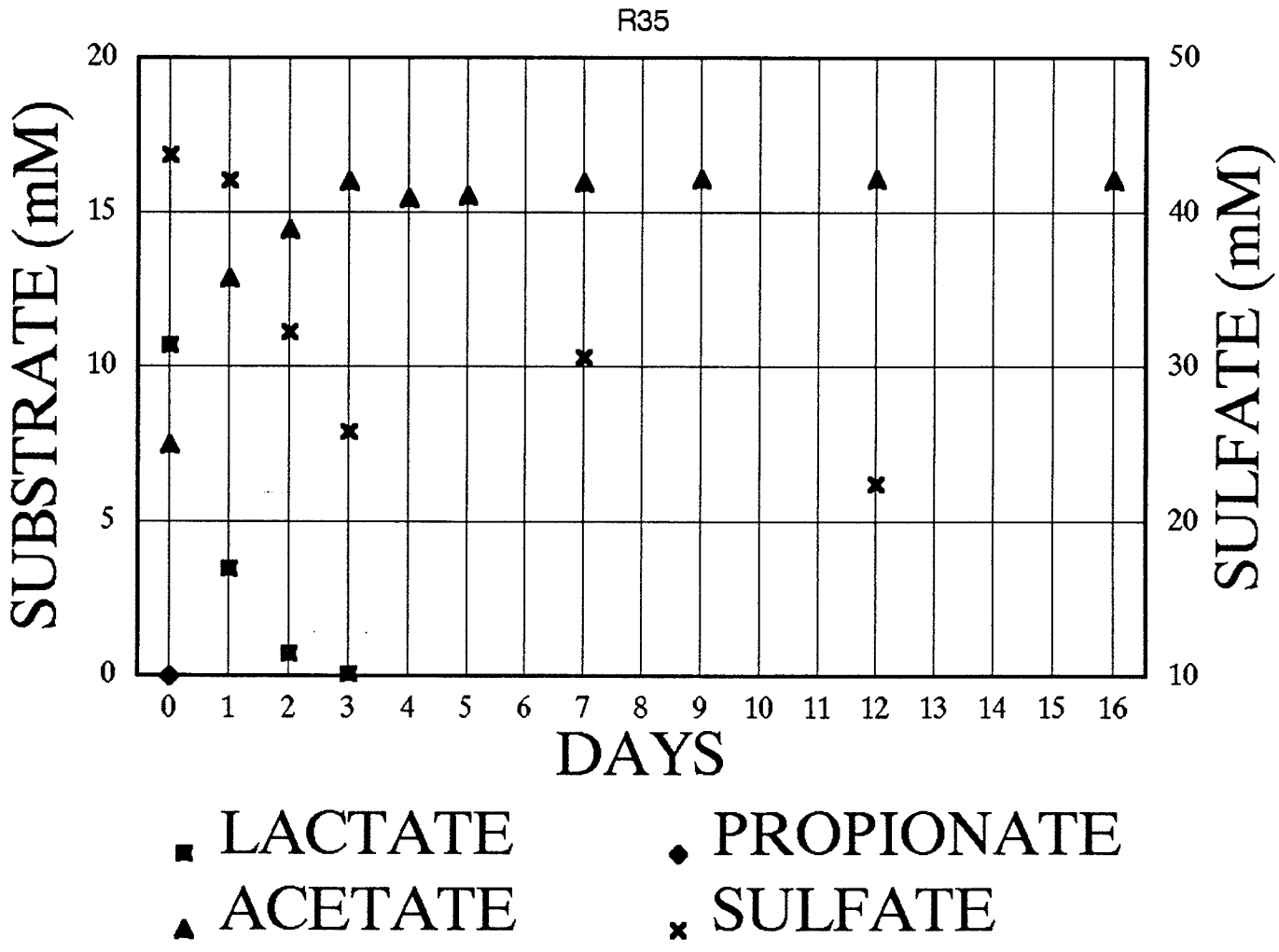


71 73
310 820
119 231

12 ml CH4

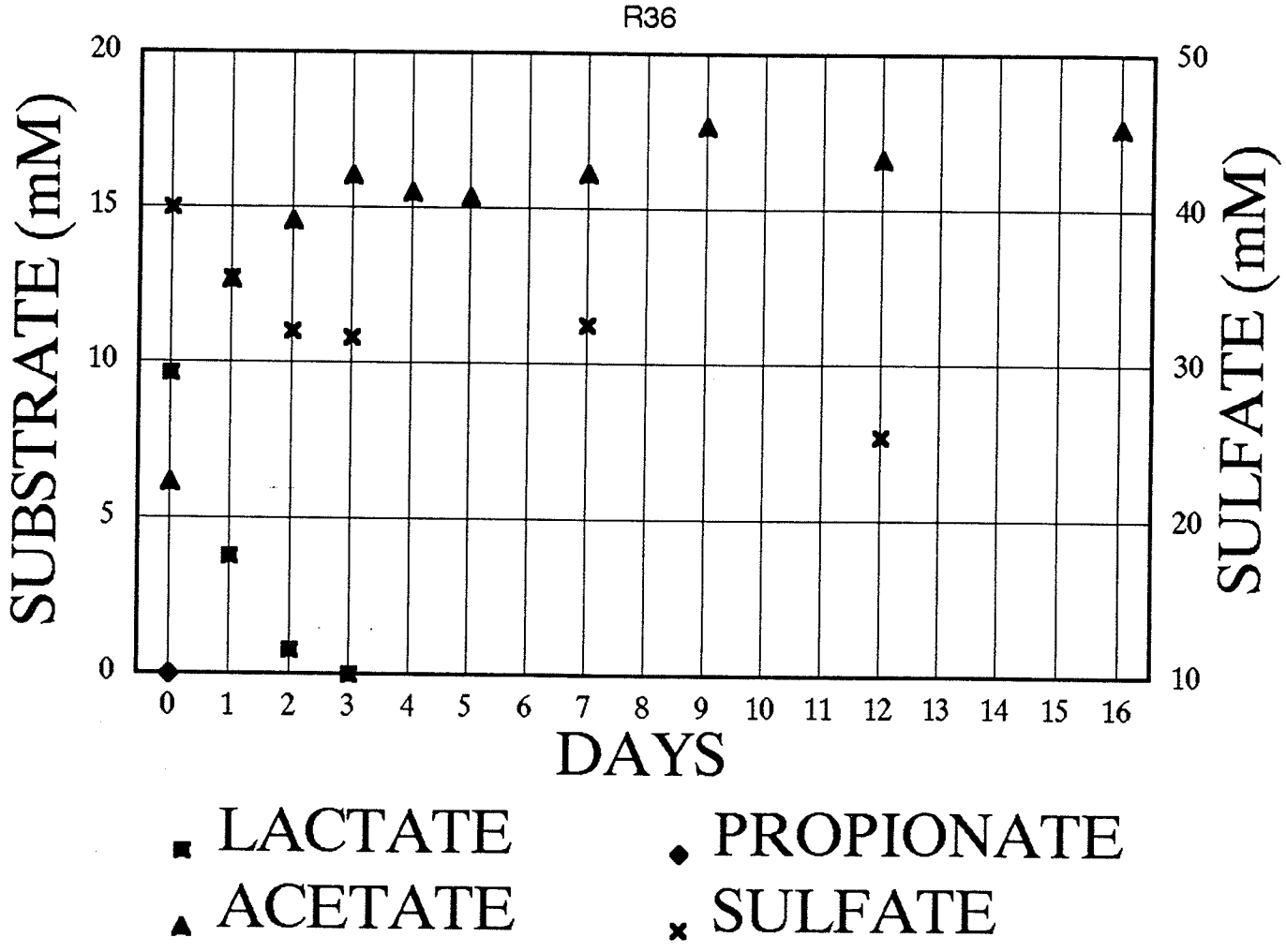


7.1 7.3
415 880
159 248
0 CH4



7.1 73
510 930
195 262

~ C44

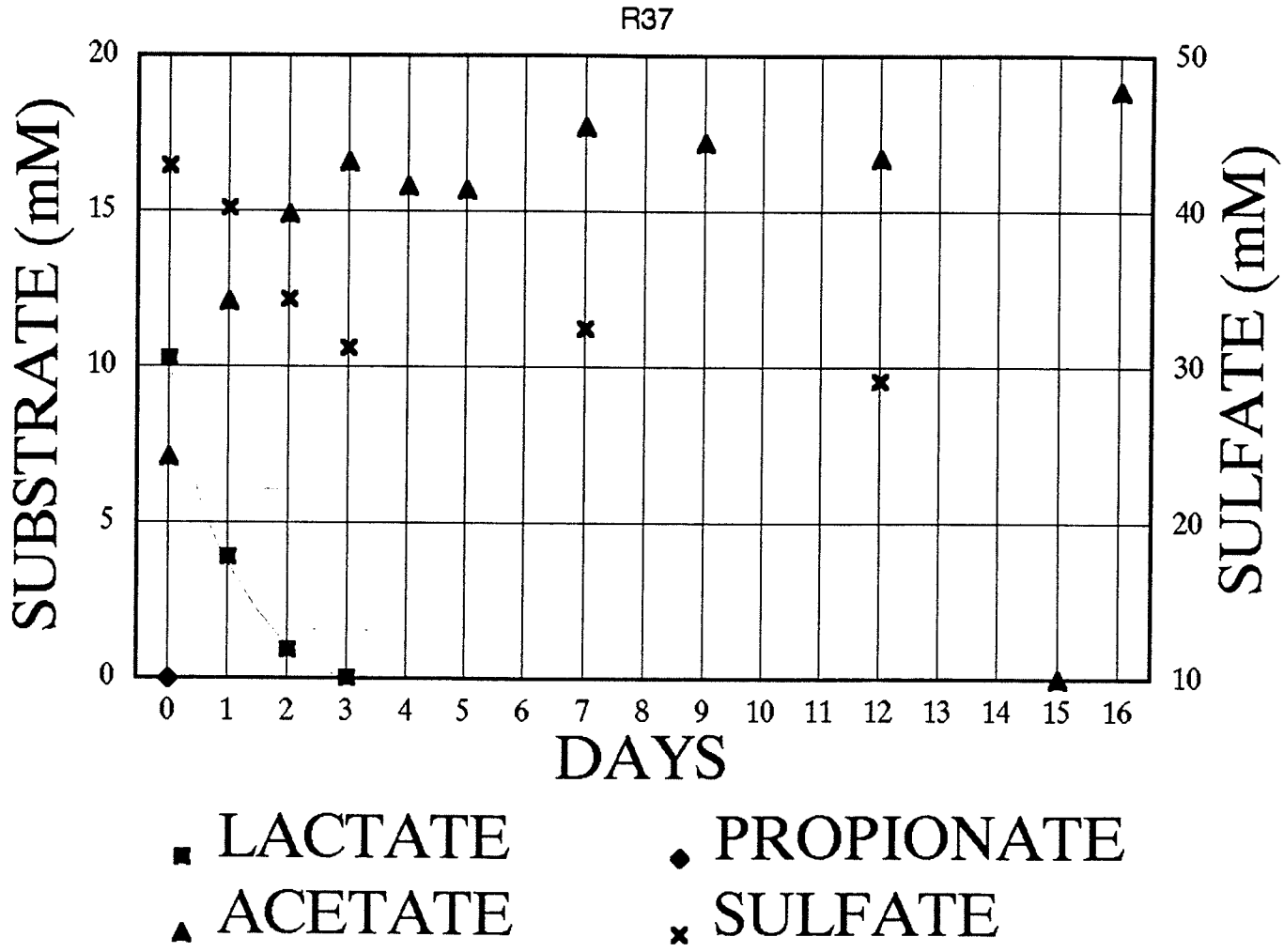


71 735

590 1080

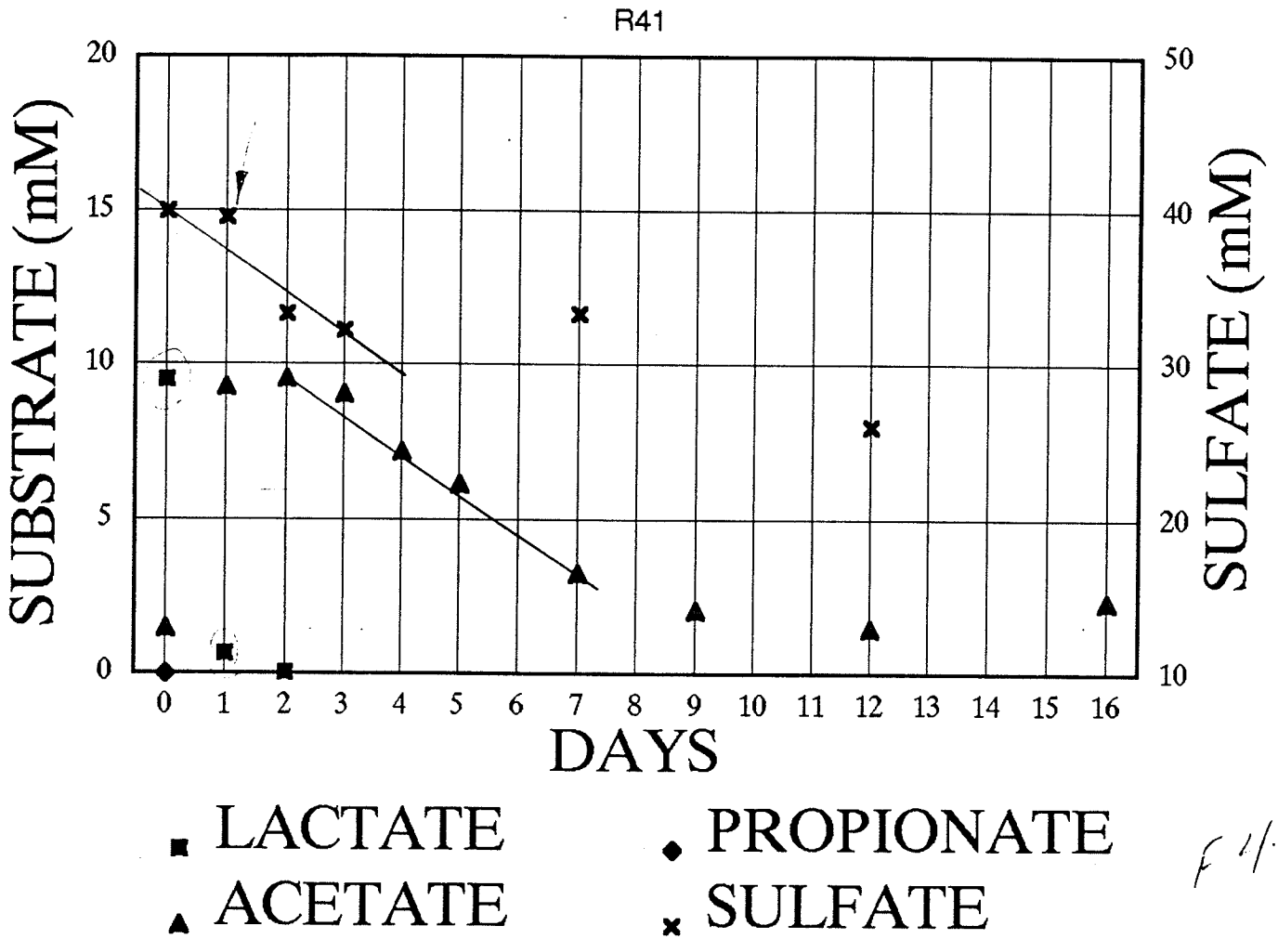
226 279

0 CH4



B 8.1
800 1340
SB 78.3

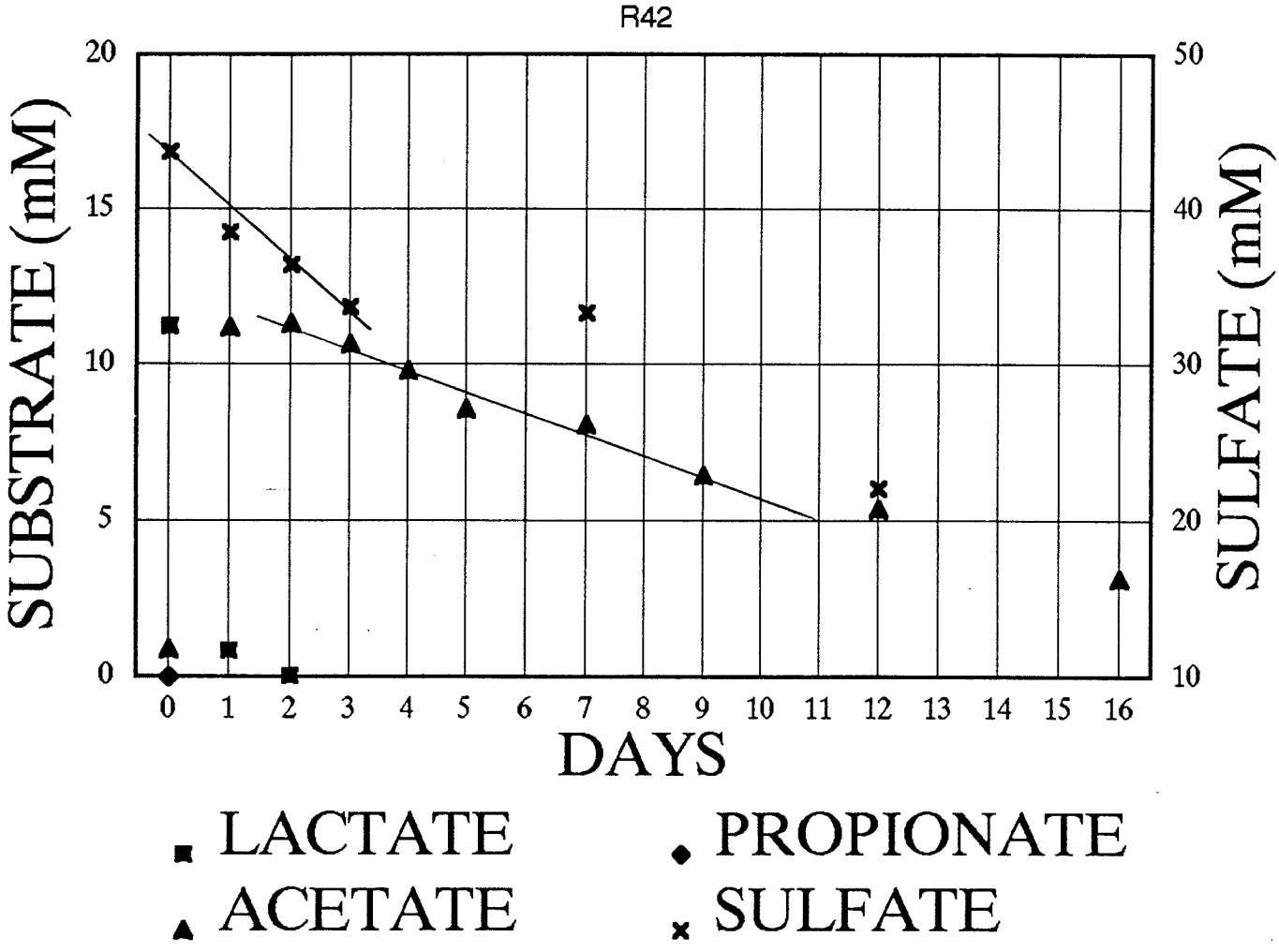
4.2 CH4



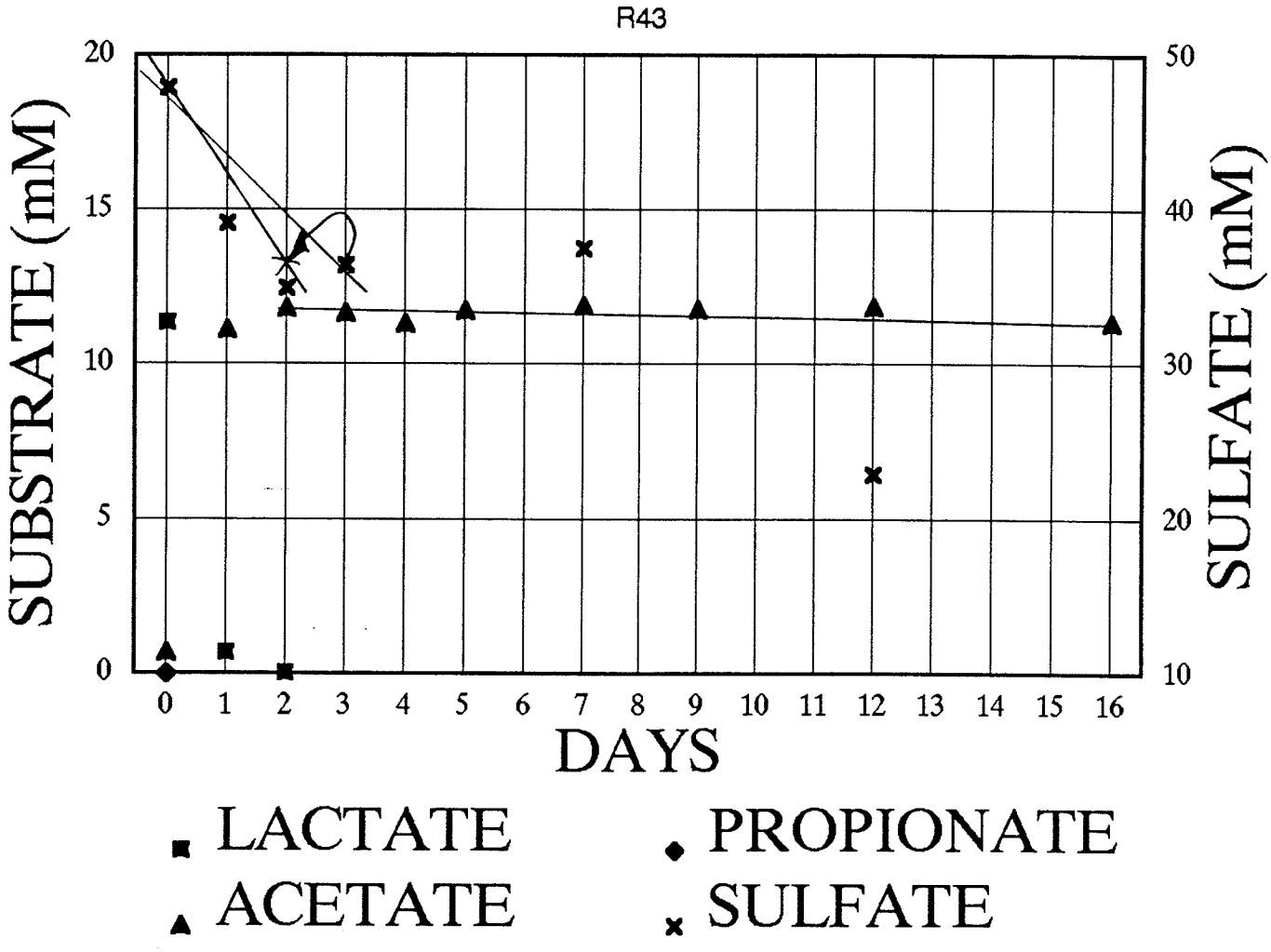
F 4.11

77 82
1110 1200
99.4 79.9

4.3 mL CH_4

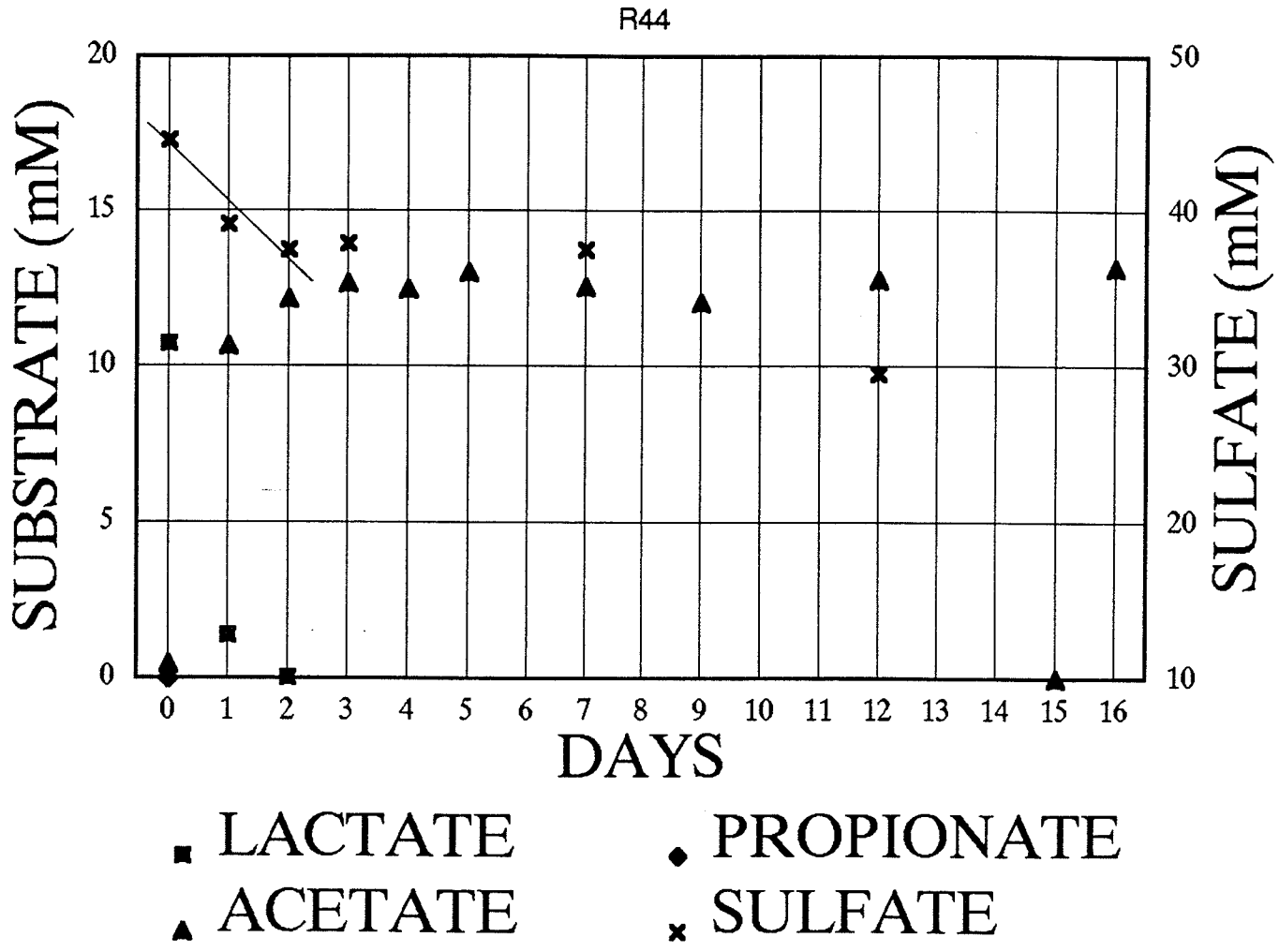


785 8.1
1380 1900
137 111
0.1 mL CH4



79 8.1
1800 2460
161 141

01 mL CH_4

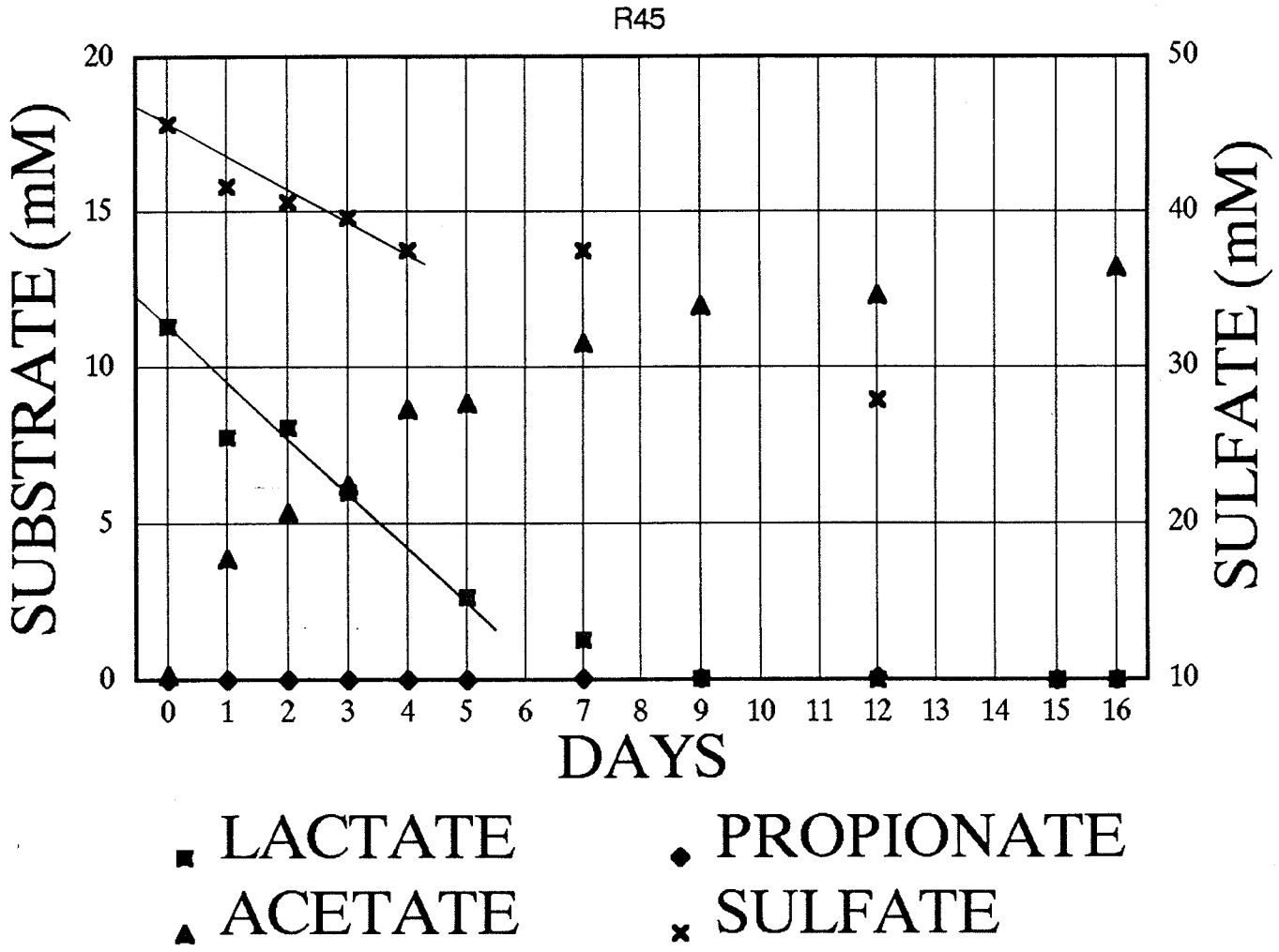


7.9 8.05

2350 2780

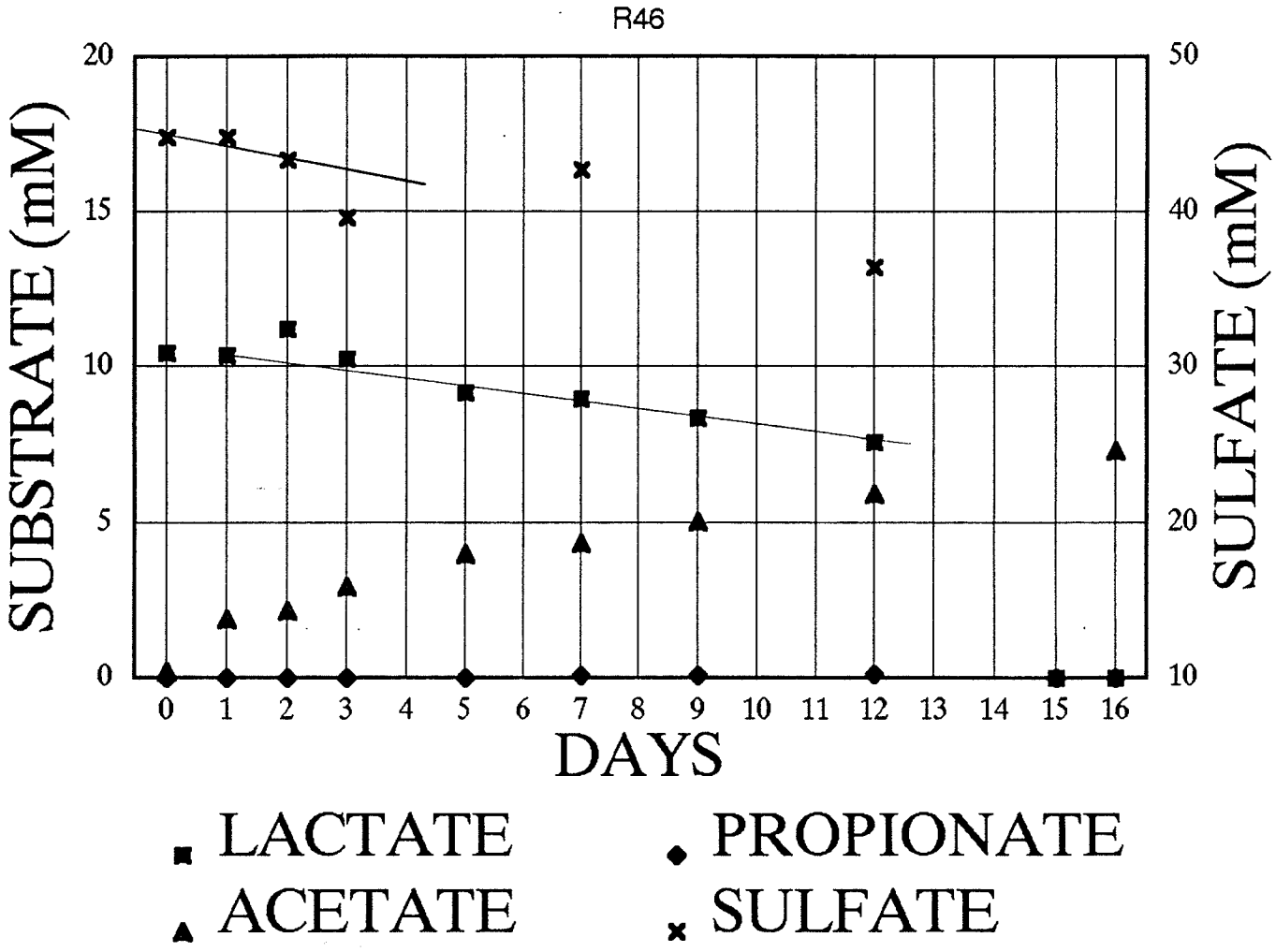
210 181

0 CH_4



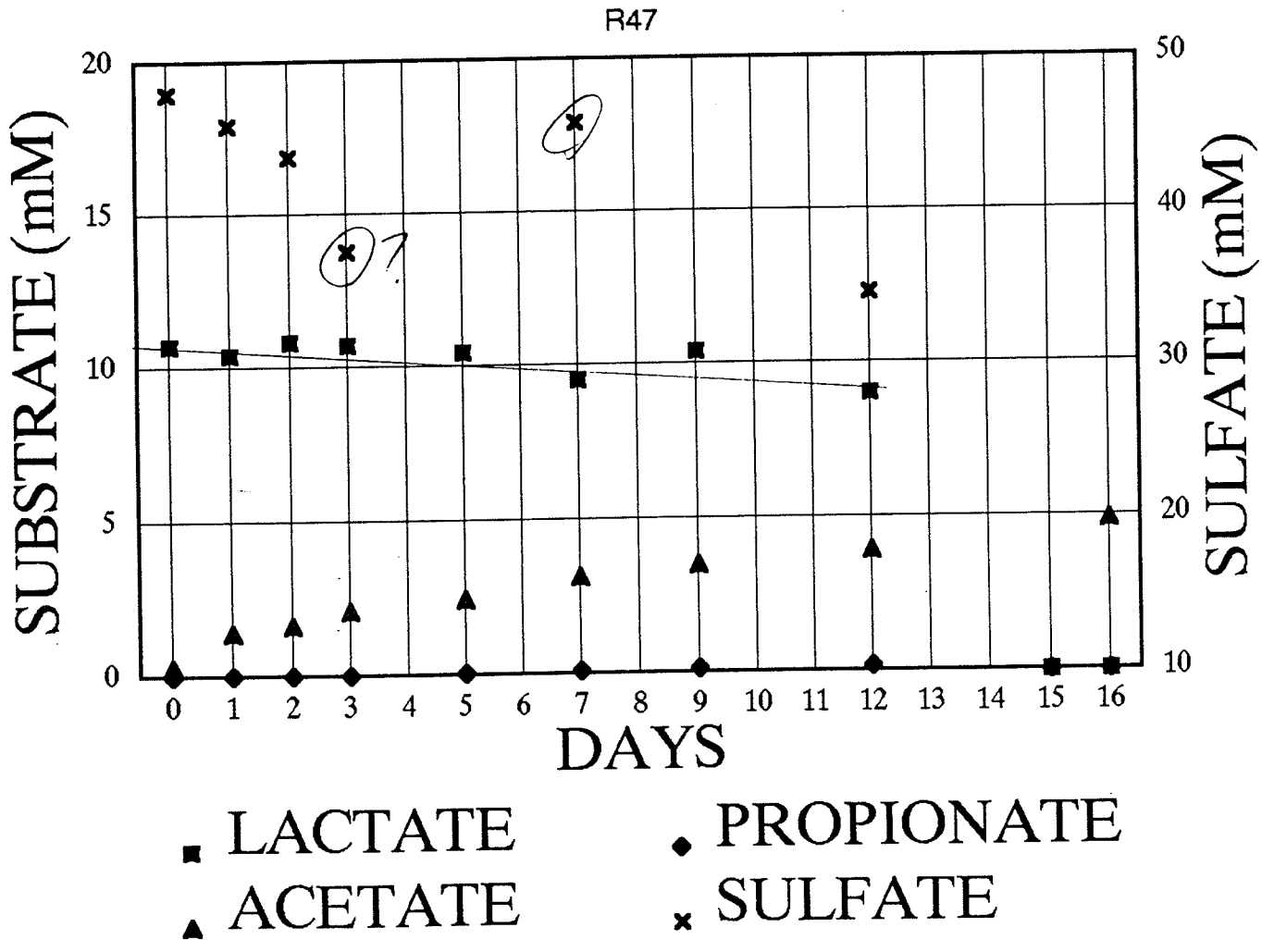
79 81
2910 3160
261 185

0 CH_4



795 8.1
3460 3460
279 208

0 CH4



APPENDIX C.

PHASE 4 RAW DATA

PHASE 4 DATA

PHASE 4 DATA REACTOR A1

DAY	HLa mg/L	HLa mM	HPr mg/L	HPr mM	HAc mg/L	HAc mM	SO4 mg/L	SO4 mM	H2 PPM	H2/4 PPM/4	DAY
0	836.00	9.29	19.00	0.26	634.00	10.57	1000.00	10.42			0
1	80.00	0.89	9.00	0.12	1309.00	21.82	370.00	3.85	15.90	3.98	1
2	0.00	0.00	13.00	0.18	1340.00	22.33	400.00	4.17	15.80	3.95	2
3			35.00	0.47	1219.00	20.32	410.00	4.27	17.20	4.30	3
4			33.00	0.45	1162.00	19.37			17.00	4.25	4
5											5
6			29.00	0.39	854.00	14.23			15.20	3.80	6
7											7
8			30.00	0.41	553.00	9.22	340.00	3.54	14.30	3.58	8

PHASE 4 DATA REACTOR A1

PHASE 4 DATA

PHASE 4 DATA REACTOR A2

DAY	HLa mg/L	HLa mM	HPr mg/L	HPr mM	HAc mg/L	HAc mM	SO4 mg/L	SO4 mM	H2 PPM		DAY
0	970.00	10.78	8.00	0.11	622.00	10.37	1000.00	10.42			0
1	80.00	0.89	9.00	0.12	1354.00	22.57	370.00	3.85	31.50	7.88	1
2	0.00	0.00	15.00	0.20	1385.00	23.08	360.00	3.75	18.40	4.60	2
3			23.00	0.31	1354.00	22.57	370.00	3.85	20.30	5.07	3
4			24.00	0.32	1246.00	20.77			19.30	4.82	4
5											5
6			22.00	0.30	1059.00	17.65			19.30	4.82	6
7											7
8			31.00	0.42	849.00	14.15	400.00	4.17	15.70	3.93	8

PHASE 4 DATA REACTOR A2

PHASE 4 DATA

PHASE 4 DATA REACTOR A3

DAY	HLa mg/L	HLa mM	HPr mg/L	HPr mM	HAc mg/L	HAc mM	SO4 mg/L	SO4 mM	H2 PPM		DAY
0	968.00	10.76	5.00	0.07	648.00	10.80	1000.00	10.42			0
1	80.00	0.89	7.00	0.09	1277.00	21.28	500.00	5.21	80.10	20.03	1
2	0.00	0.00	15.00	0.20	1413.00	23.55	420.00	4.38	28.50	7.13	2
3			24.00	0.32	1443.00	24.05	380.00	3.96	24.90	6.23	3
4			23.00	0.31	1441.00	24.02			28.20	7.05	4
5											5
6			22.00	0.30	1361.00	22.68			28.10	7.03	6
7											7
8			39.00	0.53	1382.00	23.03	400.00	4.17	23.80	5.95	8

PHASE 4 DATA REACTOR A3

PHASE 4 DATA

PHASE 4 DATA REACTOR B1 REACTOR B1

PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	1013.00	11.26	22.00	0.30	952.00	15.87	900.00	9.38			0
PHASE 4 DATA	1	464.00	5.16	26.00	0.35	1133.00	18.88	620.00	6.46	46.30	11.58	1
PHASE 4 DATA	2	0.00	0.00	35.00	0.47	1554.00	25.90	300.00	3.13	15.00	3.75	2
PHASE 4 DATA	3			21.00	0.28	1581.00	26.35	250.00	2.60	21.10	5.28	3
PHASE 4 DATA	4			18.00	0.24	1590.00	26.50			27.50	6.88	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			16.00	0.22	1371.00	22.85			26.80	6.70	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			27.00	0.36	1092.00	18.20	275.00	2.86	22.50	5.63	8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR B2											REACTOR B2
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	1054.00	11.71	19.00	0.26	905.00	15.08	880.00	9.17			0
PHASE 4 DATA	1	592.00	6.58	24.00	0.32	1041.00	17.35	740.00	7.71	49.70	12.43	1
PHASE 4 DATA	2	0.00	0.00	50.00	0.68	1714.00	28.57	300.00	3.13	16.70	4.18	2
PHASE 4 DATA	3			43.00	0.58	1744.00	29.07	250.00	2.60	21.80	5.45	3
PHASE 4 DATA	4			40.00	0.54	1760.00	29.33			27.50	6.88	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			36.00	0.49	1548.00	25.80			26.10	6.53	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			49.00	0.66	1338.00	22.30	300.00	3.13	23.80	5.95	8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR B3											REACTOR B3
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	1011.00	11.23	15.00	0.20	734.00	12.23	840.00	8.75			0
PHASE 4 DATA	1	750.00	8.33	29.00	0.39	1052.00	17.53	840.00	8.75	46.30	11.58	1
PHASE 4 DATA	2	190.00	2.11	52.00	0.70	1635.00	27.25	500.00	5.21	97.00	24.25	2
PHASE 4 DATA	3	58.00	0.64	60.00	0.81	1697.00	28.28	340.00	3.54	20.30	5.07	3
PHASE 4 DATA	4			58.00	0.78	1741.00	29.02			29.00	7.25	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			60.00	0.81	1727.00	28.78			32.20	8.05	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			73.00	0.99	1633.00	27.22	420.00	4.38	27.20	6.80	8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR C1											REACTOR C1
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		

PHASE 4 DATA

PHASE 4 DATA	0	985.00	10.94	0.00	0.00	6.00	0.10	960.00	10.00		0
PHASE 4 DATA	1	684.00	7.60	25.00	0.34	107.00	1.78	800.00	8.33	15.00	3.75	1
PHASE 4 DATA	2	0.00	0.00	181.00	2.45	259.00	4.32	680.00	7.08	24.40	6.10	2
PHASE 4 DATA	3			114.00	1.54	144.00	2.40	650.00	6.77	15.00	3.75	3
PHASE 4 DATA	4			51.00	0.69	40.00	0.67	600.00	6.25	14.10	3.53	4
PHASE 4 DATA	5			0.00	0.00	96.00	1.60	450.00	4.69			5
PHASE 4 DATA	6									12.50	3.13	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			0.00	0.00			330.00	3.44	11.60	2.90	8

PHASE 4 DATA

PHASE 4 DATA REACTOR C2 REACTOR C2

PHASE 4 DATA	DAY	HLa mg/L	HLa mM	HPr mg/L	HPr mM	HAc mg/L	HAc mM	SO4 mg/L	SO4 mM	H2 PPM		DAY
PHASE 4 DATA	0	944.00	10.49	0.00	0.00	2.00	0.03	900.00	9.38			0
PHASE 4 DATA	1	812.00	9.02	20.00	0.27	50.00	0.83	800.00	8.33	15.90	3.98	1
PHASE 4 DATA	2	0.00	0.00	387.00	5.23	241.00	4.02	850.00	8.85	22.70	5.68	2
PHASE 4 DATA	3			303.00	4.09	100.00	1.67	850.00	8.85	15.00	3.75	3
PHASE 4 DATA	4			222.00	3.00	20.00	0.33	840.00	8.75	17.00	4.25	4
PHASE 4 DATA	5			126.00	1.70	16.00	0.27	650.00	6.77			5
PHASE 4 DATA	6			29.00	0.39	17.00	0.28			13.90	3.48	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			0.00	0.00	0.00	0.00	450.00	4.69	11.60	2.90	8

PHASE 4 DATA

PHASE 4 DATA REACTOR C3 REACTOR C3

PHASE 4 DATA	DAY	HLa mg/L	HLa mM	HPr mg/L	HPr mM	HAc mg/L	HAc mM	SO4 mg/L	SO4 mM	H2 PPM		DAY
PHASE 4 DATA	0	954.00	10.60	0.00	0.00	2.00	0.03	1000.00	10.42			0
PHASE 4 DATA	1	704.00	7.82	21.00	0.28	211.00	3.52	980.00	10.21	18.50	4.63	1
PHASE 4 DATA	2	75.00	0.83	467.00	6.31	683.00	11.38	1000.00	10.42	32.10	8.02	2
PHASE 4 DATA	3			451.00	6.09	749.00	12.48	1000.00	10.42	24.10	6.03	3
PHASE 4 DATA	4			446.00	6.03	786.00	13.10	1000.00	10.42	25.30	6.33	4
PHASE 4 DATA	5			423.00	5.72	804.00	13.40	800.00	8.33			5
PHASE 4 DATA	6			442.00	5.97	875.00	14.58			24.70	6.18	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			456.00	6.16	953.00	15.88	730.00	7.60	19.80	4.95	8

PHASE 4 DATA

PHASE 4 DATA REACTOR D1 REACTOR D1

PHASE 4 DATA	DAY	HLa mg/L	HLa mM	HPr mg/L	HPr mM	HAc mg/L	HAc mM	SO4 mg/L	SO4 mM	H2 PPM		DAY
PHASE 4 DATA	0	997.00	11.08	4.00	0.05	550.00	9.17	810.00	8.44			0

PHASE 4 DATA	1	79.00	0.88	44.00	0.59	1426.00	23.77	590.00	6.15	8.90	2.22	1
PHASE 4 DATA	2	0.00	0.00	34.00	0.46	1764.00	29.40	580.00	6.04			2
PHASE 4 DATA	3			48.00	0.65	1714.00	28.57	550.00	5.73			3
PHASE 4 DATA	4			72.00	0.97	1535.00	25.58					4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			57.00	0.77	1471.00	24.52					6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			82.00	1.11	1436.00	23.93	580.00	6.04			8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR D2											REACTOR D2
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	962.00	10.69	3.00	0.04	560.00	9.33	840.00	8.75			0
PHASE 4 DATA	1	50.00	0.56	11.00	0.15	993.00	16.55	620.00	6.46			1
PHASE 4 DATA	2	0.00	0.00	24.00	0.32	1952.00	32.53	600.00	6.25			2
PHASE 4 DATA	3			35.00	0.47	1928.00	32.13	585.00	6.09			3
PHASE 4 DATA	4			41.00	0.55	1780.00	29.67					4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			40.00	0.54	1840.00	30.67					6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			53.00	0.72	1869.00	31.15	630.00	6.56			8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR D3											REACTOR D3
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	967.00	10.74	4.00	0.05	631.00	10.52	840.00	8.75			0
PHASE 4 DATA	1	46.00	0.51	15.00	0.20	1392.00	23.20	630.00	6.56			1
PHASE 4 DATA	2	0.00	0.00	17.00	0.23	1970.00	32.83	620.00	6.46			2
PHASE 4 DATA	3			40.00	0.54	2069.00	34.48	630.00	6.56			3
PHASE 4 DATA	4			37.00	0.50	1932.00	32.20					4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			41.00	0.55	1961.00	32.68					6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			56.00	0.76	1892.00	31.53	650.00	6.77			8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR E1											REACTOR E1
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	524.00	5.82	392.00	5.30	713.00	11.88	810.00	8.44			0
PHASE 4 DATA	1	67.00	0.74	385.00	5.20	980.00	16.33	700.00	7.29			1
PHASE 4 DATA	2	0.00	0.00	433.00	5.85	1089.00	18.15	625.00	6.51			2

PHASE 4 DATA	3			450.00	6.08	1004.00	16.73	620.00	6.46			3
PHASE 4 DATA	4			431.00	5.82	938.00	15.63	650.00	6.77			4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			414.00	5.59	981.00	16.35					6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			440.00	5.95	1000.00	16.67	630.00	6.56			8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR E2											REACTOR E2
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	514.00	5.71	423.00	5.72	657.00	10.95	840.00	8.75			0
PHASE 4 DATA	1	34.00	0.38	413.00	5.58	903.00	15.05	750.00	7.81			1
PHASE 4 DATA	2	0.00	0.00	474.00	6.41	1018.00	16.97	725.00	7.55			2
PHASE 4 DATA	3			467.00	6.31	915.00	15.25	700.00	7.29			3
PHASE 4 DATA	4			473.00	6.39	914.00	15.23	725.00	7.55			4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			481.00	6.50	951.00	15.85					6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			486.00	6.57	966.00	16.10	740.00	7.71			8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR E3											REACTOR E3
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	482.00	5.36	366.00	4.95	574.00	9.57	890.00	9.27			0
PHASE 4 DATA	1	44.00	0.49	407.00	5.50	901.00	15.02	825.00	8.59			1
PHASE 4 DATA	2	0.00	0.00	491.00	6.64	1070.00	17.83	770.00	8.02			2
PHASE 4 DATA	3			511.00	6.91	1095.00	18.25	750.00	7.81			3
PHASE 4 DATA	4			474.00	6.41	1037.00	17.28	730.00	7.60			4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			500.00	6.76	1113.00	18.55					6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			491.00	6.64	1184.00	19.73	770.00	8.02			8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR F1											REACTOR F1
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	836.00	9.29	55.00	0.74	649.00	10.82	775.00	8.07			0
PHASE 4 DATA	1	52.00	0.58	60.00	0.81	1144.00	19.07	620.00	6.46	6.30	1.58	1
PHASE 4 DATA	2	0.00	0.00	82.00	1.11	1261.00	21.02	620.00	6.46	11.50	2.88	2
PHASE 4 DATA	3			78.00	1.05	1147.00	19.12	620.00	6.46	12.70	3.18	3
PHASE 4 DATA	4			79.00	1.07	1096.00	18.27			14.80	3.70	4

PHASE 4 DATA	5										5
PHASE 4 DATA	6		99.00	1.34	935.00	15.58			12.50	3.13	6
PHASE 4 DATA	7										7
PHASE 4 DATA	8		83.00	1.12	564.00	9.40	630.00	6.56	10.90	2.73	8
PHASE 4 DATA											
PHASE 4 DATA	REACTOR F2										REACTOR F2
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2	DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM	
PHASE 4 DATA											
PHASE 4 DATA	0	922.00	10.24	10.00	0.14	629.00	10.48	850.00	8.85		0
PHASE 4 DATA	1	54.00	0.60	18.00	0.24	1301.00	21.68	725.00	7.55	8.10	2.03	1
PHASE 4 DATA	2	0.00	0.00	24.00	0.32	1360.00	22.67	725.00	7.55	15.00	3.75	2
PHASE 4 DATA	3			25.00	0.34	1439.00	23.98	720.00	7.50	16.50	4.13	3
PHASE 4 DATA	4			24.00	0.32	1303.00	21.72			18.50	4.63	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			27.00	0.36	1203.00	20.05			15.20	3.80	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			36.00	0.49	1008.00	16.80	725.00	7.55	16.40	4.10	8
PHASE 4 DATA											
PHASE 4 DATA	REACTOR F3										REACTOR F3
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2	DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM	
PHASE 4 DATA											
PHASE 4 DATA	0	1018.00	11.31	3.00	0.04	564.00	9.40	850.00	8.85		0
PHASE 4 DATA	1	115.00	1.28	6.00	0.08	1164.00	19.40	800.00	8.33	20.20	5.05	1
PHASE 4 DATA	2	0.00	0.00	14.00	0.19	1422.00	23.70	750.00	7.81	27.00	6.75	2
PHASE 4 DATA	3			14.00	0.19	1431.00	23.85	750.00	7.81	23.50	5.88	3
PHASE 4 DATA	4			18.00	0.24	1484.00	24.73			27.50	6.88	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			20.00	0.27	1507.00	25.12			24.70	6.18	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			34.00	0.46	1452.00	24.20	750.00	7.81	21.10	5.28	8
PHASE 4 DATA											
PHASE 4 DATA	REACTOR G1											REACTOR G1
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2	H2/30	DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	1010.00	11.22	23.00	0.31	987.00	16.45	625.00	6.51			0
PHASE 4 DATA	1	492.00	5.47	44.00	0.59	1193.00	19.88	375.00	3.91	67.10	2.24	1
PHASE 4 DATA	2	0.00	0.00	42.00	0.57	1712.00	28.53	200.00	2.08	22.70	0.76	2
PHASE 4 DATA	3			38.00	0.51	1607.00	26.78	160.00	1.67	34.00	1.13	3
PHASE 4 DATA	4			40.00	0.54	1766.00	29.43			54.50	1.82	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			36.00	0.49	1600.00	26.67			220.00	7.33	6

PHASE 4 DATA	7											7
PHASE 4 DATA	8			54.00	0.73	1469.00	24.48	150.00	1.56	579.00	19.30	8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR G2											REACTOR G2
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2	H2/3	DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	1005.00	11.17	21.00	0.28	933.00	15.55	650.00	6.77			0
PHASE 4 DATA	1	616.00	6.84	38.00	0.51	1215.00	20.25	460.00	4.79	30.60	10.20	1
PHASE 4 DATA	2	0.00	0.00	76.00	1.03	1685.00	28.08	250.00	2.60	28.70	9.57	2
PHASE 4 DATA	3			60.00	0.81	1710.00	28.50	225.00	2.34	38.60	12.87	3
PHASE 4 DATA	4			83.00	1.12	1768.00	29.47			52.90	17.63	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			80.00	1.08	1728.00	28.80			58.90	19.63	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			91.00	1.23	1622.00	27.03	175.00	1.82	52.40	17.47	8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR G3											REACTOR G3
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2	H2/8	DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	1022.00	11.36	24.00	0.32	962.00	16.03	700.00	7.29			0
PHASE 4 DATA	1	580.00	6.44	34.00	0.46	1119.00	18.65	550.00	5.73	54.90	6.86	1
PHASE 4 DATA	2	254.00	2.82	42.00	0.57	1535.00	25.58	420.00	4.38	144.00	18.00	2
PHASE 4 DATA	3	0.00	0.00	50.00	0.68	1726.00	28.77	340.00	3.54	97.30	12.16	3
PHASE 4 DATA	4			55.00	0.74	1793.00	29.88			157.00	19.63	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			55.00	0.74	1693.00	28.22			120.00	15.00	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			72.00	0.97	1686.00	28.10	285.00	2.97	82.30	10.29	8
PHASE 4 DATA												
PHASE 4 DATA	REACTOR H1											REACTOR H1
PHASE 4 DATA	DAY	HLa	HLa	HPr	HPr	HAc	HAc	SO4	SO4	H2		DAY
PHASE 4 DATA		mg/L	mM	mg/L	mM	mg/L	mM	mg/L	mM	PPM		
PHASE 4 DATA												
PHASE 4 DATA	0	1007.00	11.19	20.00	0.27	910.00	15.17	3400.00	35.42			0
PHASE 4 DATA	1	450.00	5.00	41.00	0.55	1331.00	22.18	3000.00	31.25	22.80	5.70	1
PHASE 4 DATA	2	0.00	0.00	11.00	0.15	1690.00	28.17	2880.00	30.00	15.80	3.95	2
PHASE 4 DATA	3			0.00	0.00	1585.00	26.42	2240.00	23.33	18.00	4.50	3
PHASE 4 DATA	4			0.00	0.00	1919.00	31.98			20.00	5.00	4
PHASE 4 DATA	5											5
PHASE 4 DATA	6			0.00	0.00	1649.00	27.48			21.00	5.25	6
PHASE 4 DATA	7											7
PHASE 4 DATA	8			0.00	0.00	1532.00	25.53	2800.00	29.17	15.70	3.93	8

PHASE 4 DATA

PHASE 4 DATA REACTOR H2

PHASE 4 DATA DAY	HLa mg/L	HLa mM	HPr mg/L	HPr mM	HAc mg/L	HAc mM	SO4 mg/L	SO4 mM	H2 PPM		REACTOR H2 DAY
PHASE 4 DATA 0	969.00	10.77	25.00	0.34	1029.00	17.15	3640.00	37.92			0
PHASE 4 DATA 1	500.00	5.56	40.00	0.54	1234.00	20.57	3400.00	35.42	30.60	7.65	1
PHASE 4 DATA 2	0.00	0.00	53.00	0.72	1783.00	29.72	3000.00	31.25	25.20	6.30	2
PHASE 4 DATA 3			0.00	0.00	1802.00	30.03	2600.00	27.08	23.30	5.83	3
PHASE 4 DATA 4			0.00	0.00	1938.00	32.30			28.20	7.05	4
PHASE 4 DATA 5											5
PHASE 4 DATA 6			0.00	0.00	1772.00	29.53			29.10	7.28	6
PHASE 4 DATA 7											7
PHASE 4 DATA 8			0.00	0.00	1640.00	27.33	3000.00	31.25	22.50	5.63	8

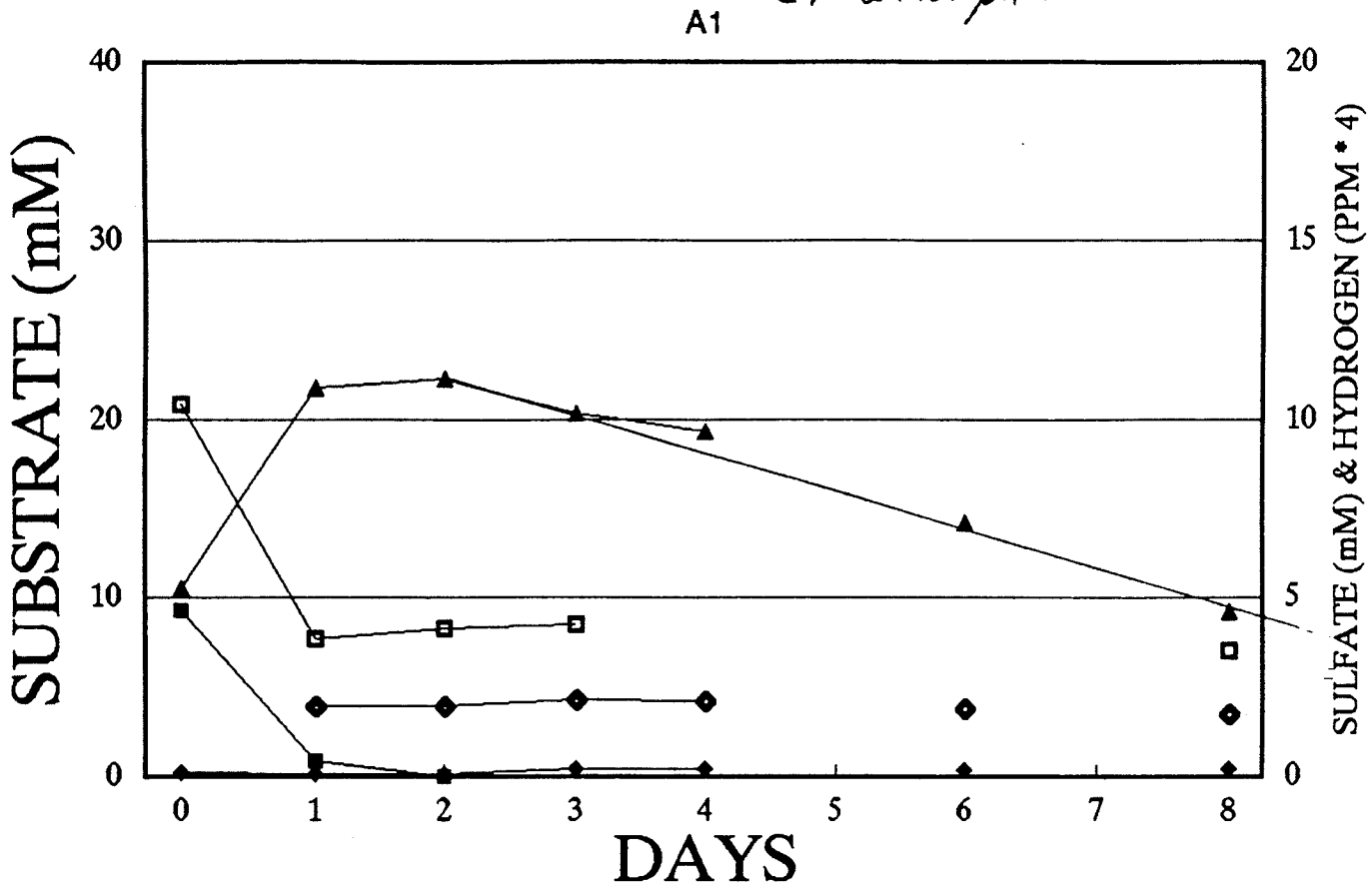
PHASE 4 DATA

PHASE 4 DATA REACTOR H3

PHASE 4 DATA DAY	HLa mg/L	HLa mM	HPr mg/L	HPr mM	HAc mg/L	HAc mM	SO4 mg/L	SO4 mM	H2 PPM		REACTOR H3 DAY
PHASE 4 DATA 0	1010.00	11.22	23.00	0.31	997.00	16.62	3800.00	39.58			0
PHASE 4 DATA 1	618.00	6.87	35.00	0.47	1119.00	18.65	3500.00	36.46	41.10	10.28	1
PHASE 4 DATA 2	300.00	3.33	45.00	0.61	1543.00	25.72	3240.00	33.75	98.10	24.53	2
PHASE 4 DATA 3	0.00	0.00	0.00	0.00	1636.00	27.27	2600.00	27.08	37.10	9.27	3
PHASE 4 DATA 4			54.00	0.73	1865.00	31.08			70.80	17.70	4
PHASE 4 DATA 5											5
PHASE 4 DATA 6			49.00	0.66	1767.00	29.45			102.30	25.58	6
PHASE 4 DATA 7											7
PHASE 4 DATA 8			60.00	0.81	1684.00	28.07	3200.00	33.33	85.70	21.43	8

PHASE 4

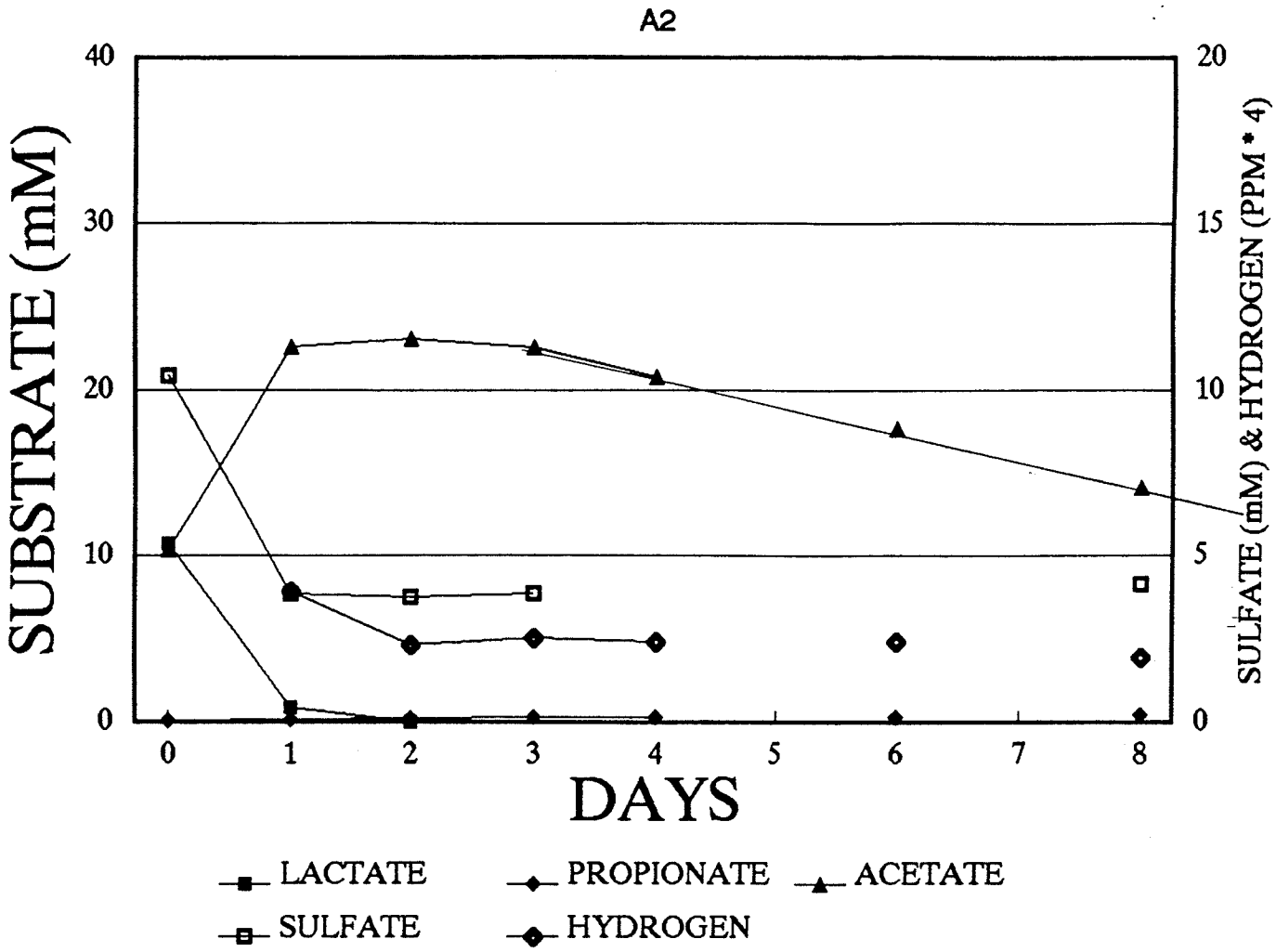
C7 - control pH 7



Day 0 / Day 1 ≡ (0/1)

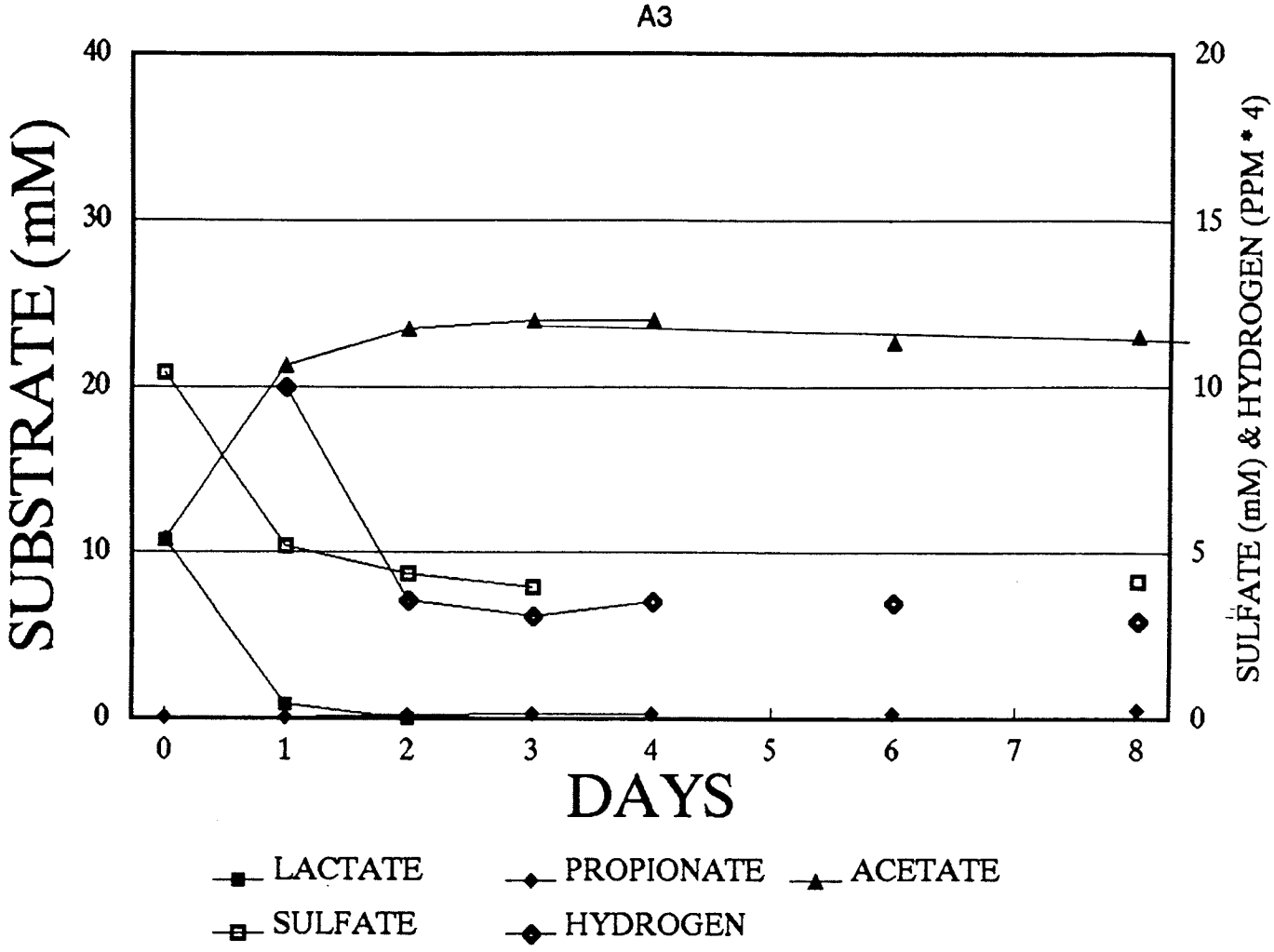
	pH (0/1)	S^{2-}	H_2S	% CH_4	mL Gas	mL CH_4
ST.	6.9/7.0	400				
EMS	7.1	440			214	
AVG.						

- H_2S removal immediate in all reactors, not due to S^{2-}
- no H_2 build-up after all Le removed.



	pH (0/1)	TS	H_2S	% CH_4	mL CH_4
ST.	7.17	500			
EN.	7.1	560		16	
AVG					

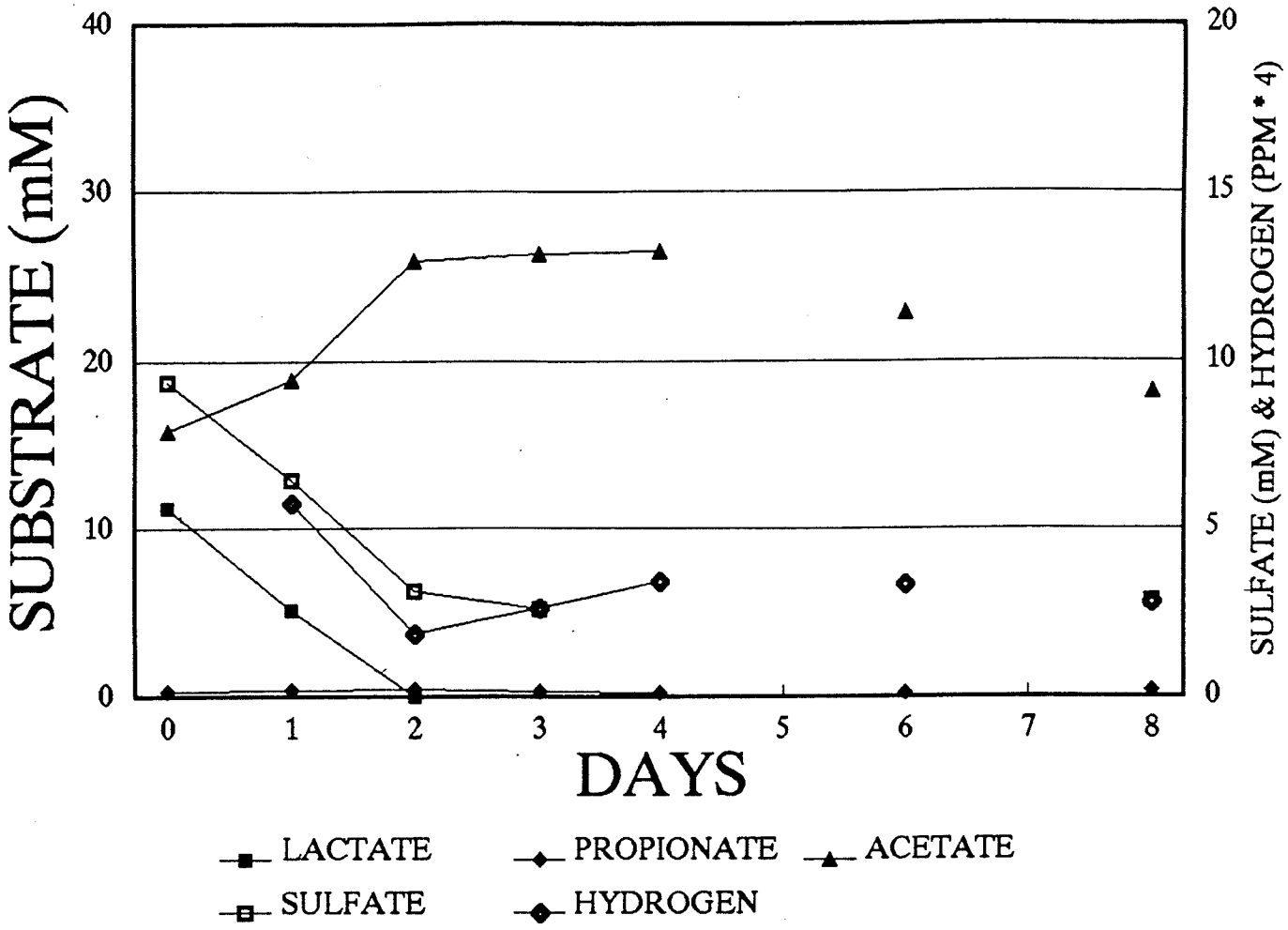
- slight H_2 build-up after 1d.



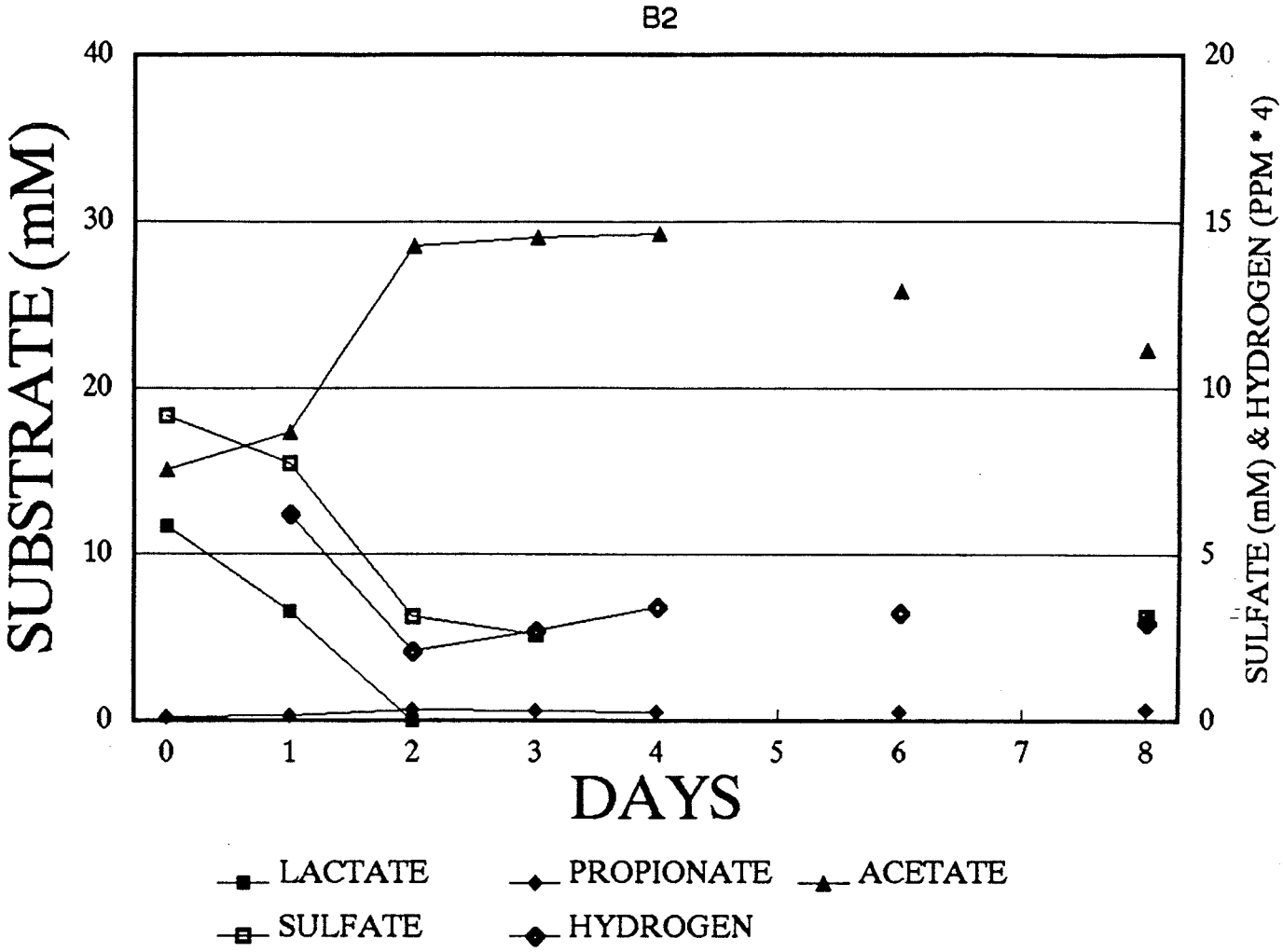
	pH (0.1)	TS	H_2S	%CH_4	mL CH_4
ST	7.1/7.1	640			
EV.	7.2	700		0.3	
AVG					

- larger H_2 build-up after 1 d.

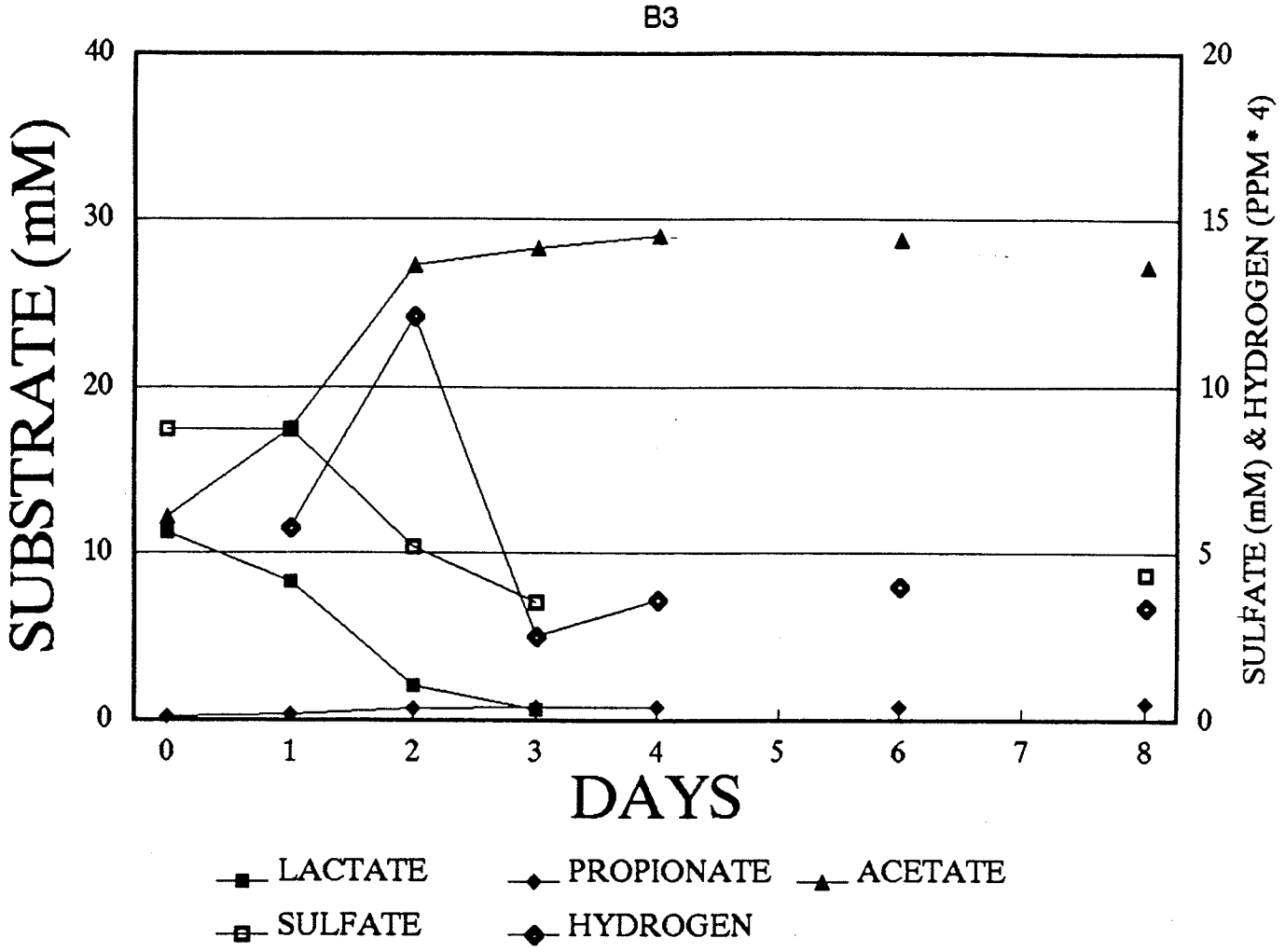
B1 pH 8↓7



	pH (0/1)	TS	H_2S	% CH_4	mL CH_4
ST.	7.2/7.2	1.40			
EX.	7.4	4.40		17.1	
Inc.					

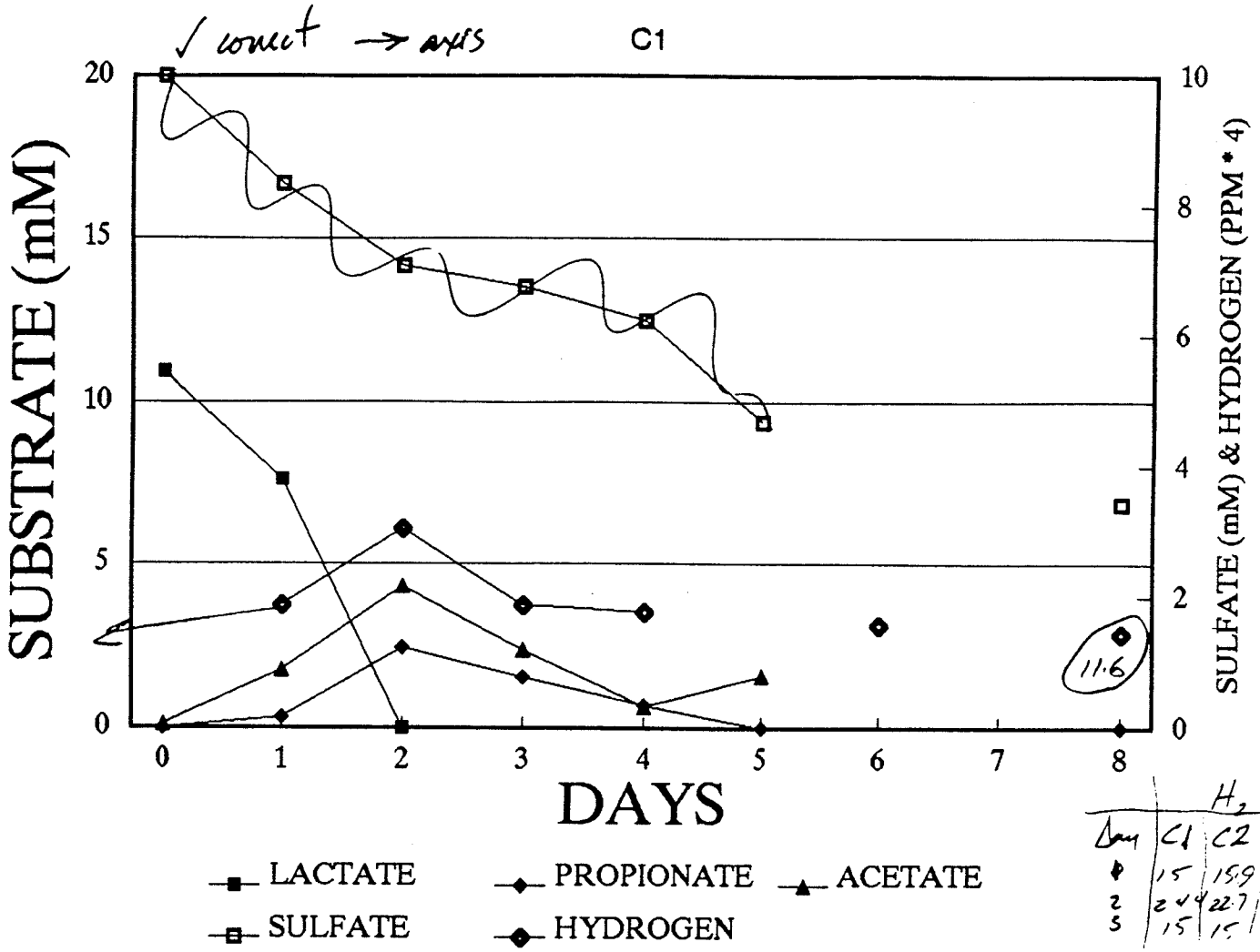


	H (0/1)	T3	H_2S	% CH_4	mL CH_4
ST.	7.2/7.2	240			
EN.	7.3	480		10.6	
AVG.					



	pH (o/i)	TS	H_2S	o/o CH_4	mL CH_4
ST.	7.2/7.2	480			
END	7.4	640		1.9	
AVG.					

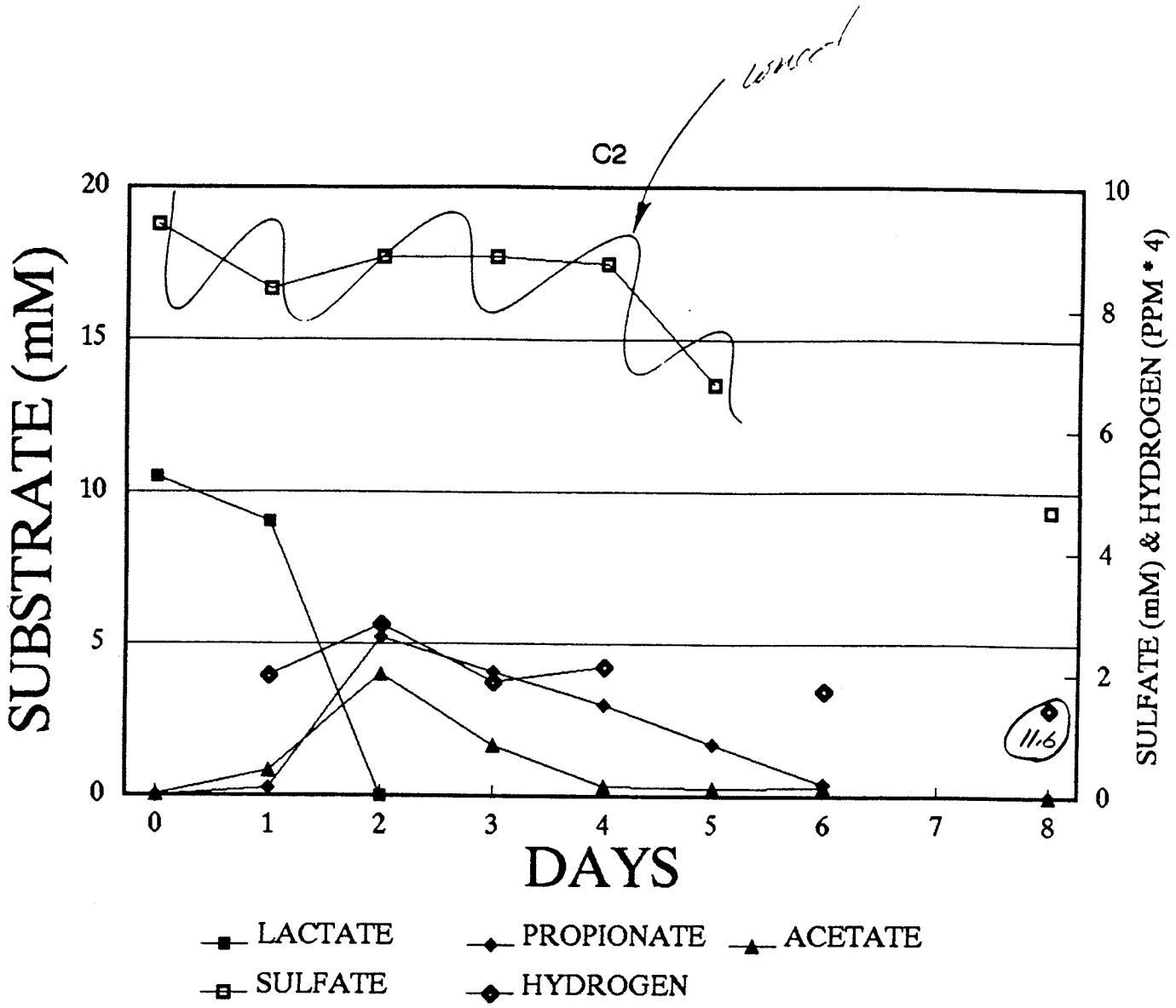
4



	H_2		
Day	C1	C2	C3
0	15	15.9	13.3
2	24	22.7	12.1
5	15	15	24.1

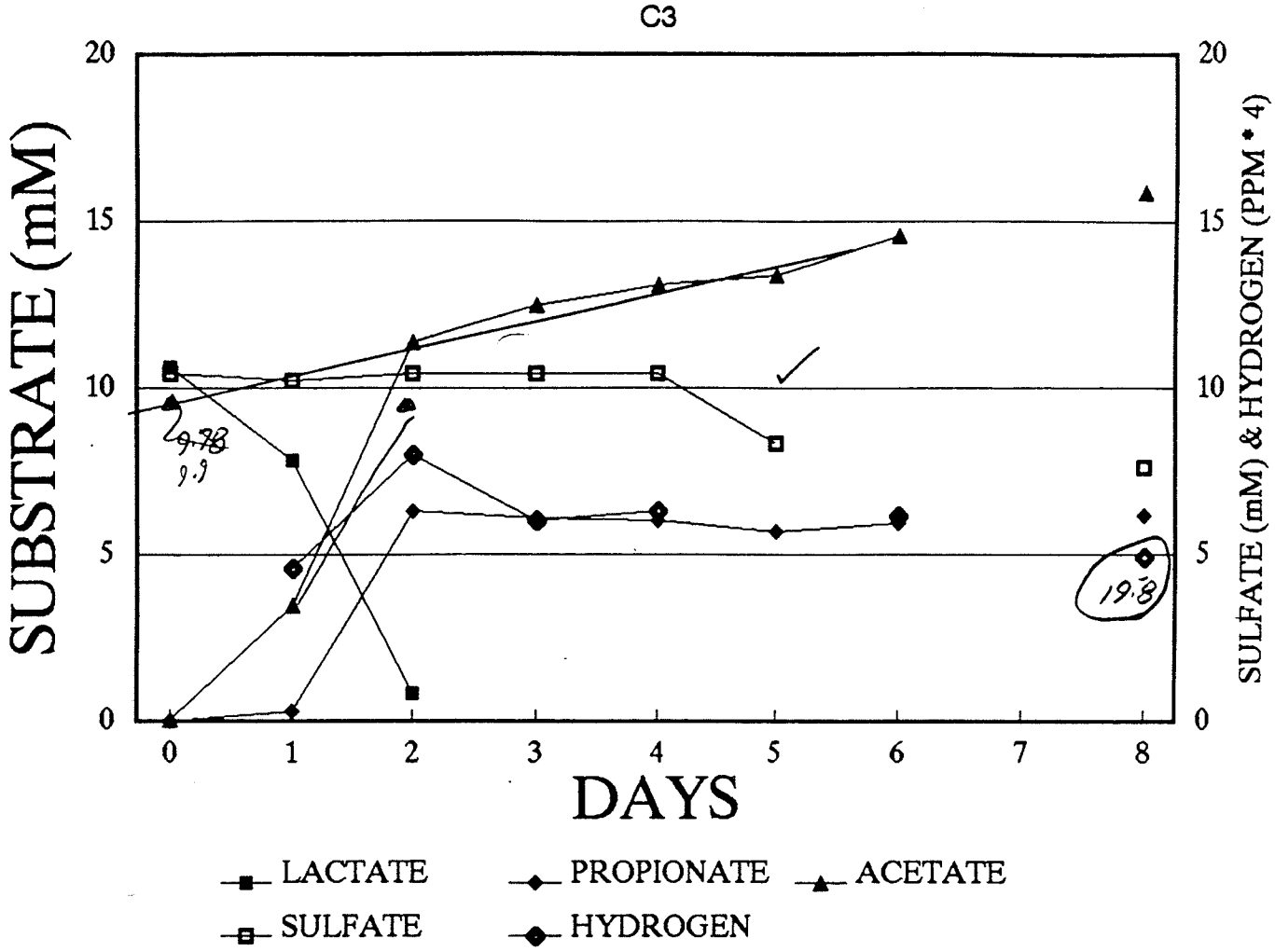
	pH (0/1)	TS	H_2S	%CH_4	mL CH_4
ST.	7.1/7.2	120			
END	7.2	300		36.5	
AVG					

F 4.2



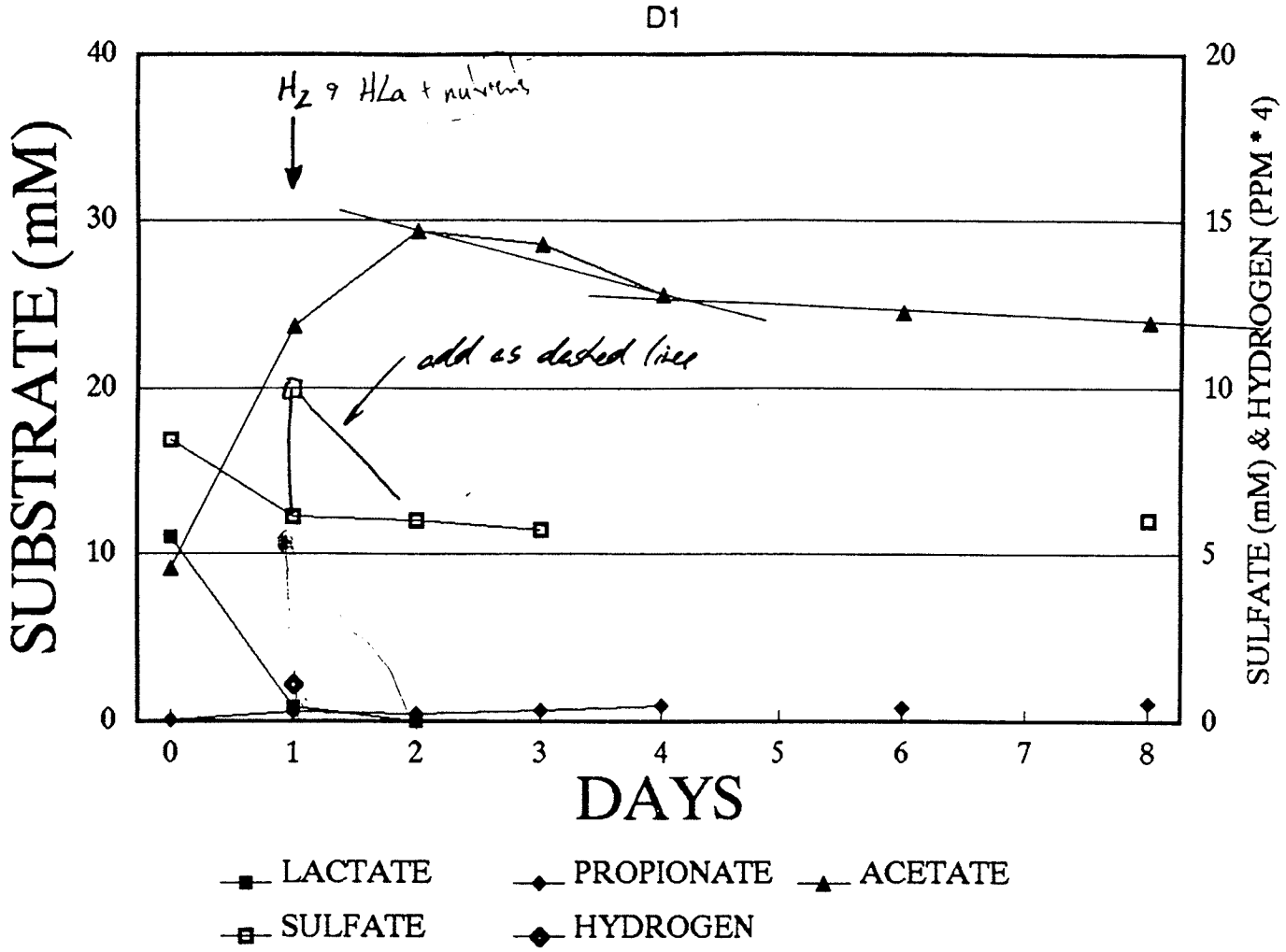
	pH	B	H_2S	%CH4	mL CH4
	0/1				
ST.	7.1/7.2	240			
ENS	7.2	300		25.7	
AVG.					

F 4.22



	pH (6/1)	TS	H_2S	% CH_4	mL CH_4
ST.	7.1/7.2	680			
END	7.2	480		13.3	
AVG					

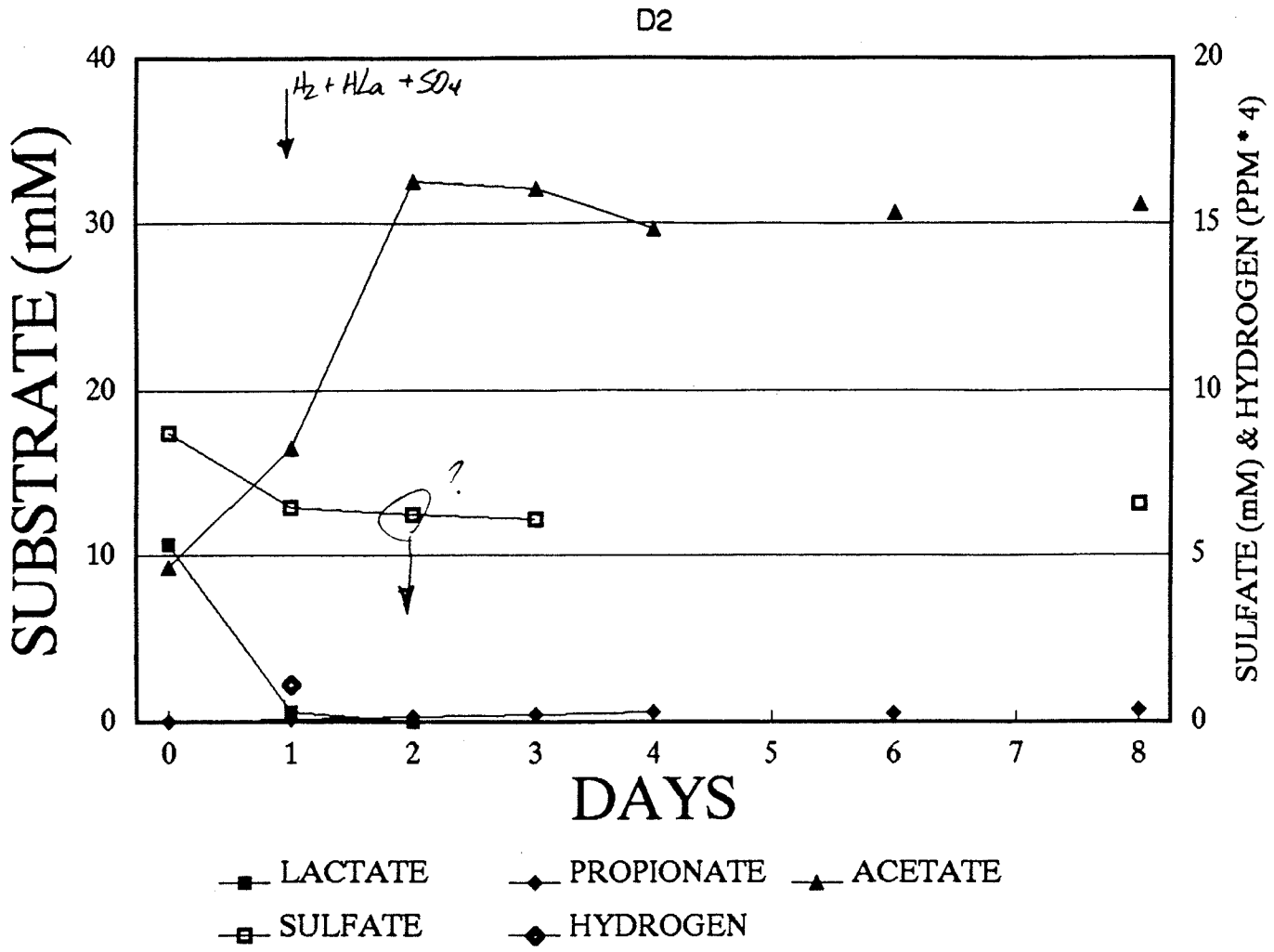
F4-20



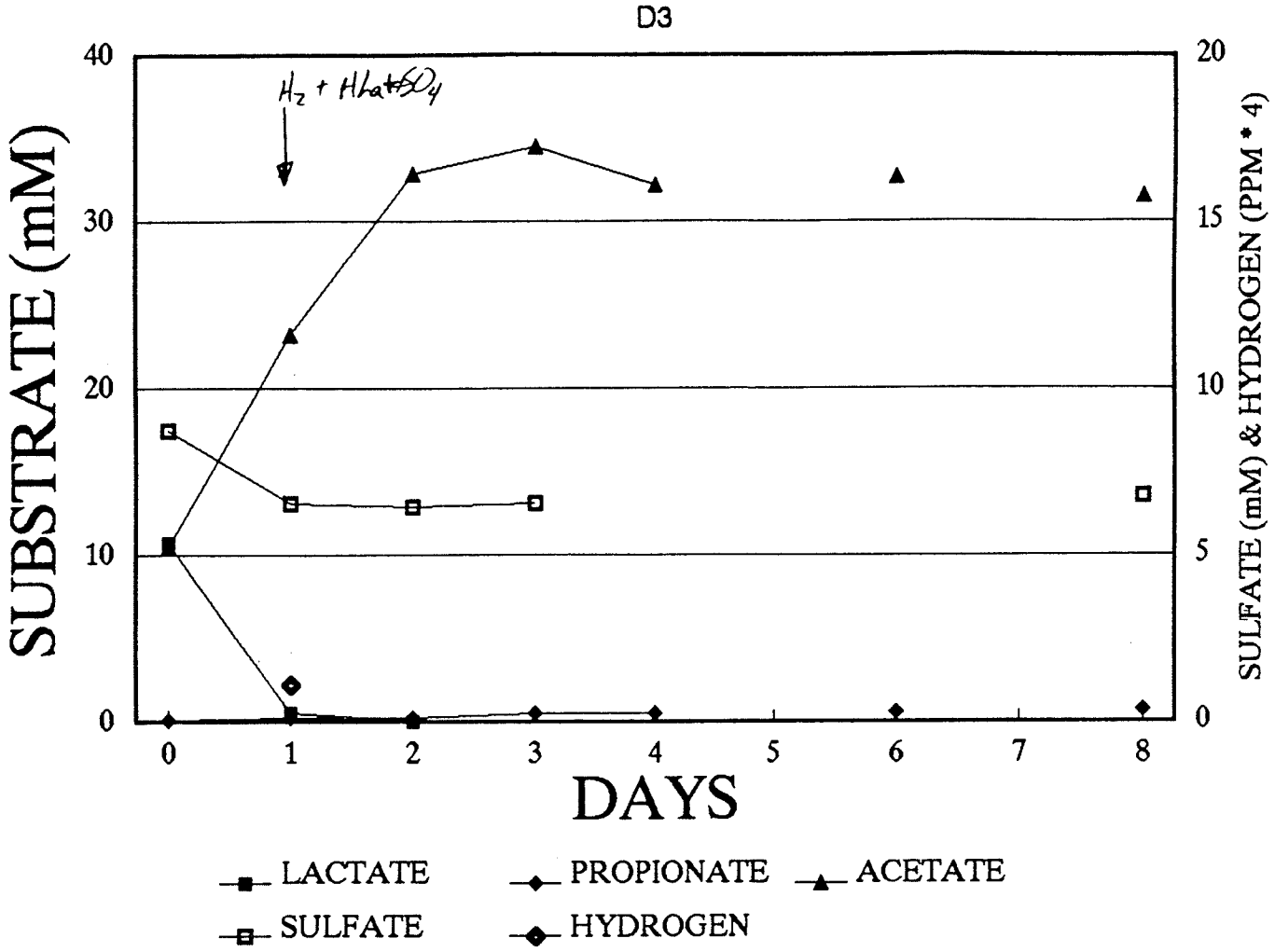
	pH (0/1)	B	H_2S	% CH_4	mL CH_4
ST.	7.1/7.1	400			
END	7.3	686		20.8	
AVG.					

- H_2 did not affect HLa degradation.

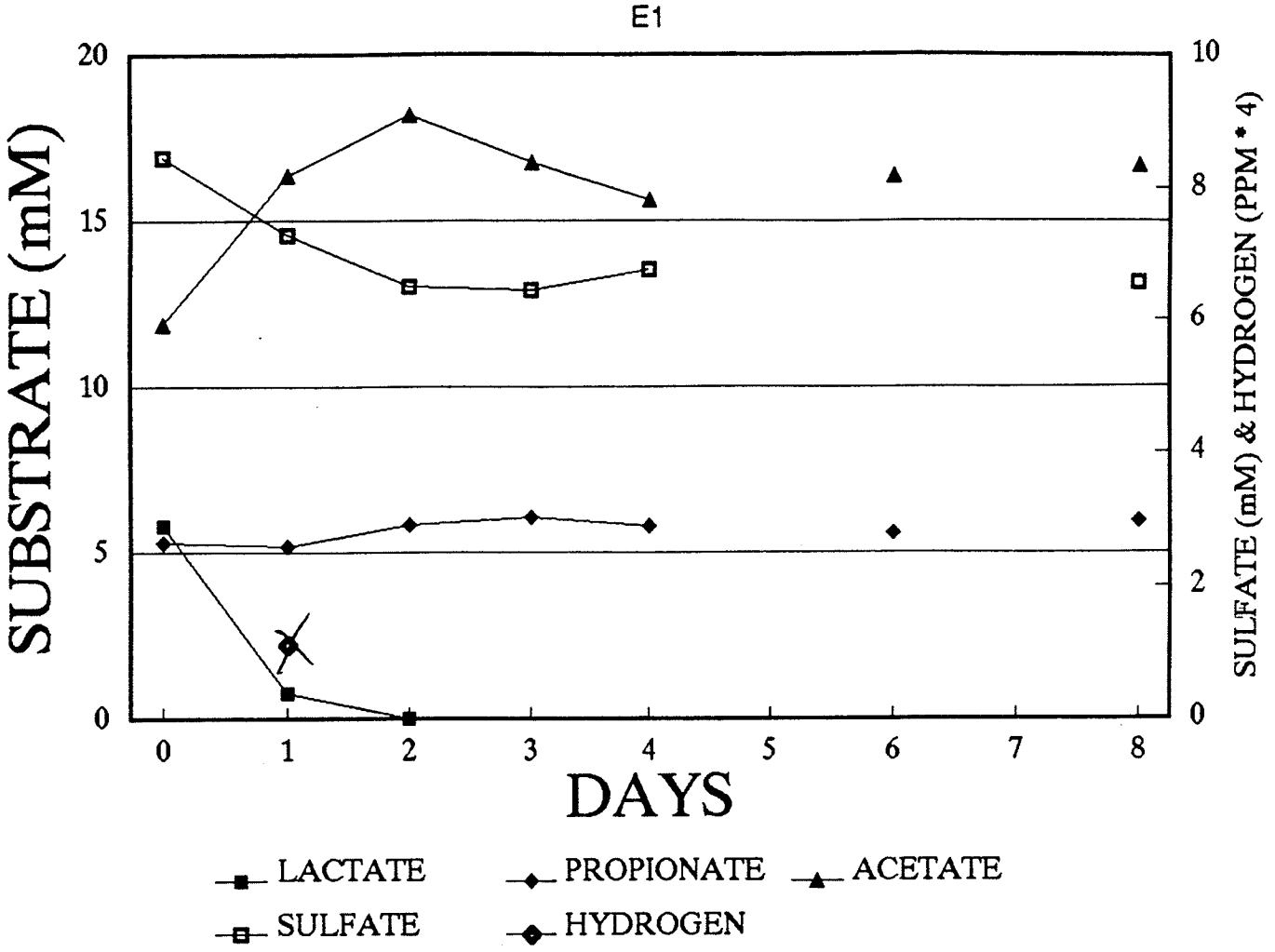
F 4.25



	pH (0/1)	TS	H_2S	CO_2	CH_4
ST.	7.1/7.1	520			
END	7.2	800		6.8	
AVG.					

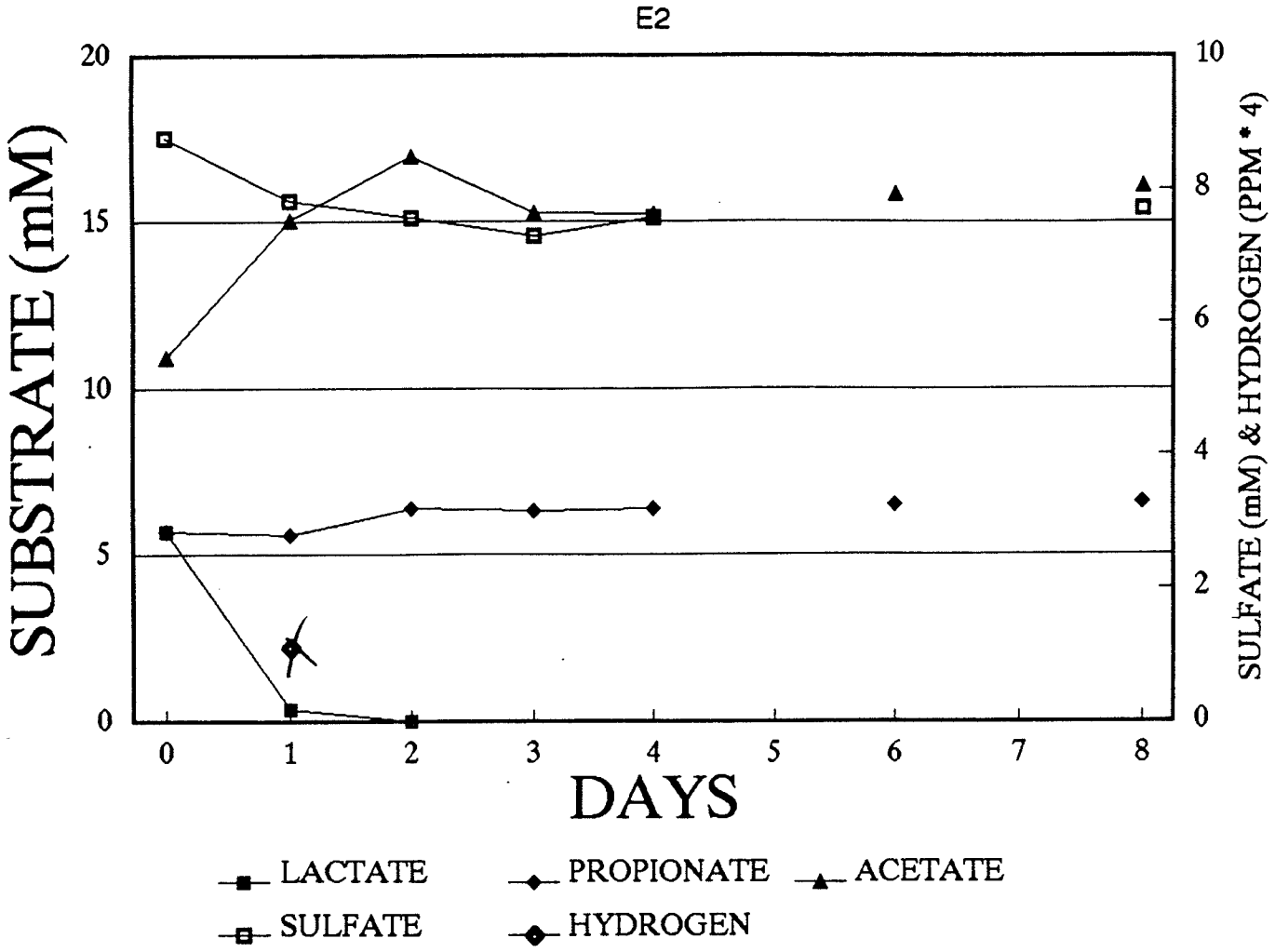


	pH (0/1)	TS	H_2S	%CH_4	-L CH_4
ST.	7.1/7.1	560			
END	7.3	960		trace	
AVG.					

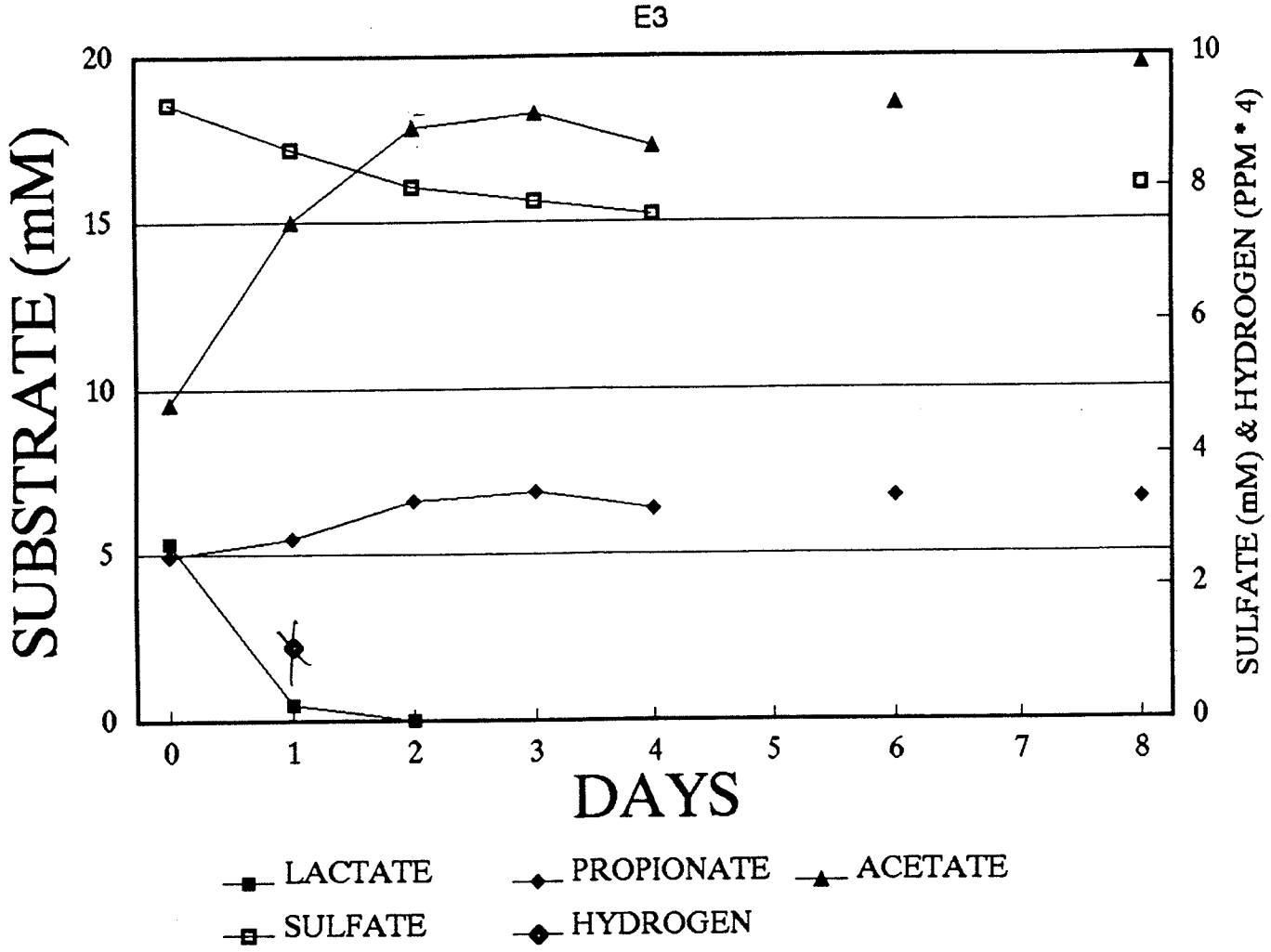


pH (6/1)	B	H_2S	% CH_4	mL CH_4
70/72	440			
72	560		14.9	

11.15

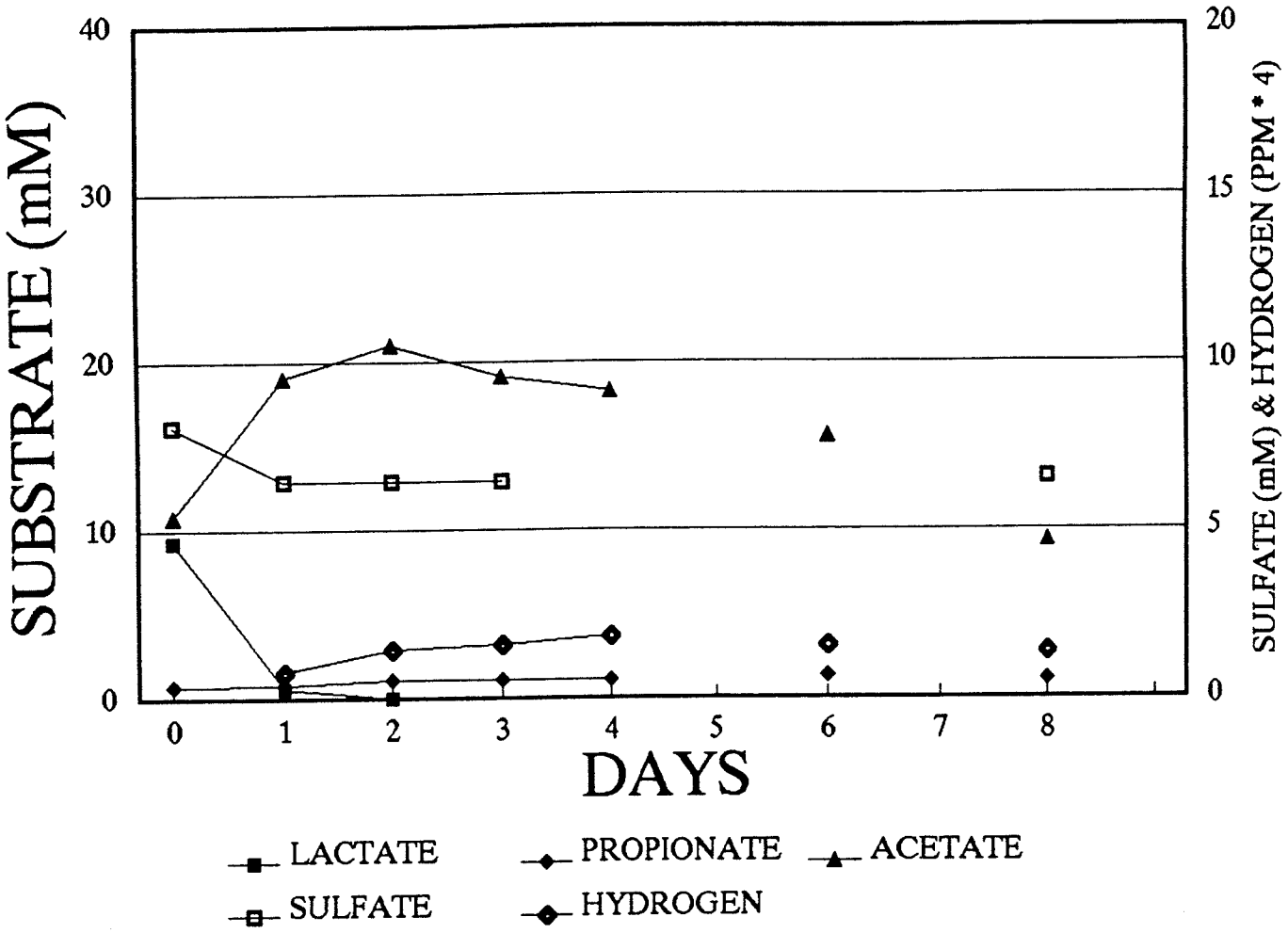


pH (0/1)	TS	H_2S	% CH_4	mL CH_4
7.0/7.2	480			
7.4	800		10.6	



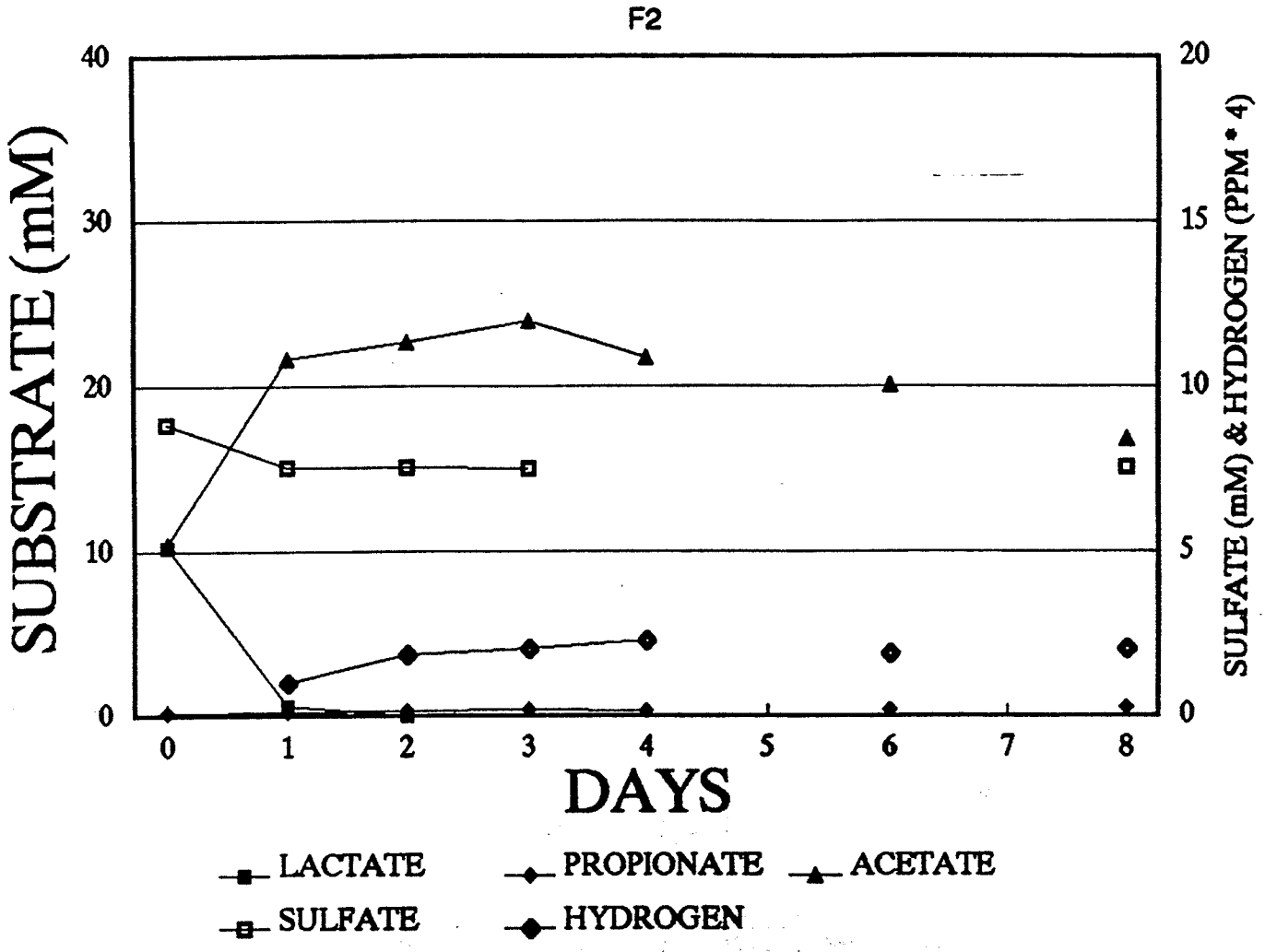
pH (0/1)	B	H_2S	o/o CH_4	nL CH_4
7.1/7.2	720			
7 3	80	84		trace

F1 Racemic La

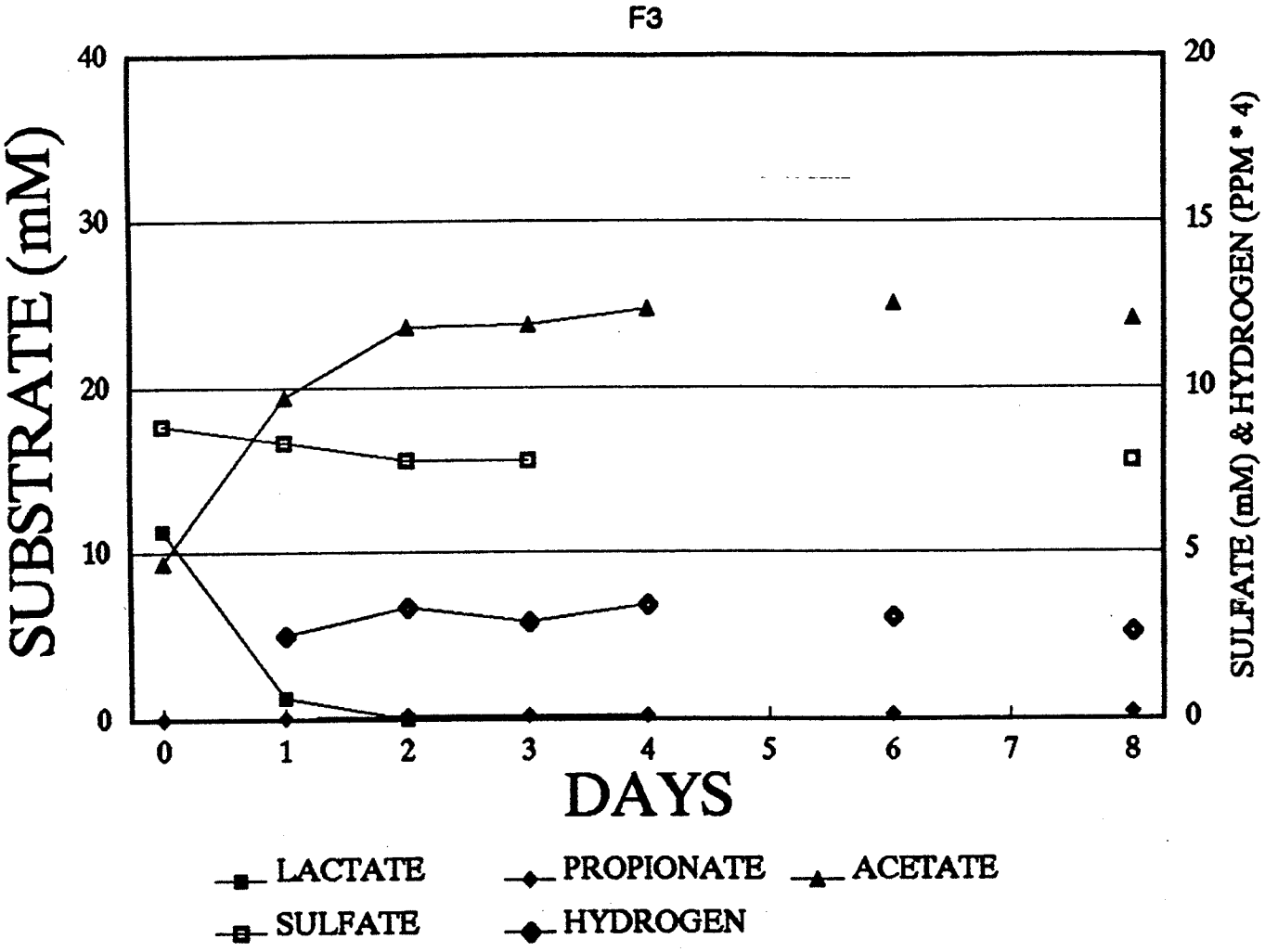


pH (0/1)	B	H_2S	% CH_4	mL CH_4
7.0/7.0	360			
7.2	480			208

F 4.27



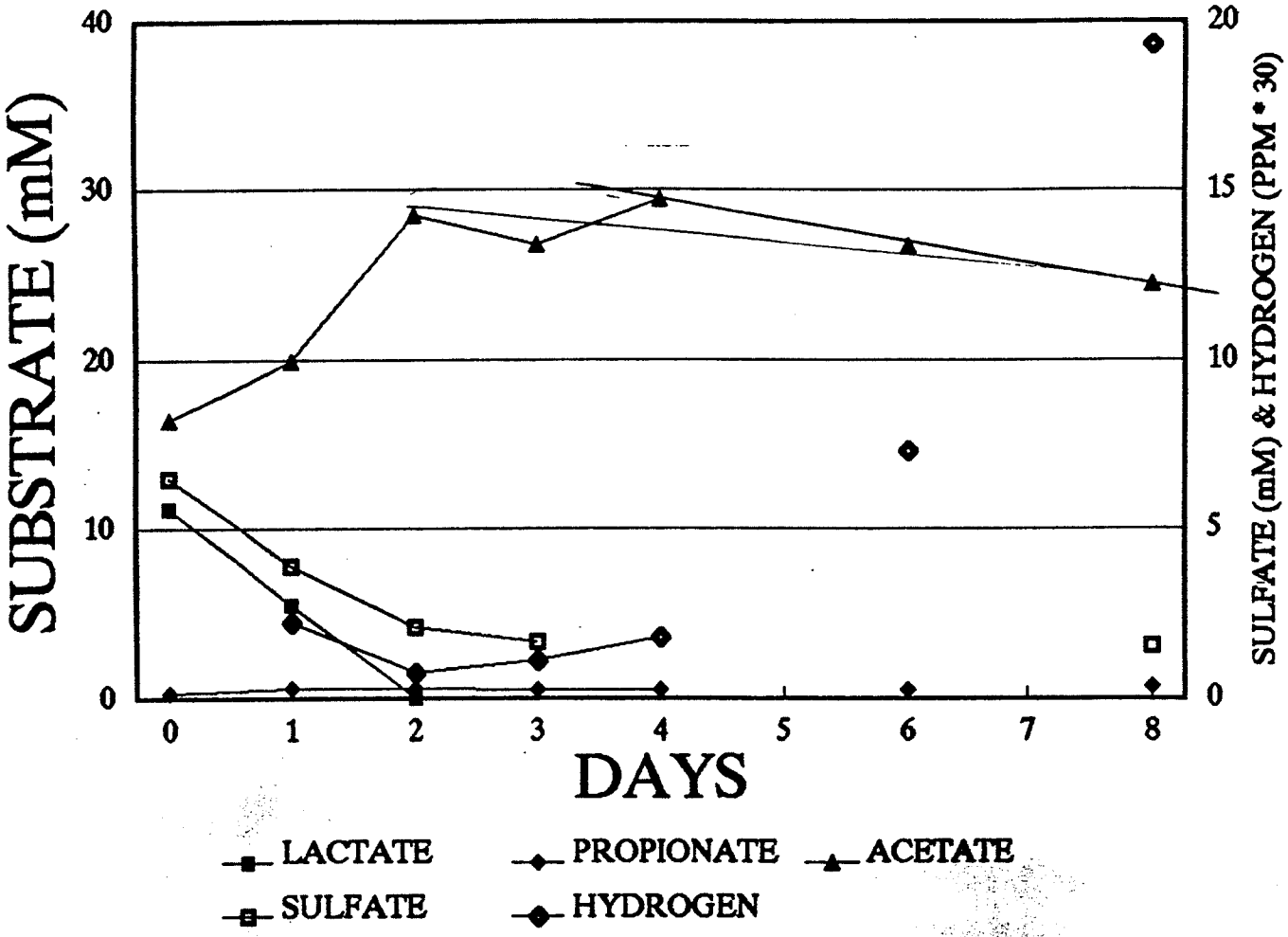
pH (0/1)	TS	H_2S	o/o CH_4	mL CH_4
7.1/7.1	440			
7.2	740		12.2	



7.1/7.1 600

7.2 840 0

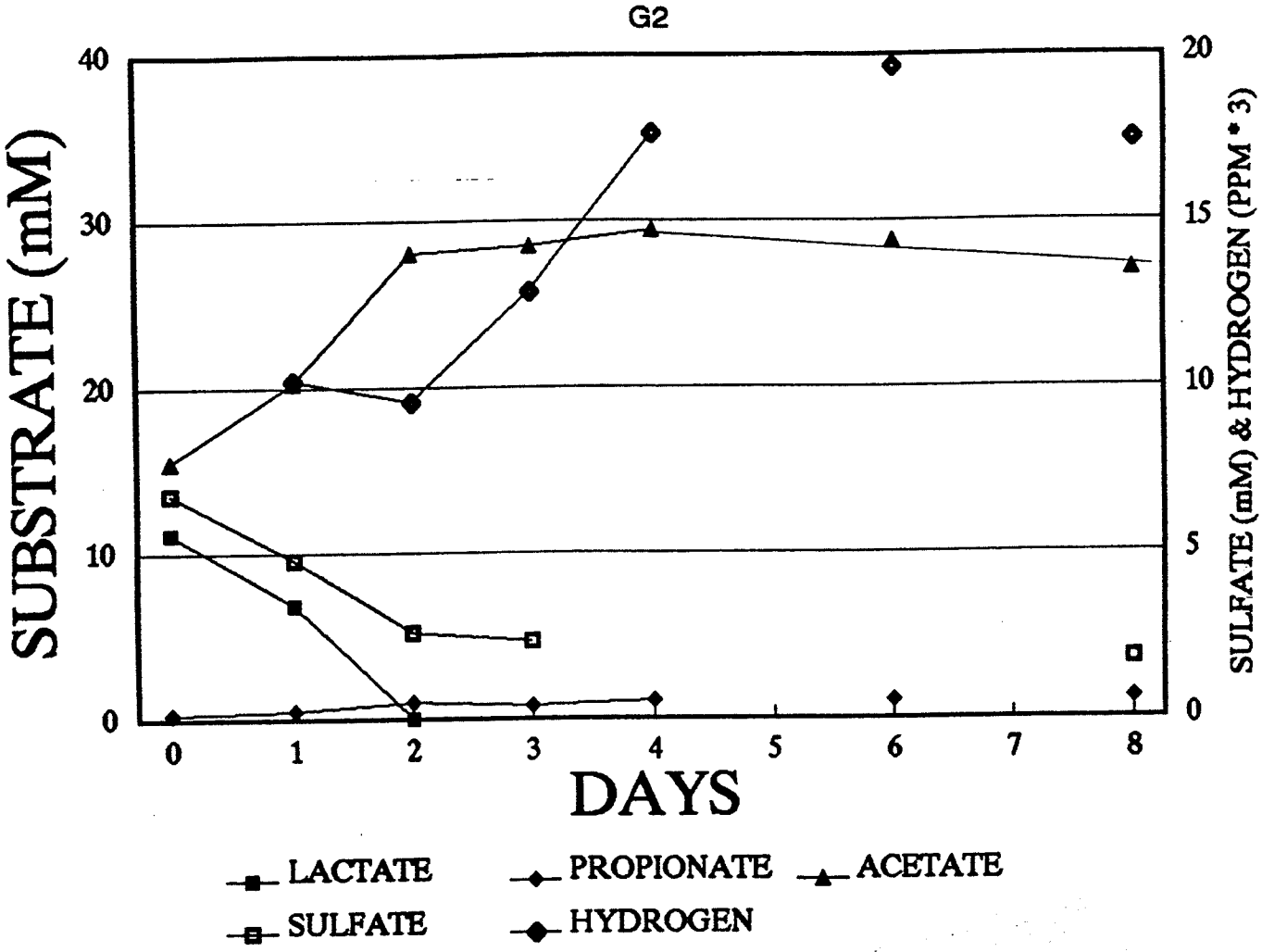
G1 CB - control pH8



8.2/8.1 400

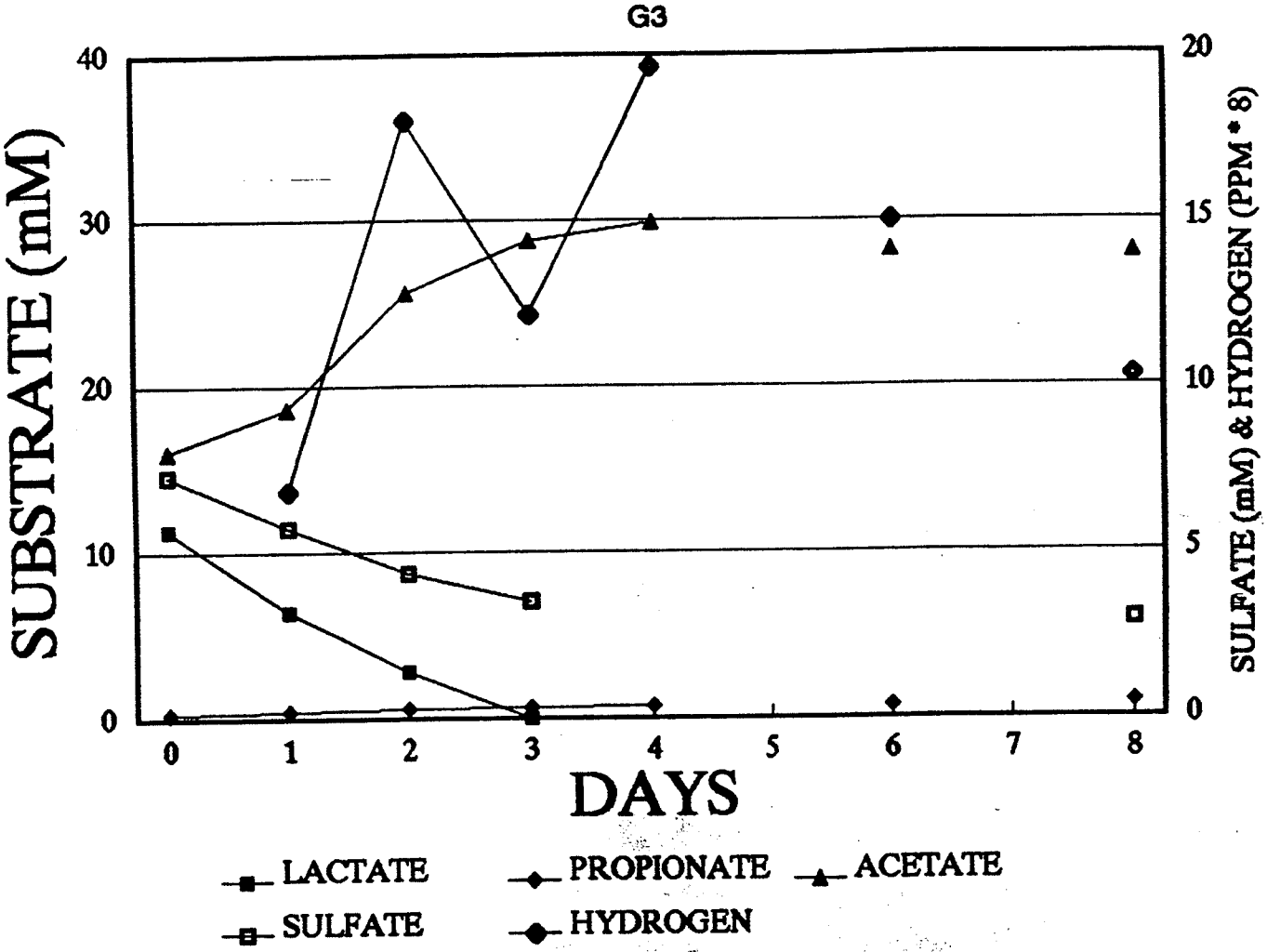
8.0 680 12.2

- H_2 removed in 2 days
- H_2 build-up after several days.



8.3/8.1 1000

8.1 1160 4.6

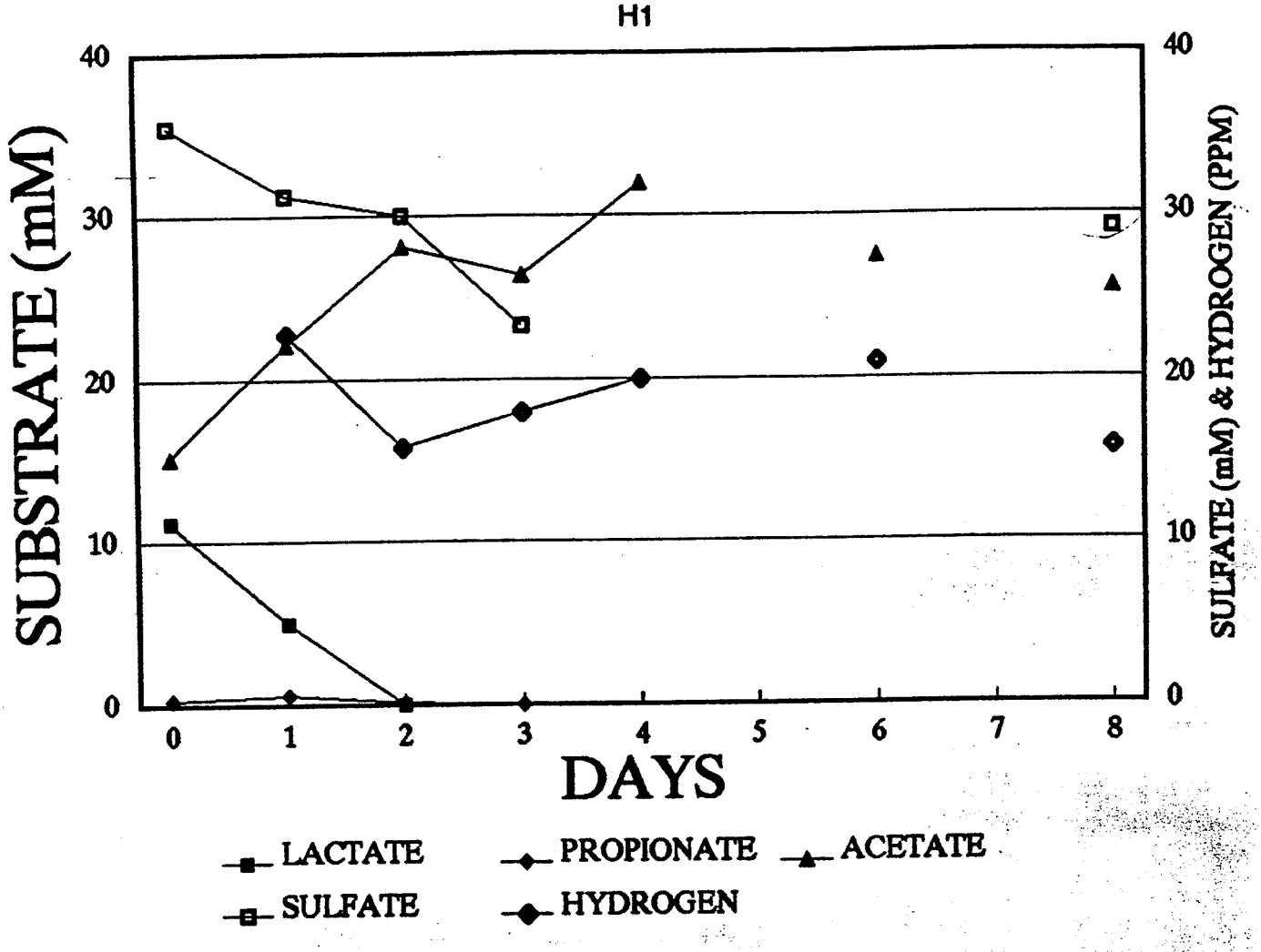


8.3/8.1 2000

8.1 2000

1.4

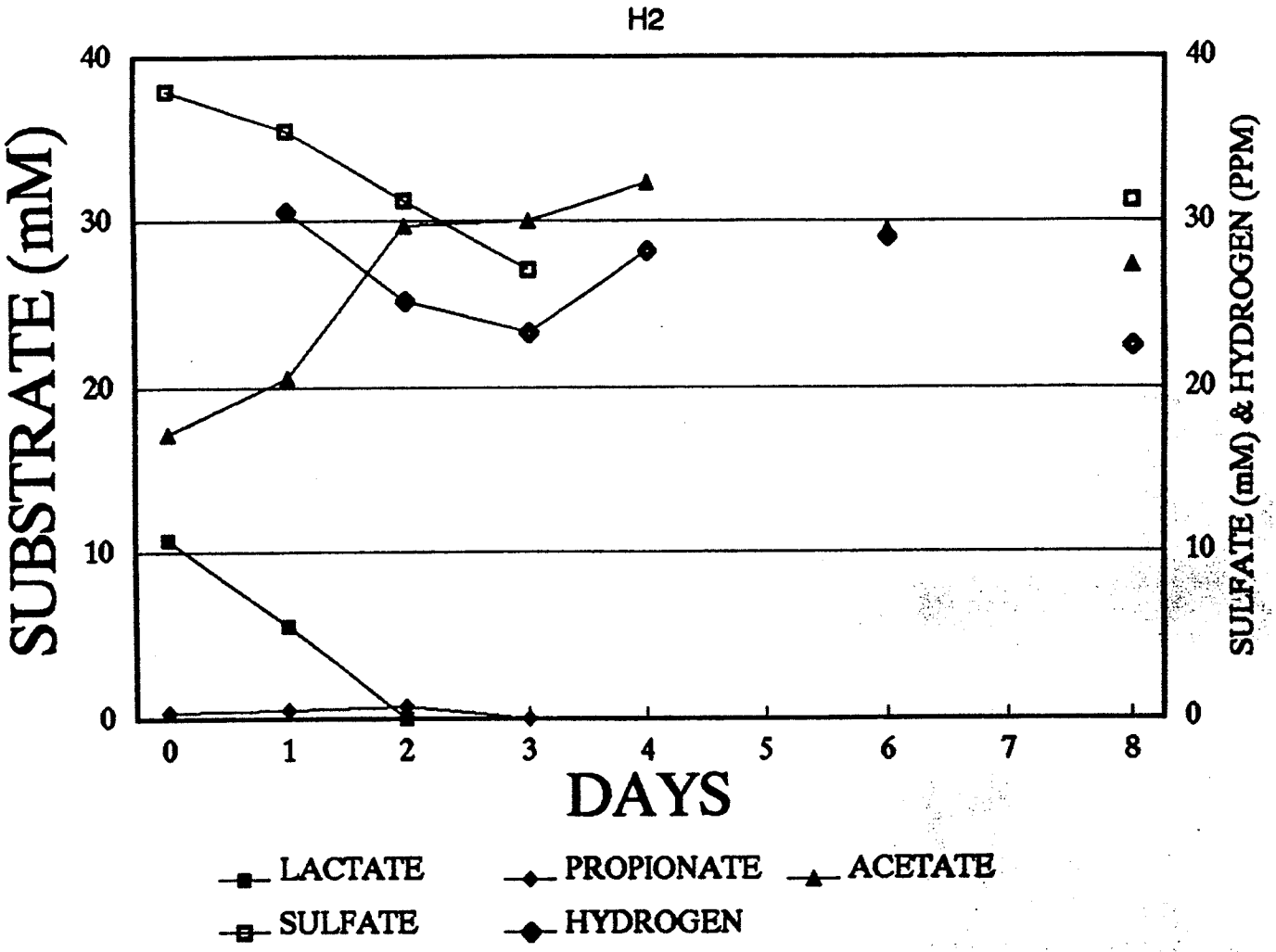
Hyl SO_4^{2-}



8.3/8.1 400

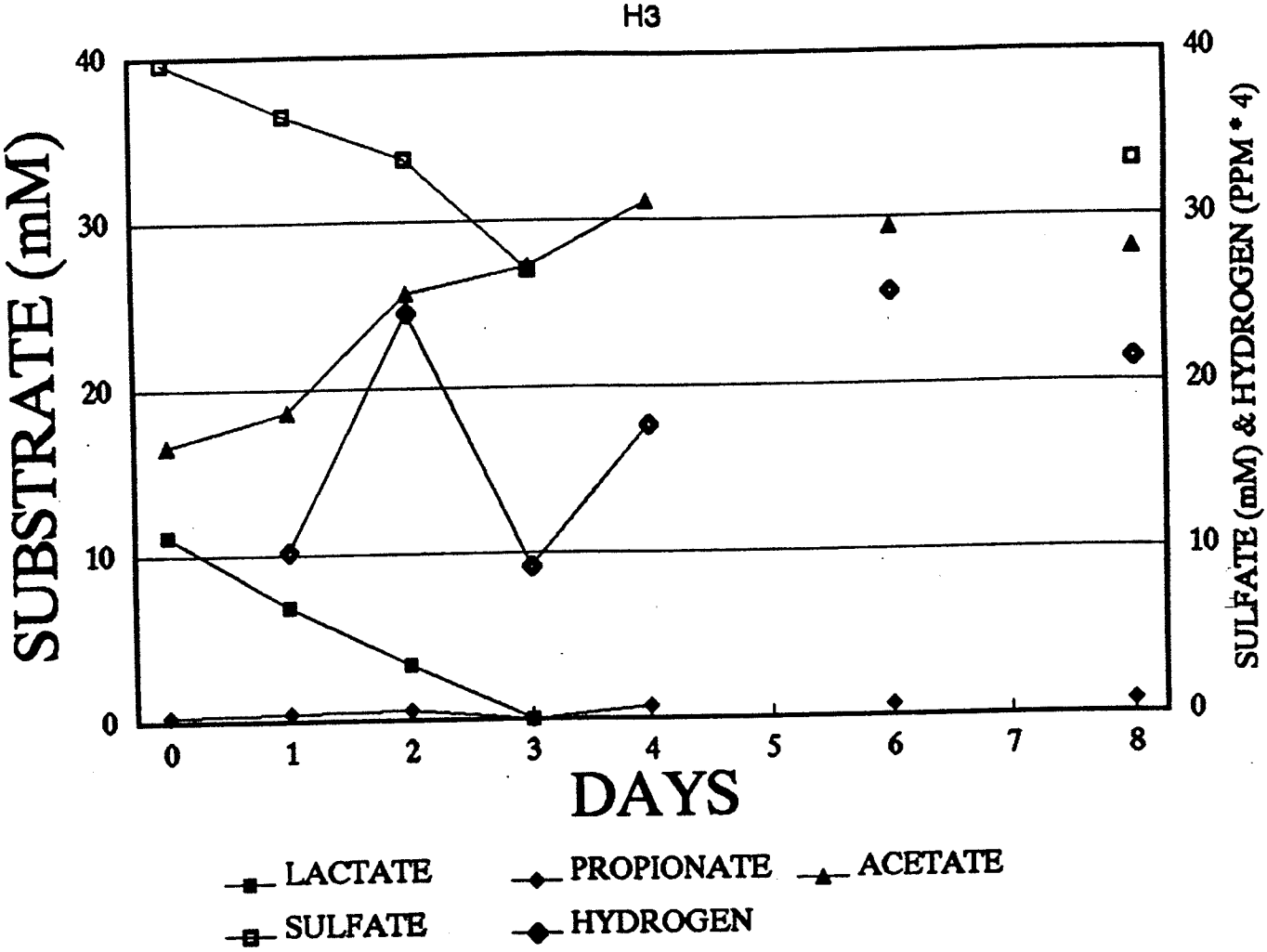
8.1 960 7.3

SO_4 values on Day 3 & 8



8.3/8.2 1000

8.1 1240 3.0



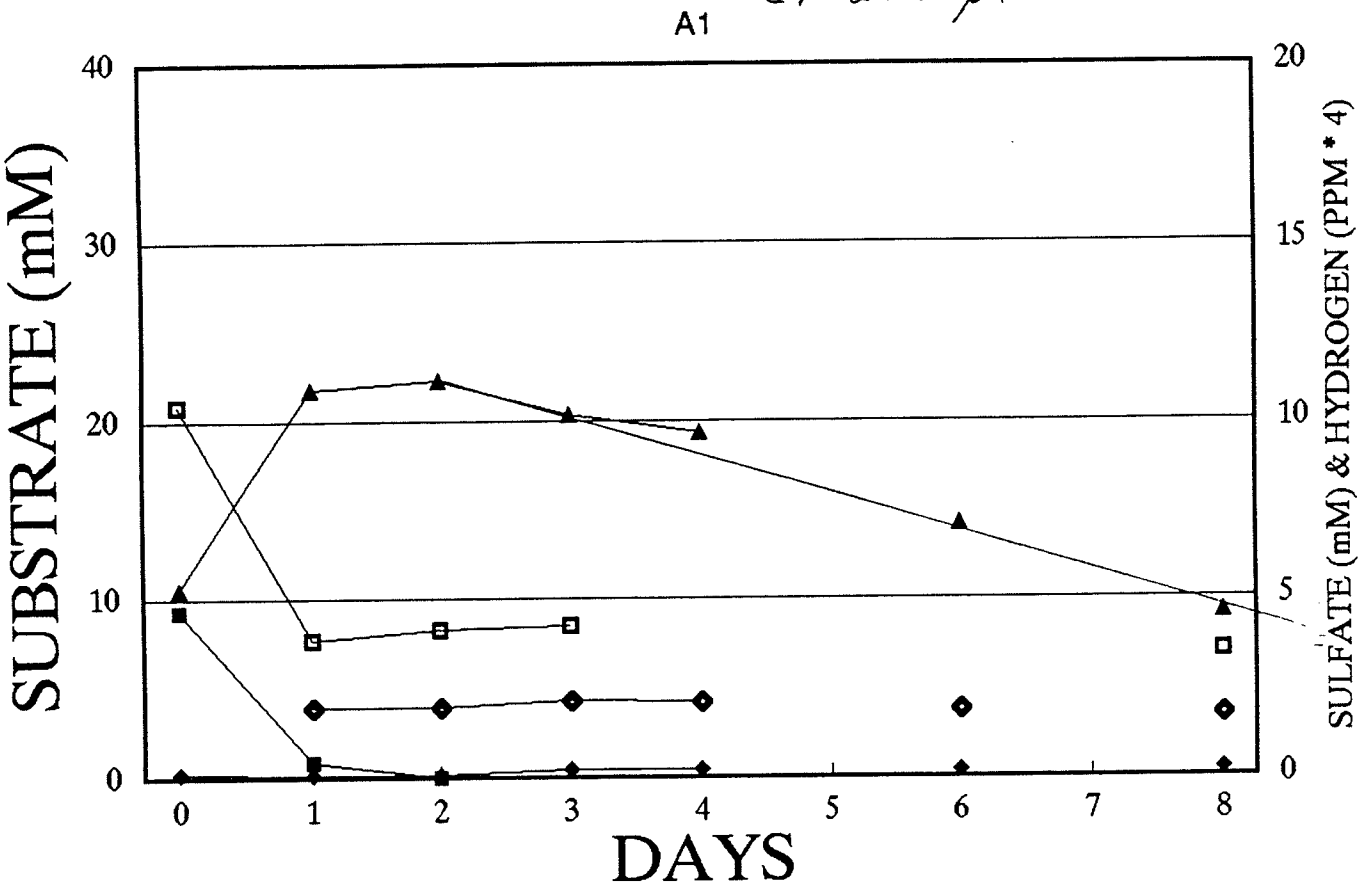
8.3/82 2040

8.1 2080 0.9

App. C
Phase 4

PHASE 4

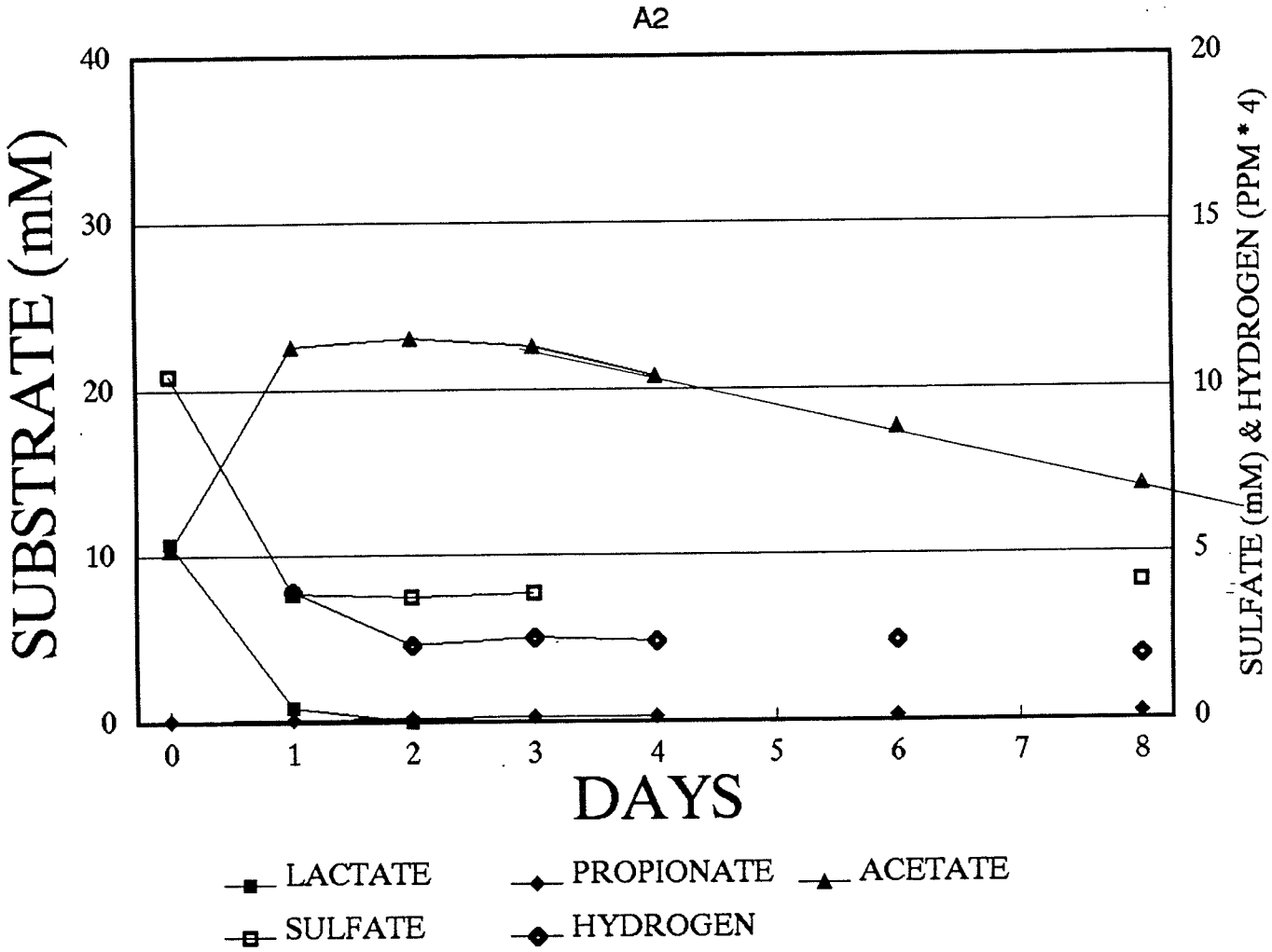
C7 - control pH7



Day 0 / Day 1 ≡ (0/1)

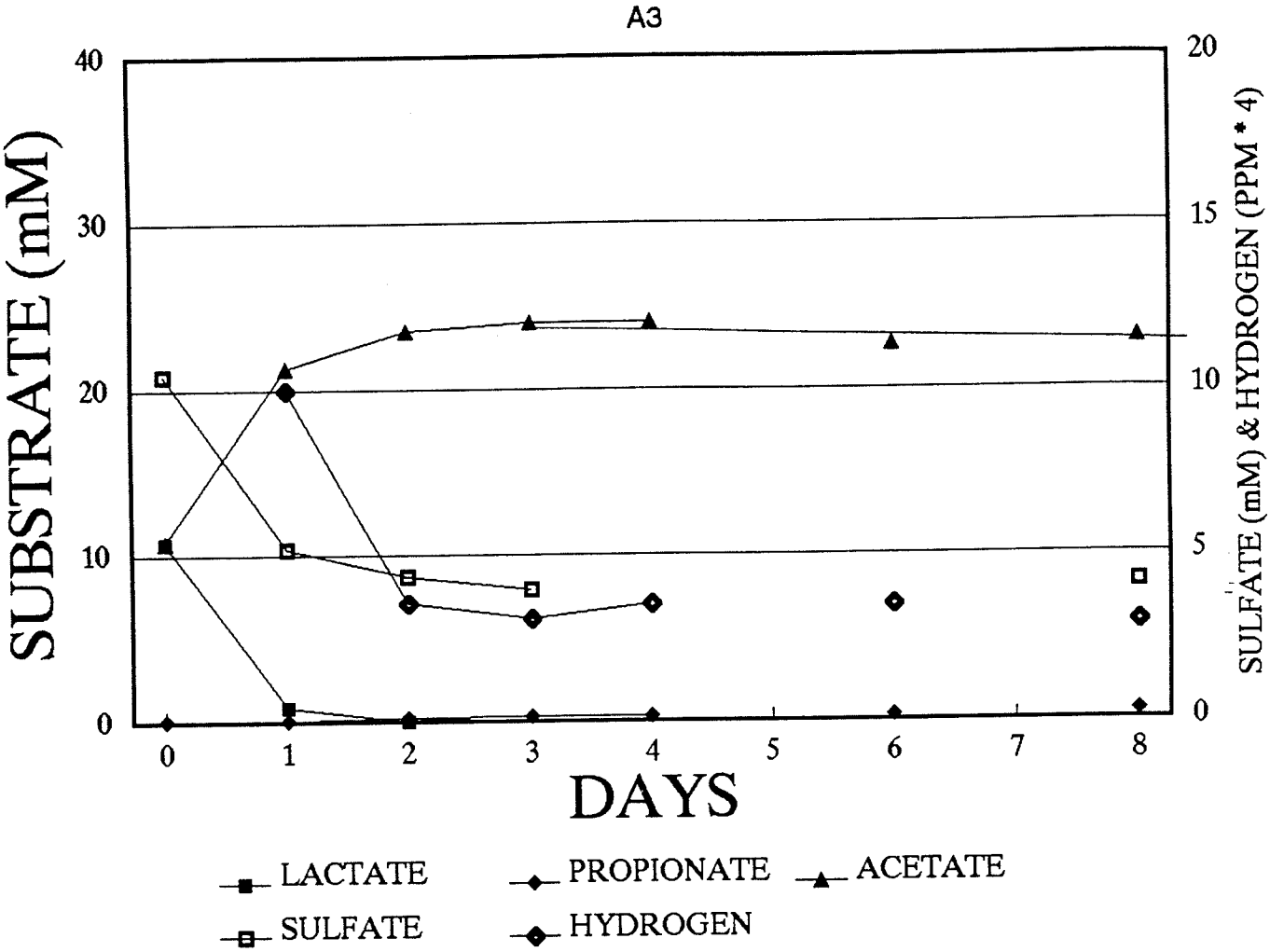
	pH (0/1)	S^{2-}	H_2S	% CH4	mL Gas	mL CH4
ST.	6.9/7.0	400				
EMS	7.1	440		21.4		
HVG.						

- H2a removal immediate in all reactors, no S^{2-}
- no H_2 build-up after all S^{2-} removed.



	pH (0/1)	TS	H_2S	% CH_4	mL CH_4
ST.	7.17	500			
EN.	7.1	560		16	
AVG					

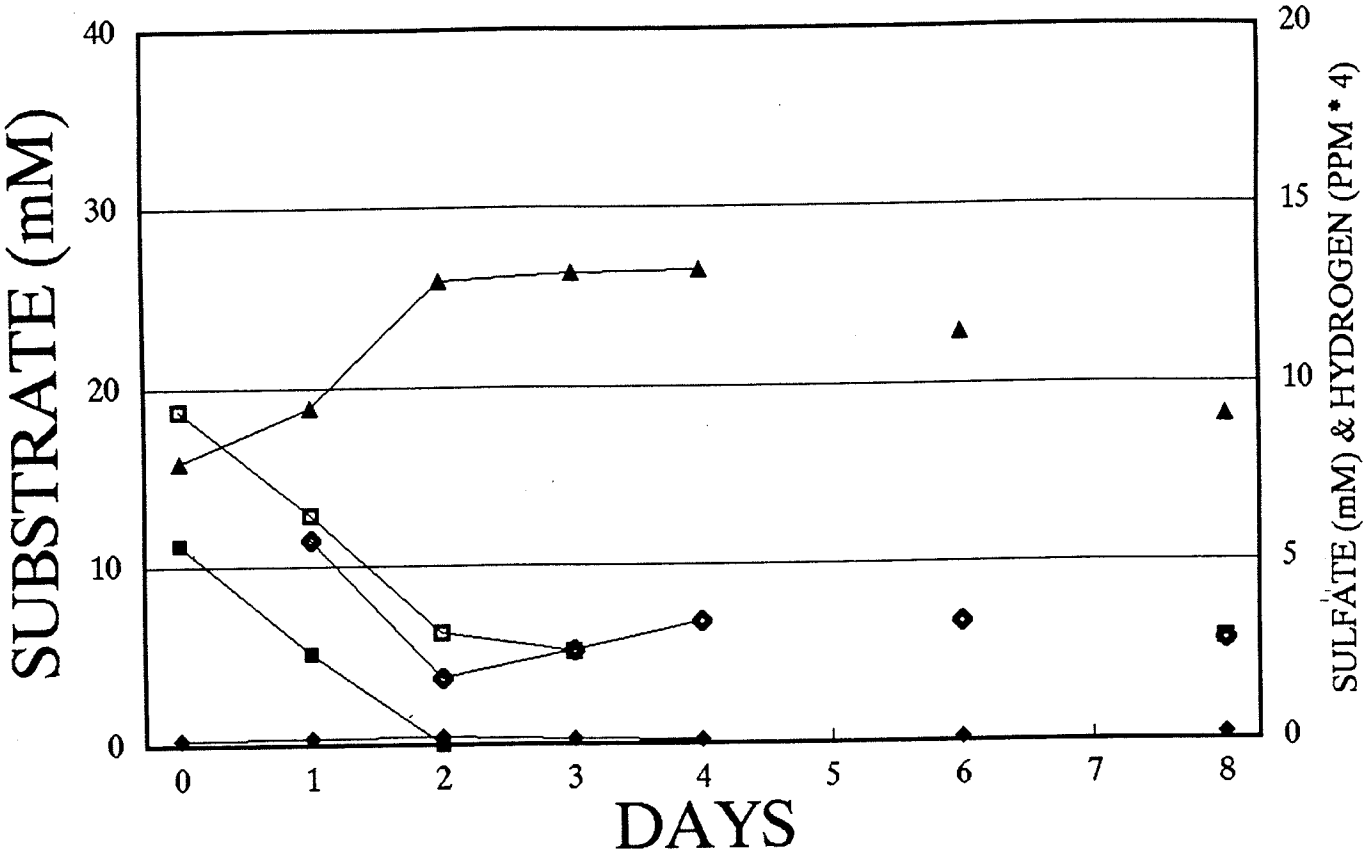
- slight H_2 build-up after 1d.



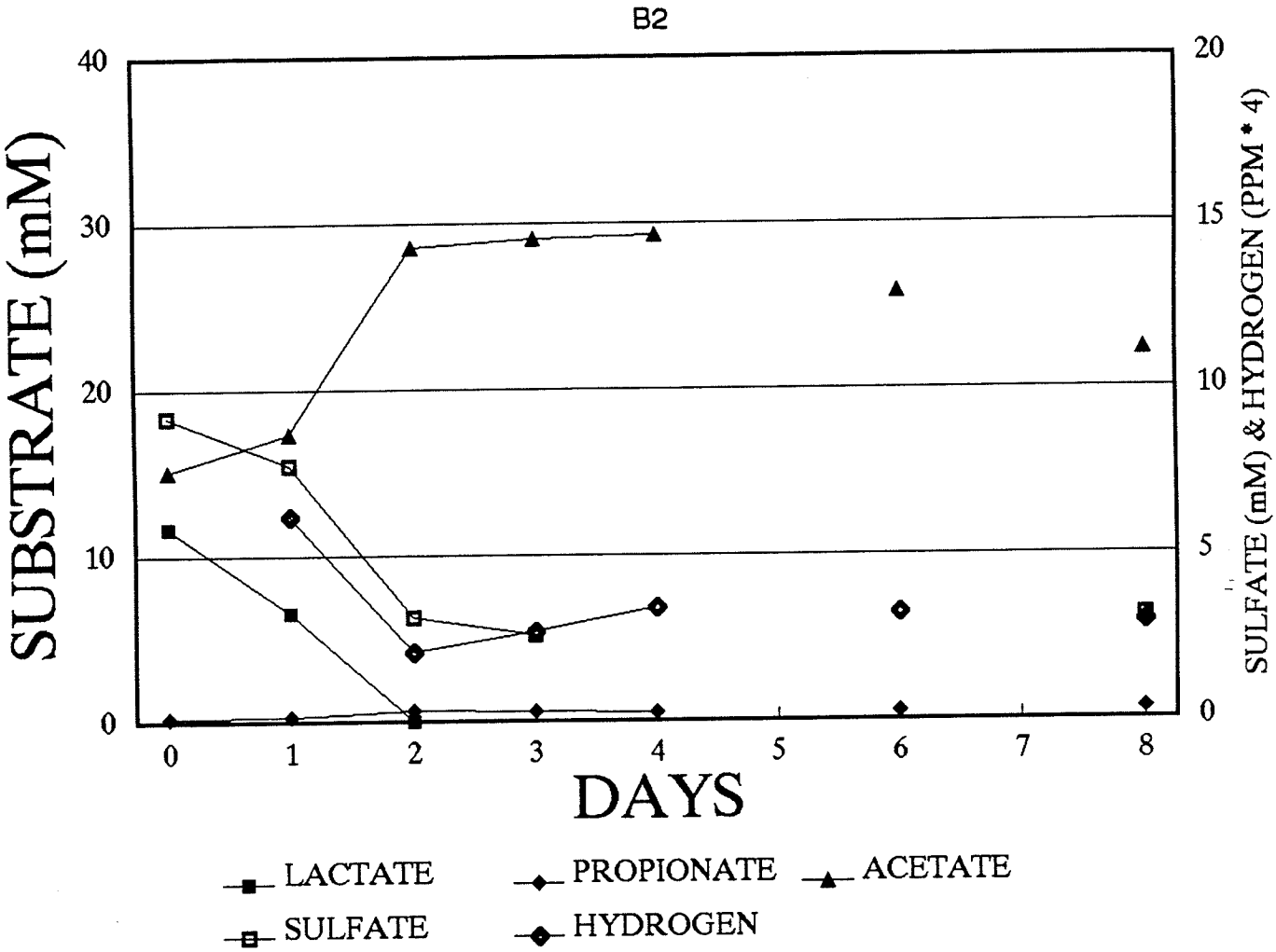
	pH	TS	H_2S	%CH_4	mL CH_4
	(0.1)				
ST	7.1/7.1	640			
EV.	7.2	700		0.3	
AVG					

- large H_2 build-up after 1 d.

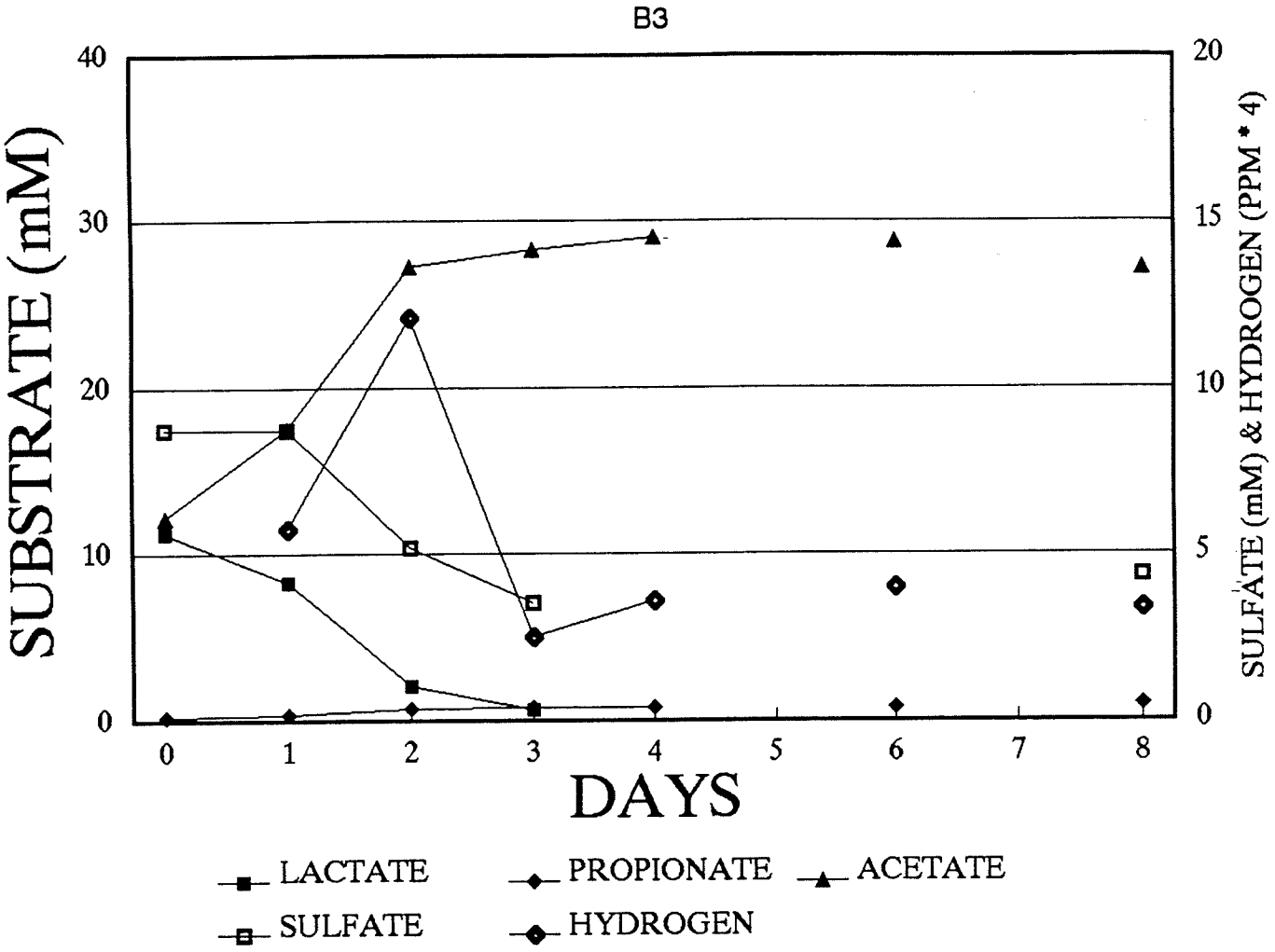
B1 pH 8↓7



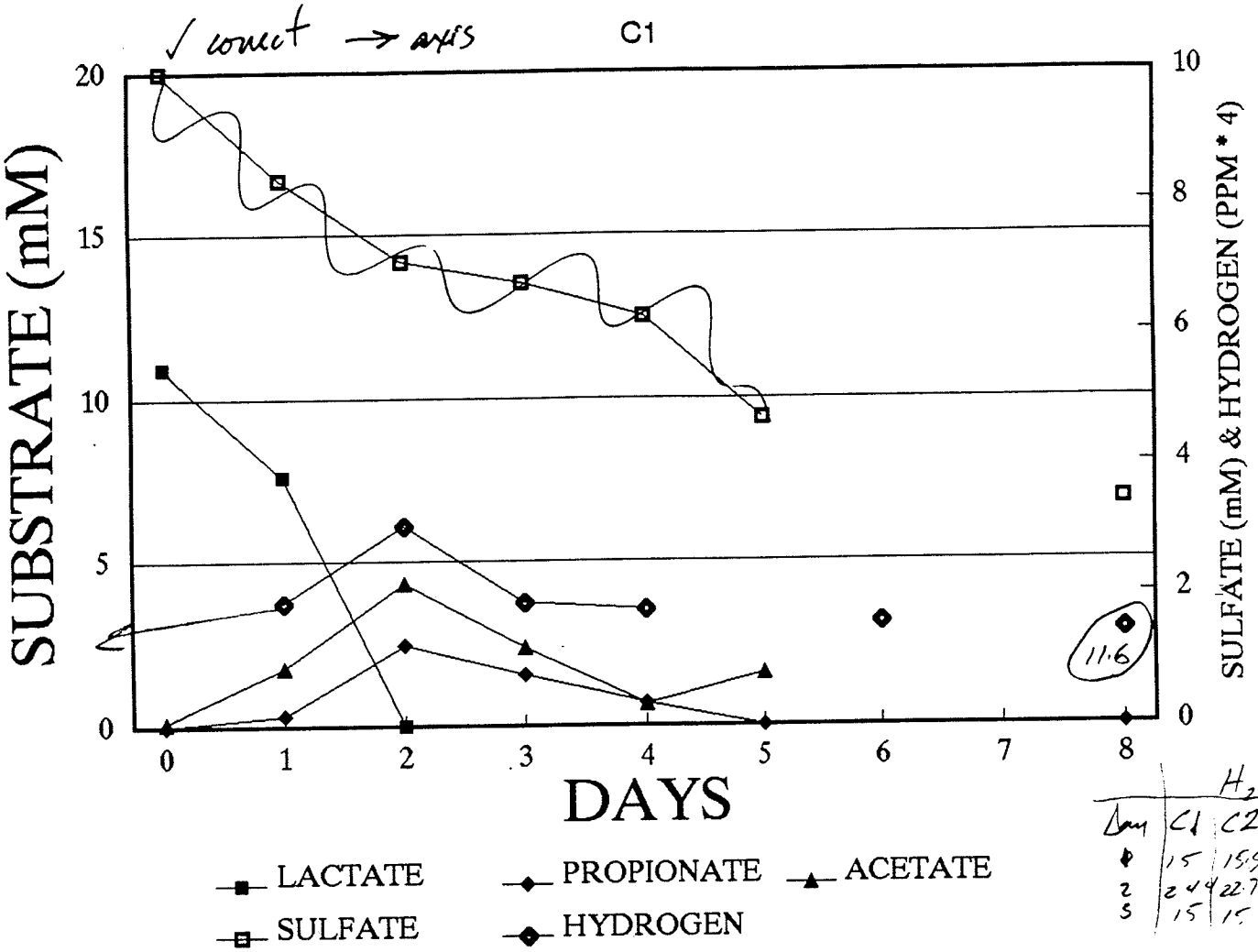
	pH (0/1)	TS	H_2S	% CH_4	mL CH_4
ST.	7.2/7.2	140			
EX.	7.4	440		17.1	
Inc.					



	H (0/1)	T3	H2S	% CH4	mL CH4
ST.	7.2/7.2	240			
EN.	7.3	480		10.6	
AVG.					



	pH (o/i)	TS	H_2S	o/o CH_4	mL CH_4
ST.	7.2/7.2	480			
END	7.4	640		1.9	
AVG.					

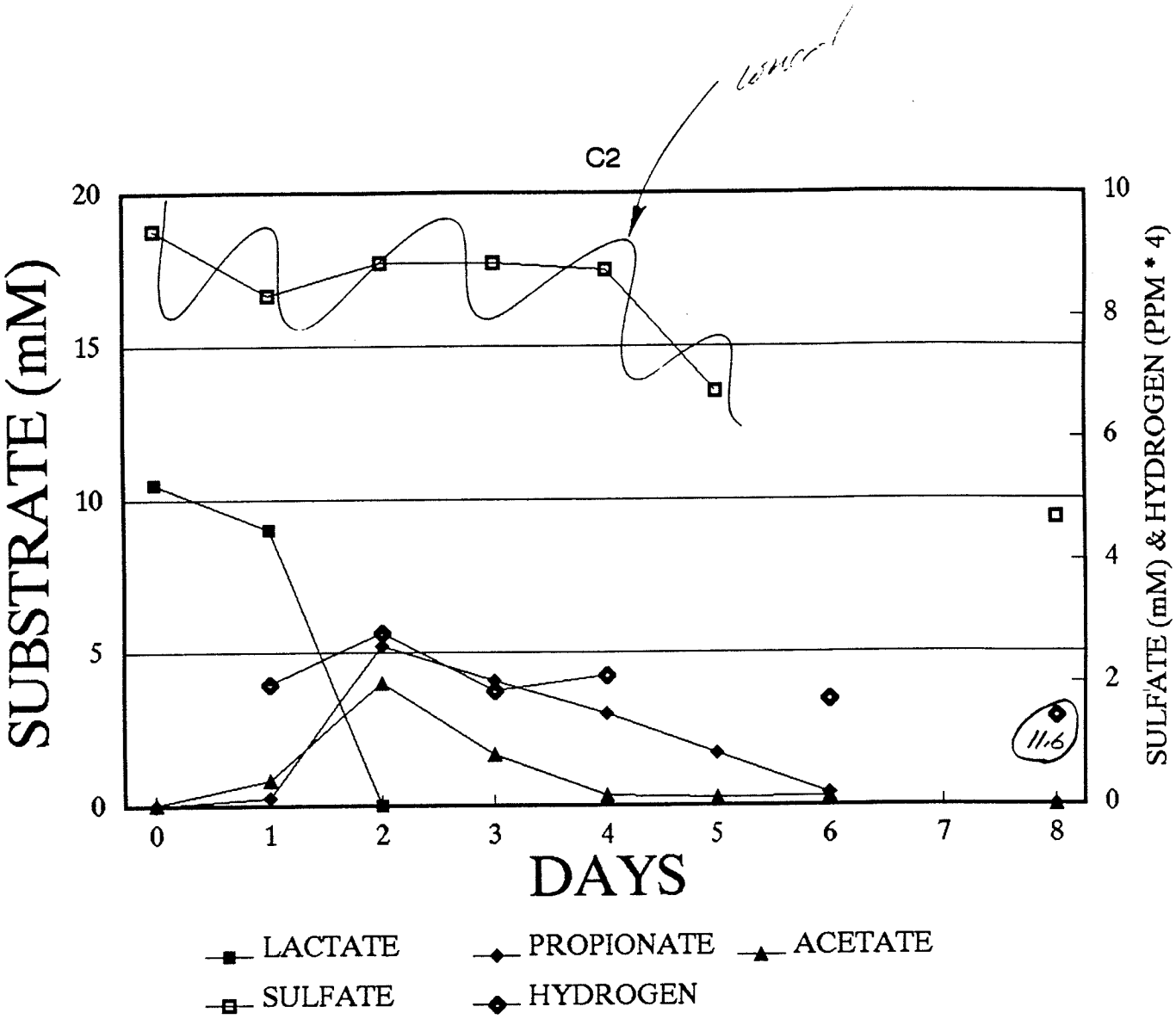


H_2

Day	C1	C2	C3
0	15	15.9	
2	24	22.7	
5	15	15	24

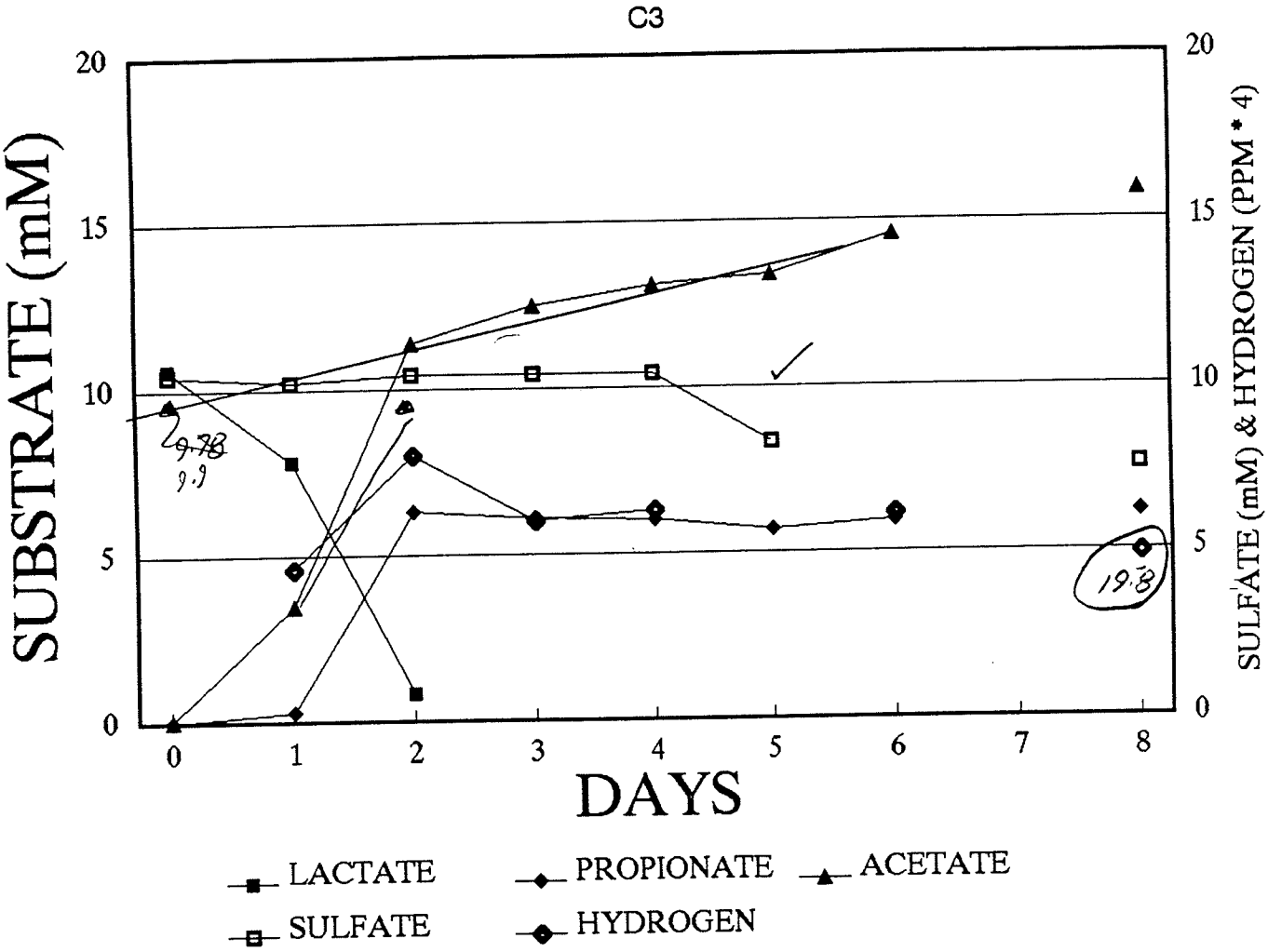
	pH (0/1)	TS	H_2S	%CH_4	mL CH_4
ST.	7.1/7.2	120			
END	7.2	300		36.5	
AVG.					

F4.2



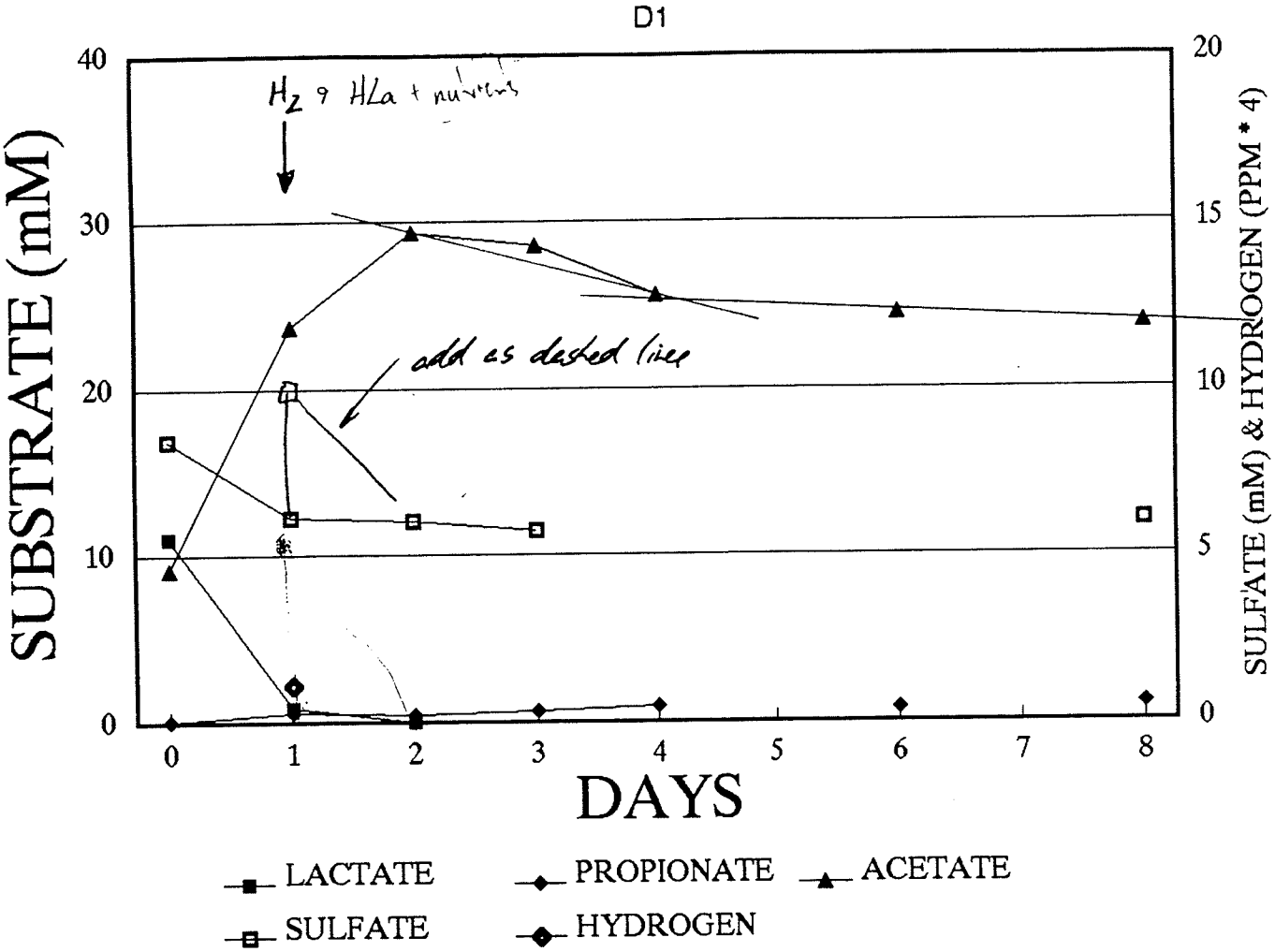
	pH	B	H_2S	% CH_4	mL CH_4
	0/1				
ST.	7.1/7.2	240			
END	7.2	300		25.7	
Avg.					

F 4.22



	pH (0/1)	TS	H_2S	% CH_4	mL CH_4
ST.	7.1/7.2	680			
END	7.2	480		13.3	
AVG					

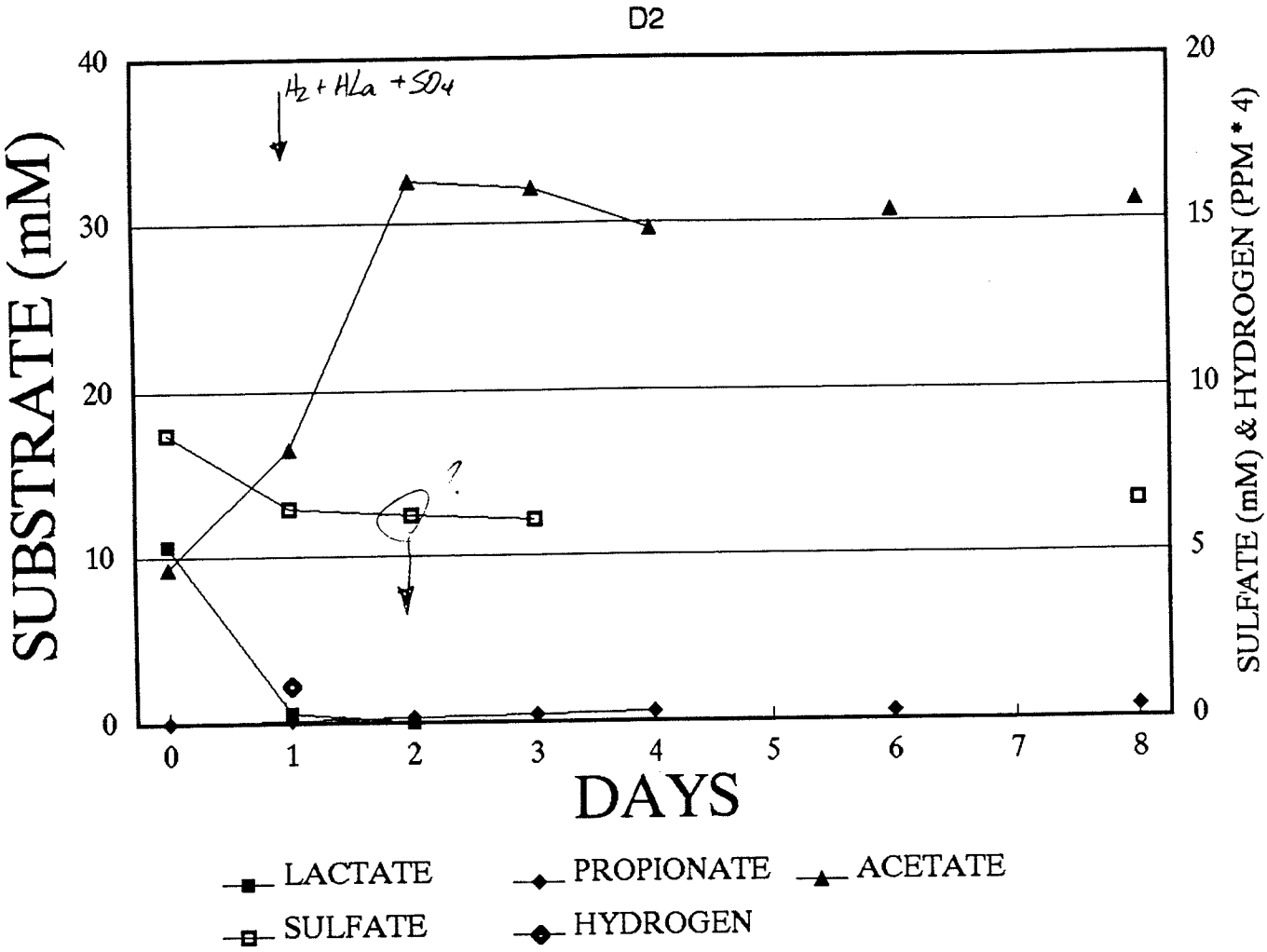
F 4-20



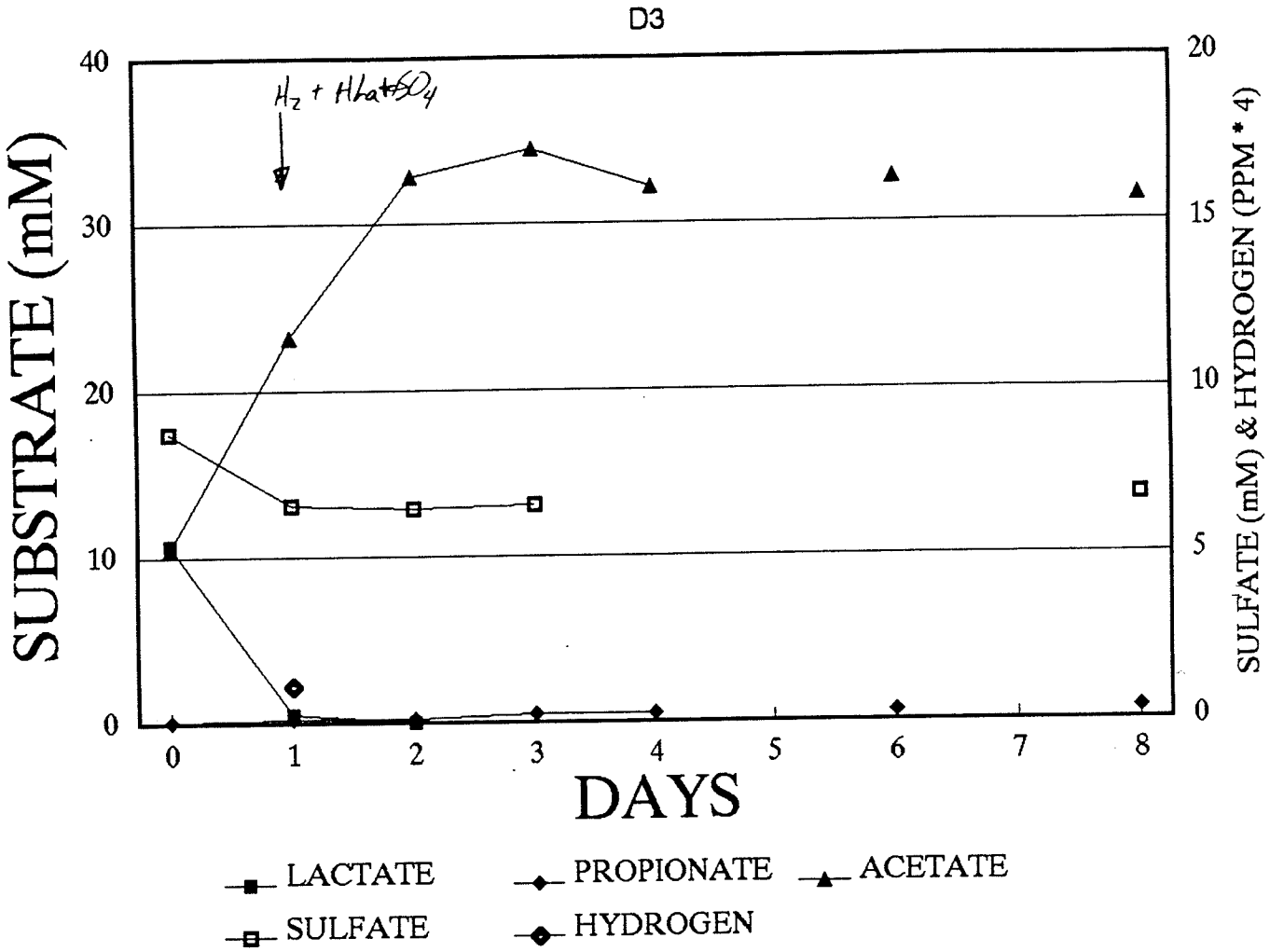
	pH (0/1)	B	H_2S	% CH_4	mL CH_4
ST.	7.1/7.1	400			
END	7.3	680		20.8	
AVG.					

- H_2 did not affect HLa degradation.

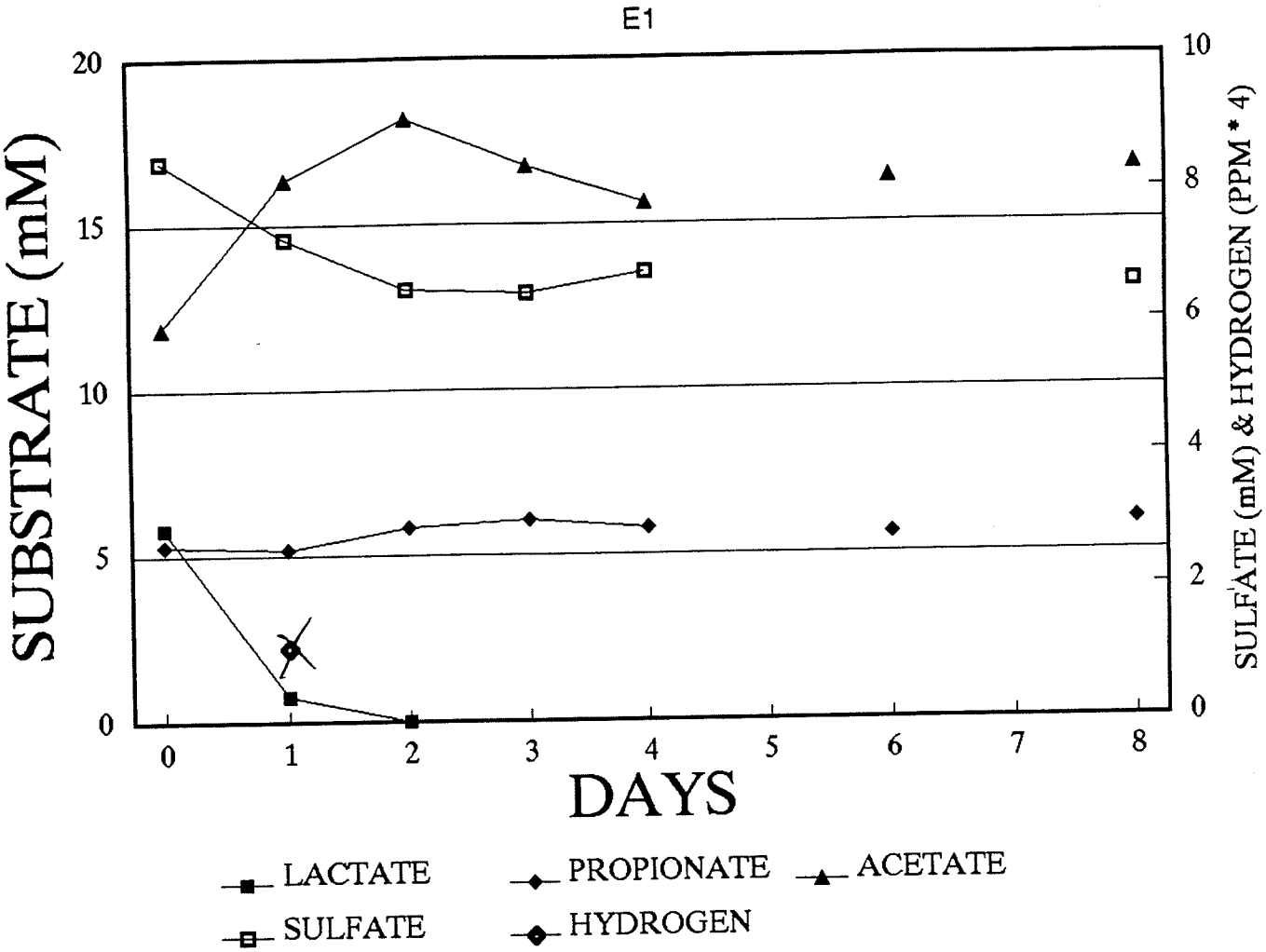
F 4.25



	pH (o/i)	TS	H_2S	odour	colour
ST.	7.1/7.1	520			
END	7.2	800		6.8	
HOL.					

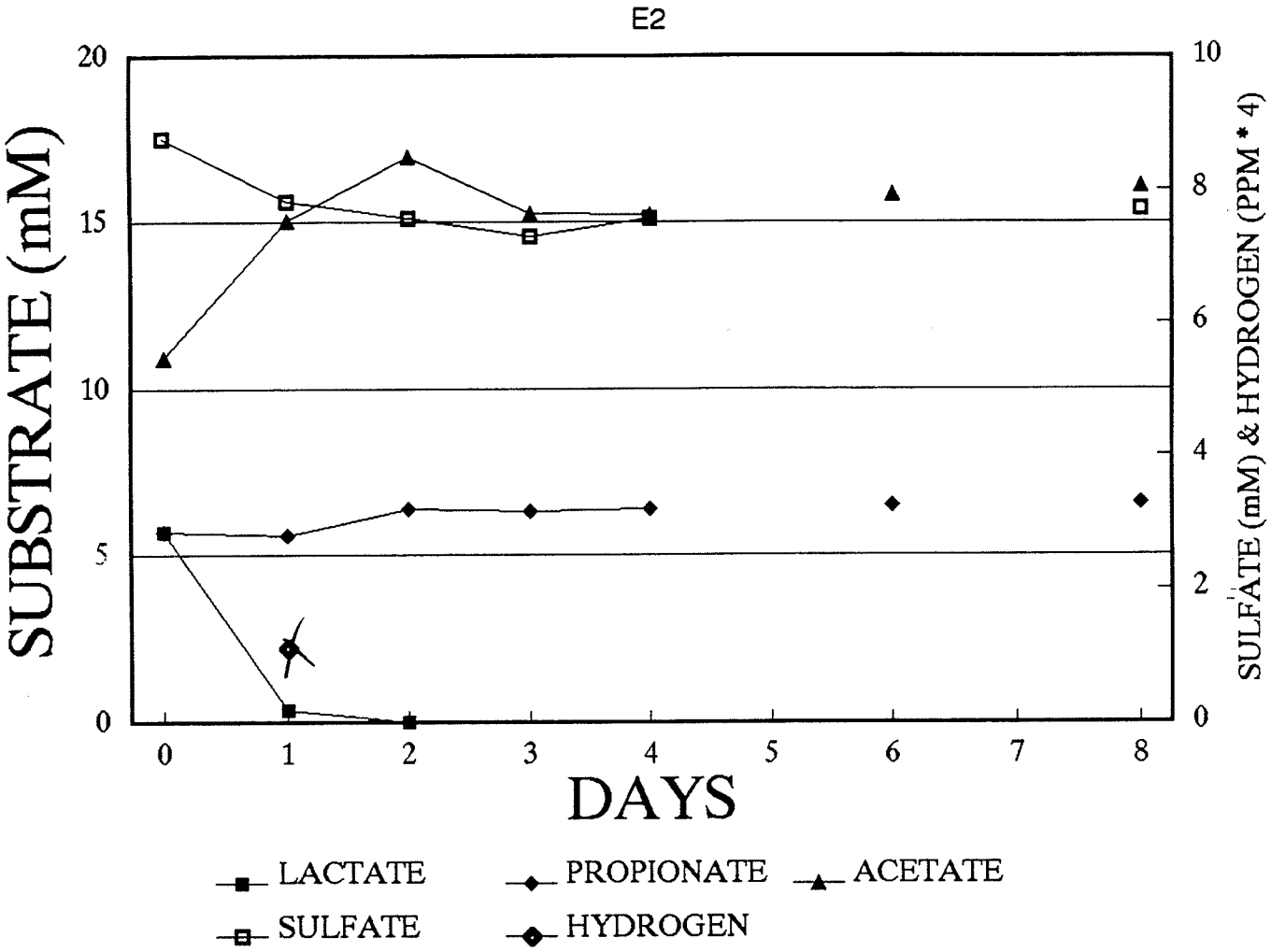


	pH (o/i)	TB	H_2S	%CH_4	-L CH_4
ST.	7.1/7.1	560			
ONB	7.3	960		trace	
ANC.					

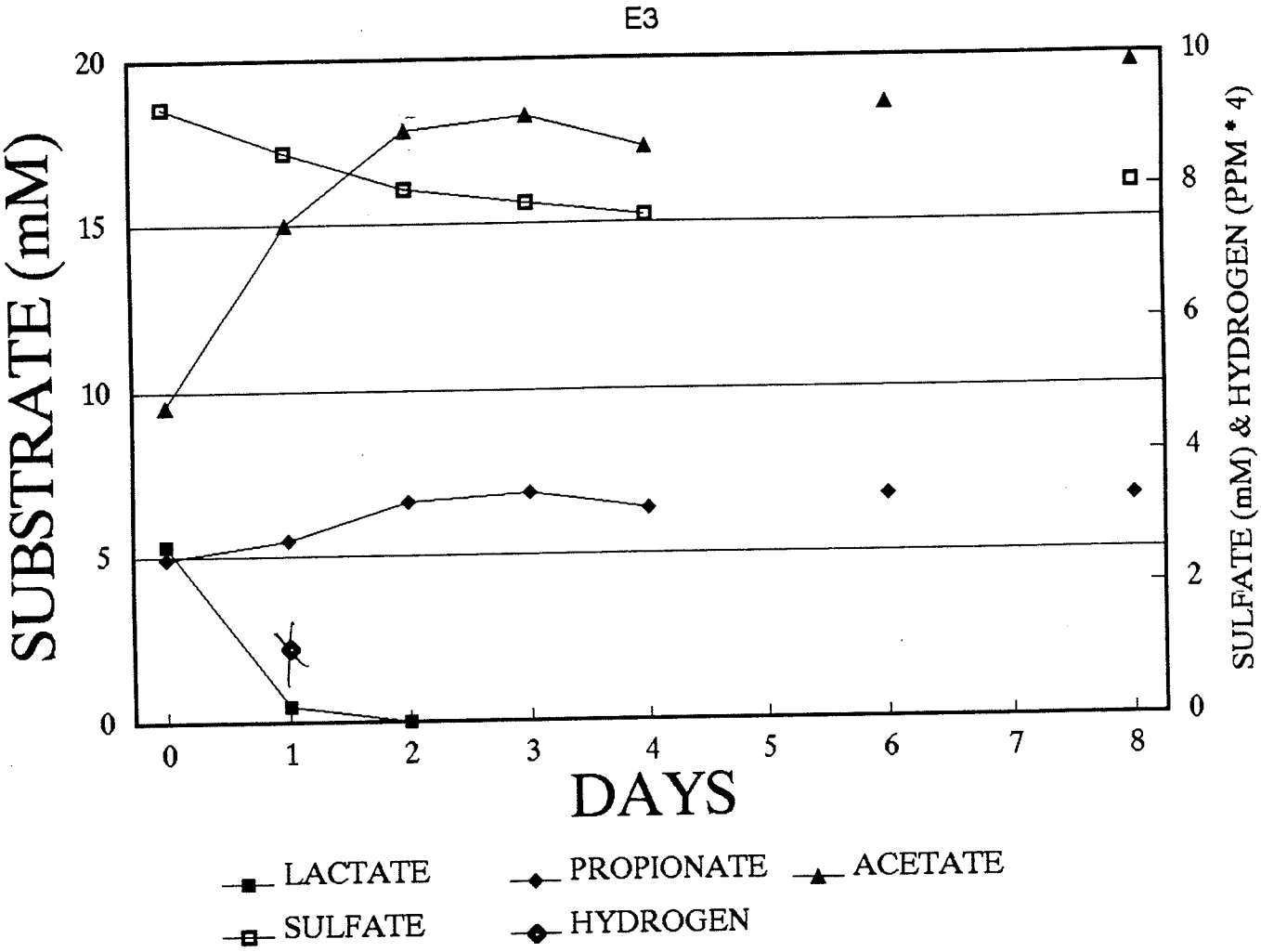


pH (o/i)	TS	H_2S	o/o CH_4	mL CH_4
7.0/7.2	440			
7.2	560		14.9	

11.15

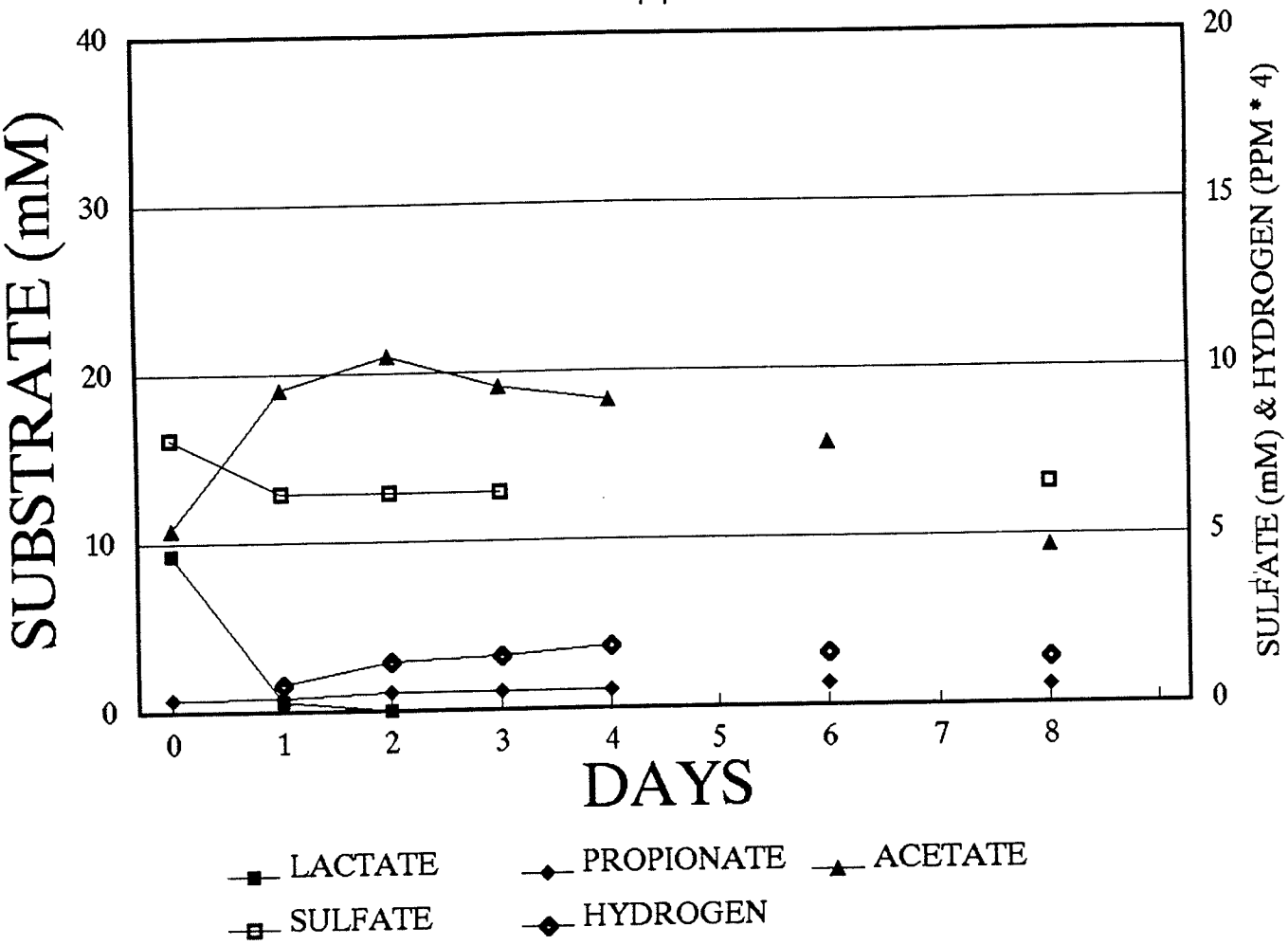


pH (o/i)	B	H_2S	o/o CH_4	mL CH_4
7.0/7.2	480			
7.4	800		10.6	



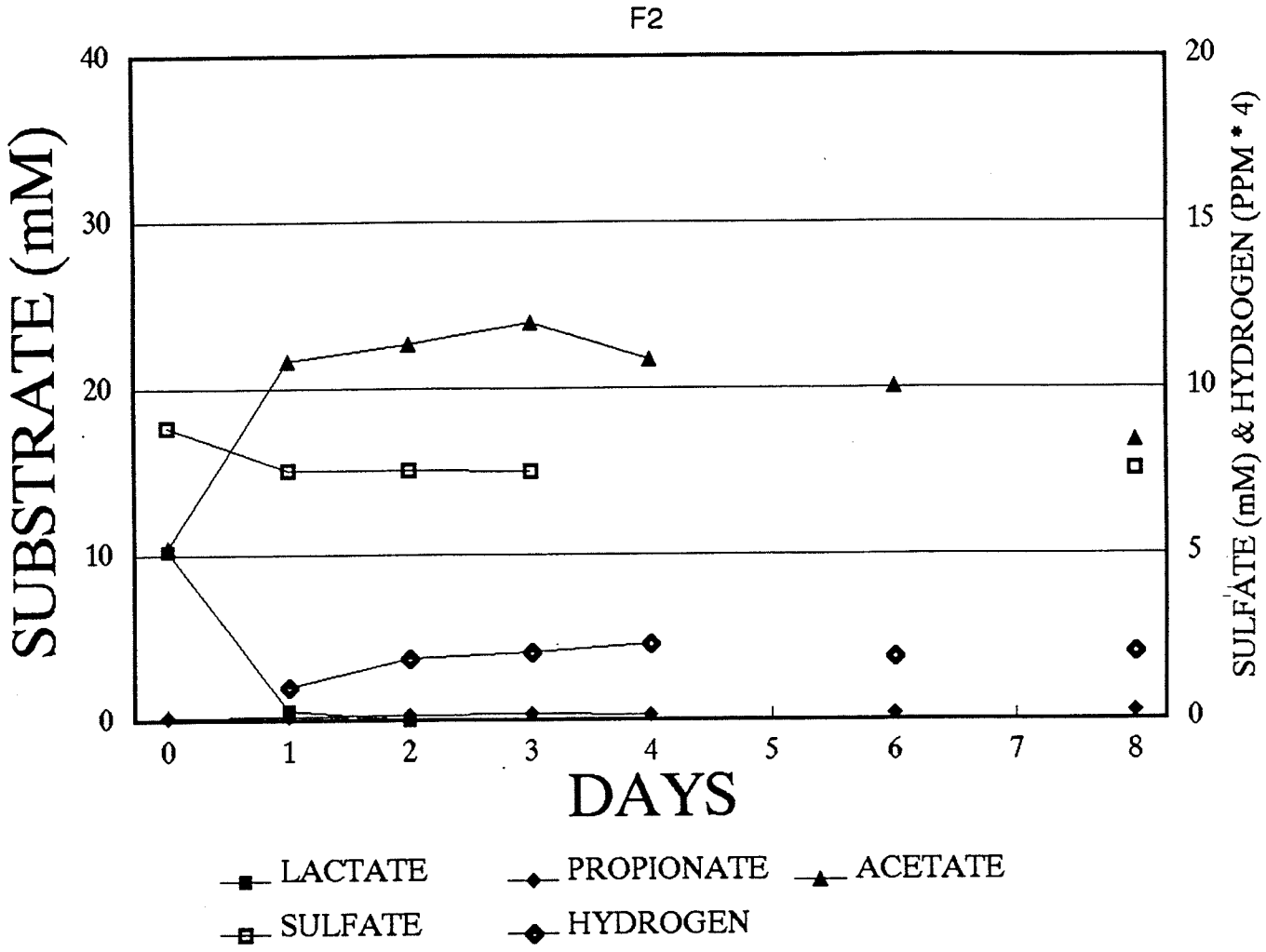
pH (0/1)	B	H_2S	o/o CH_4	nL CH_4
7.1/7.2	720			
73	808		trace	

F1 Reservoir

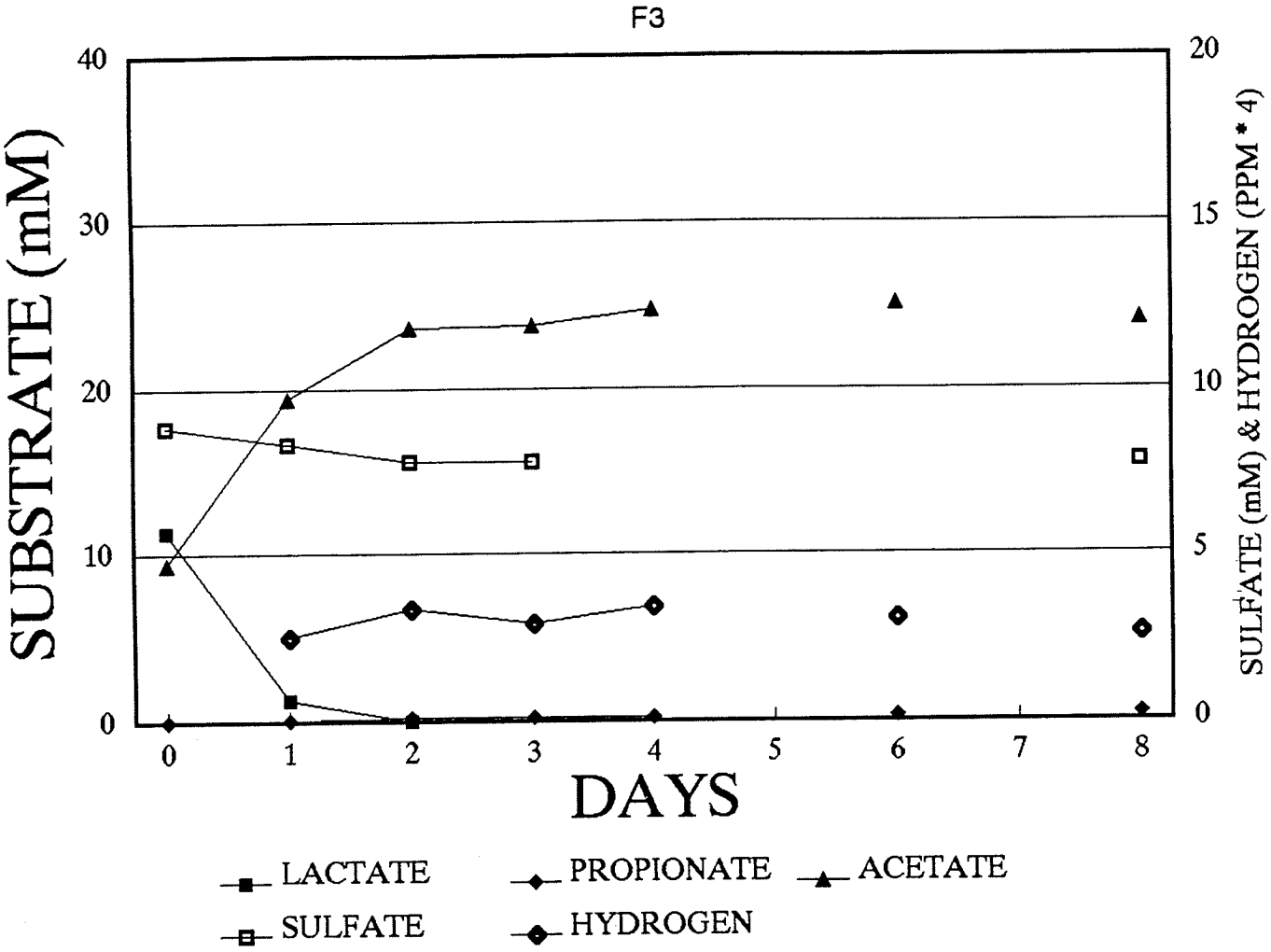


pH (0/1)	B	H_2S	% CH_4	mL CH_4
7.0/7.0	360			
7.2	480			208

F 4.27



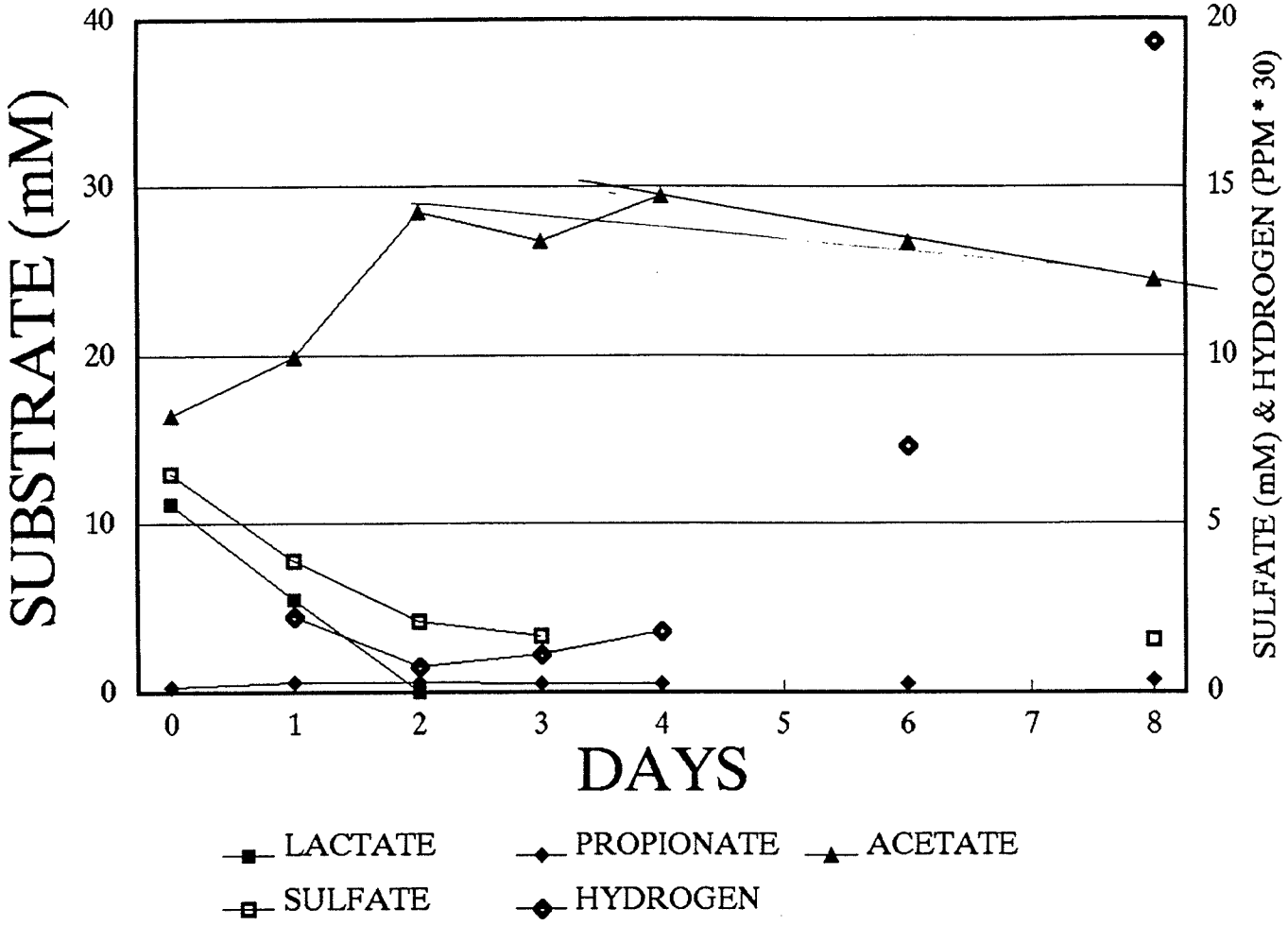
pH (o/i)	TS	H_2S	o/o CH_4	mL CH_4
7.1/7.1	440			
7.2	740		12.2	



7.1/7.1 600

7.2 840 0

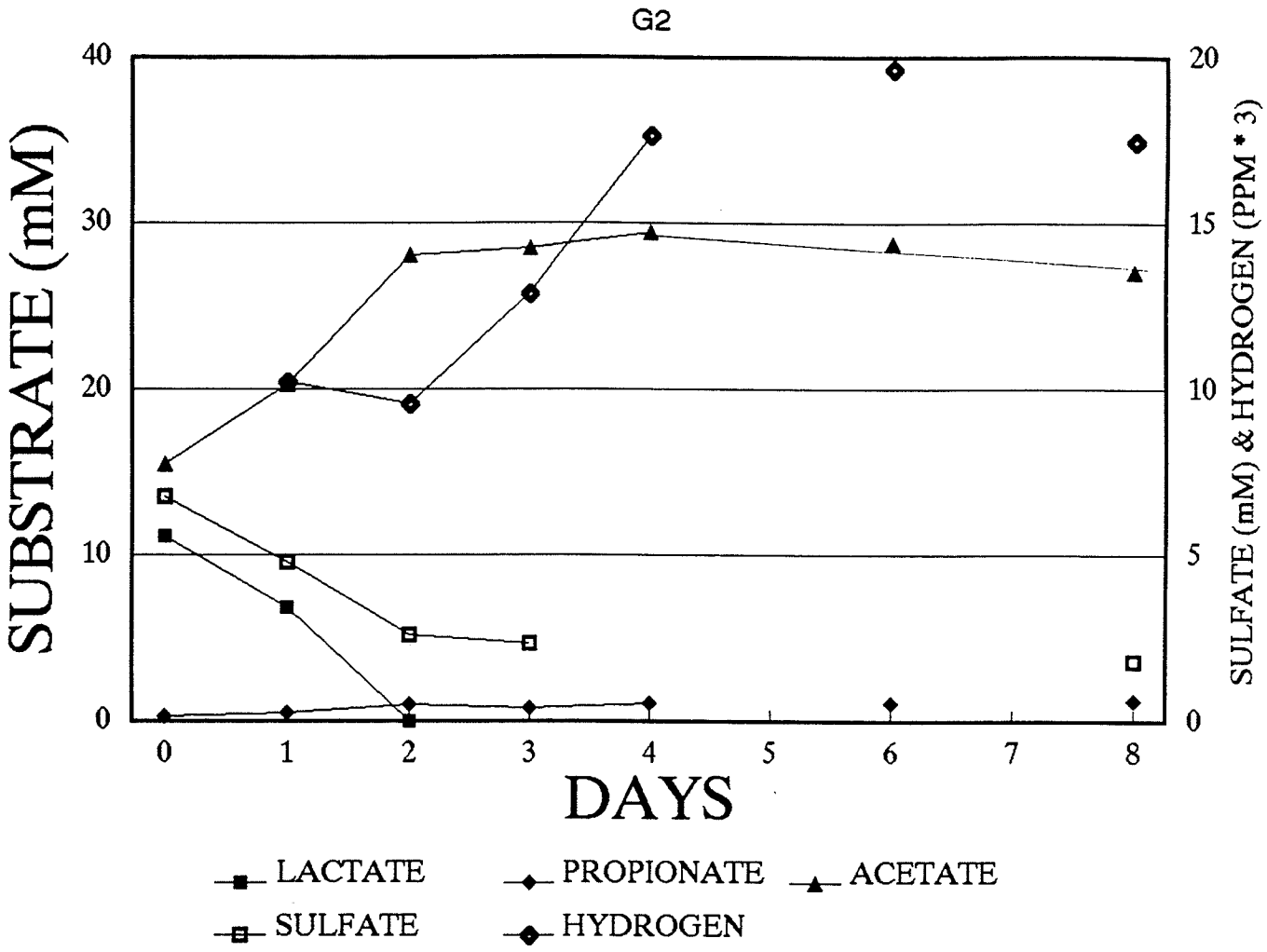
G1 C8 - control pH 8



8.2/8.1 400

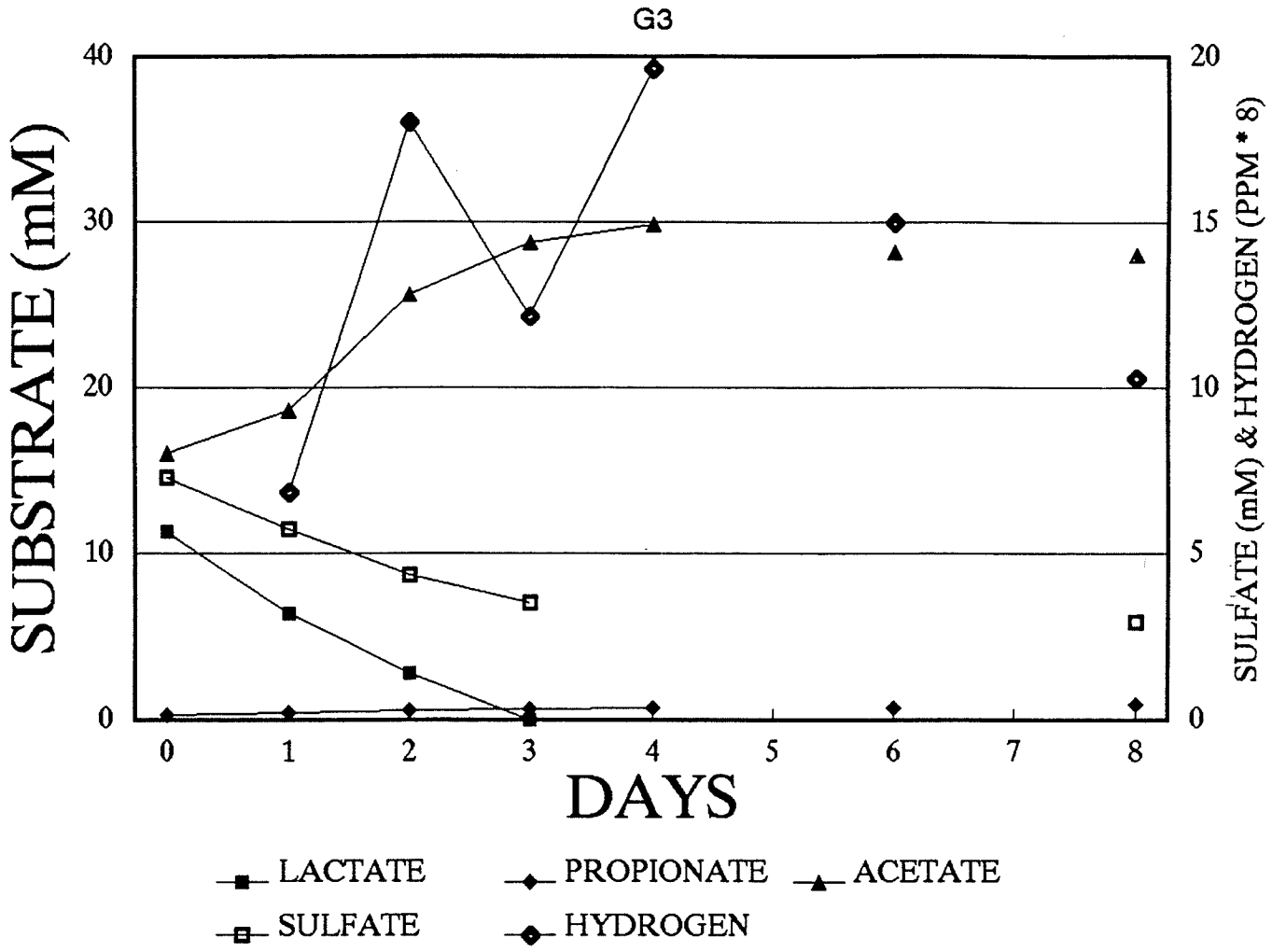
8.0 680 12.2

- H_2 removed in 2 days
- H_2 build-up after several days.



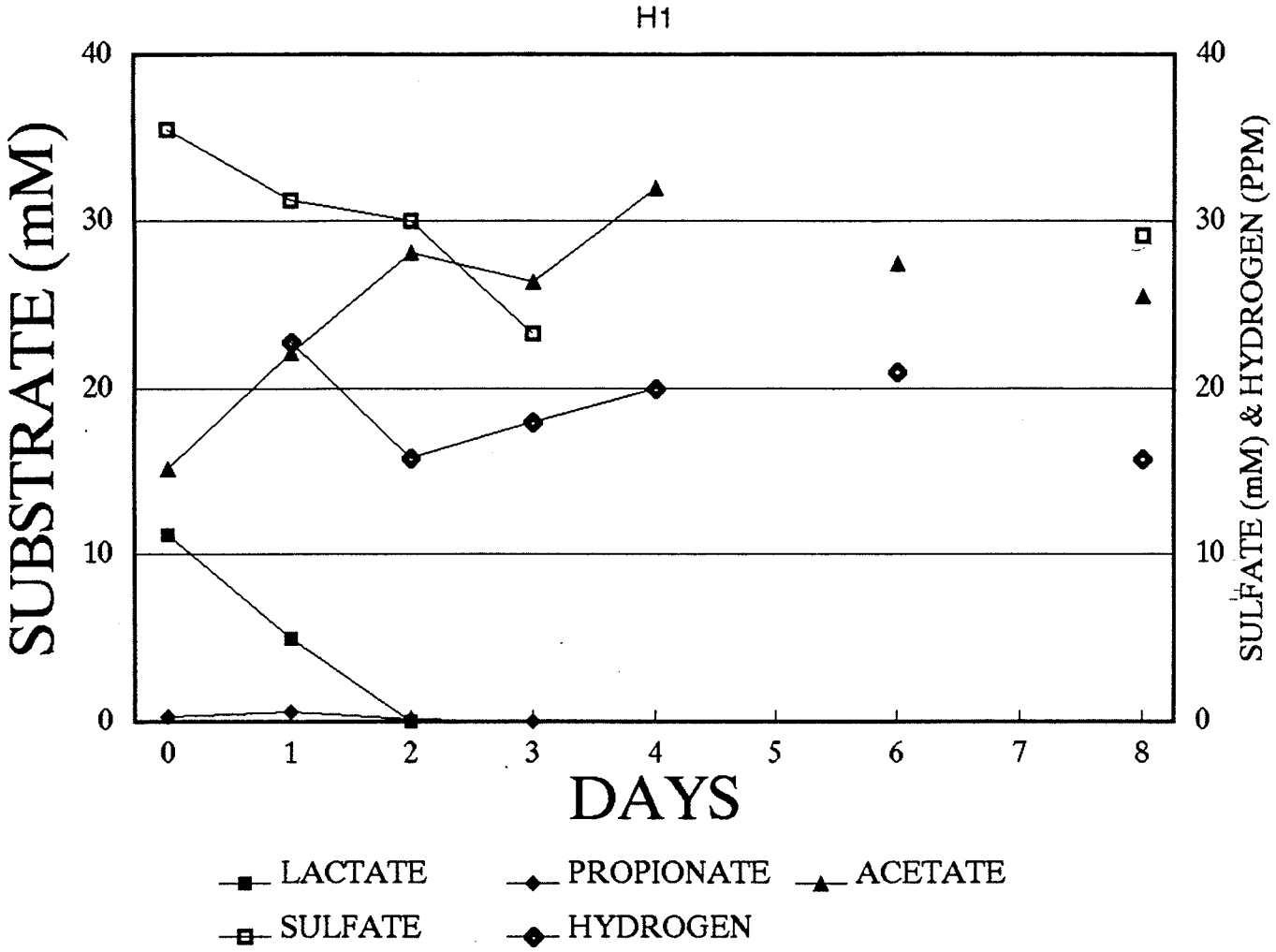
8.3/8.1 1000

8.1 1160 4.6



8.3/8.1 2000

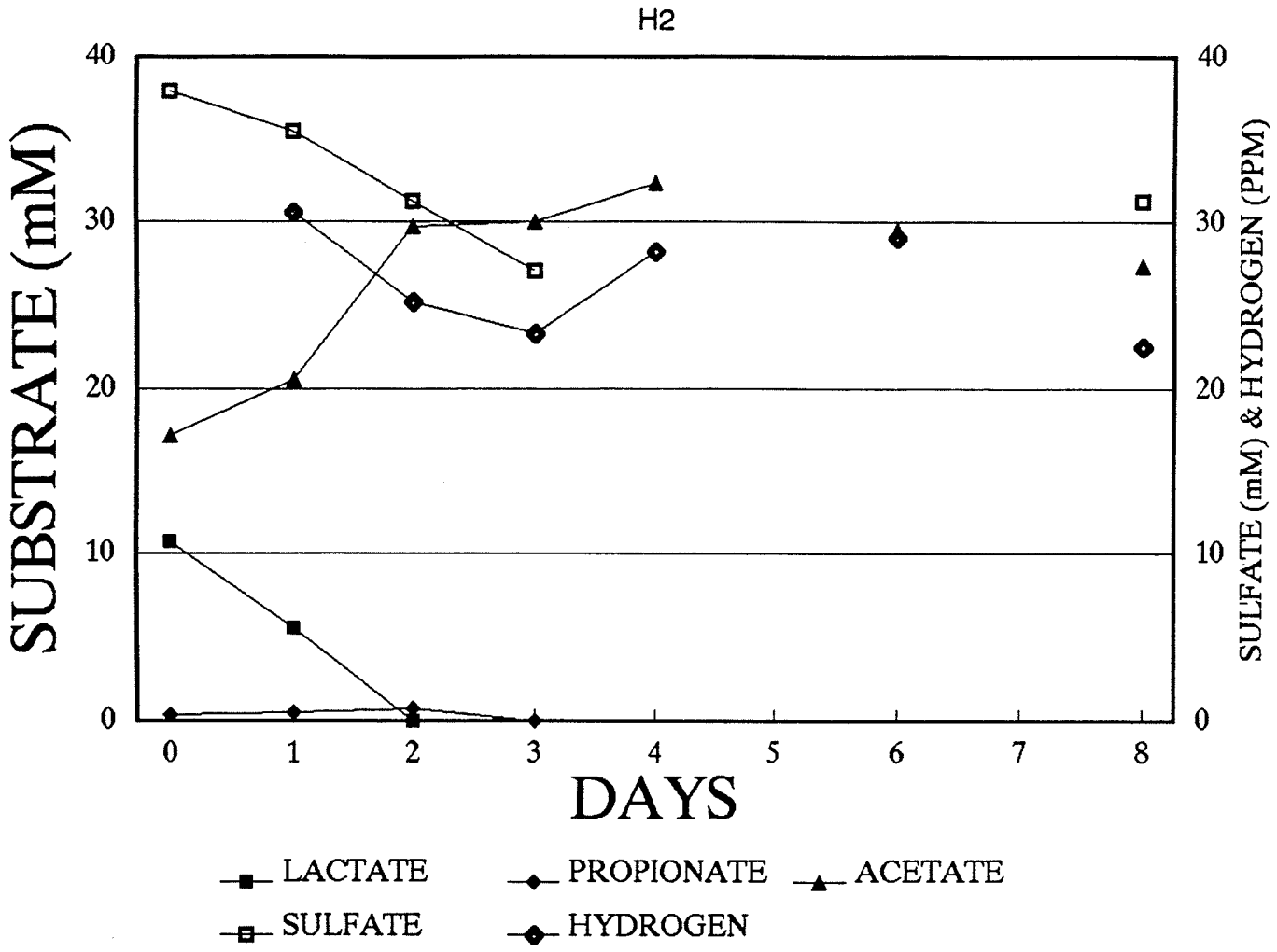
8.1 2000 1.4



83/8.1 400

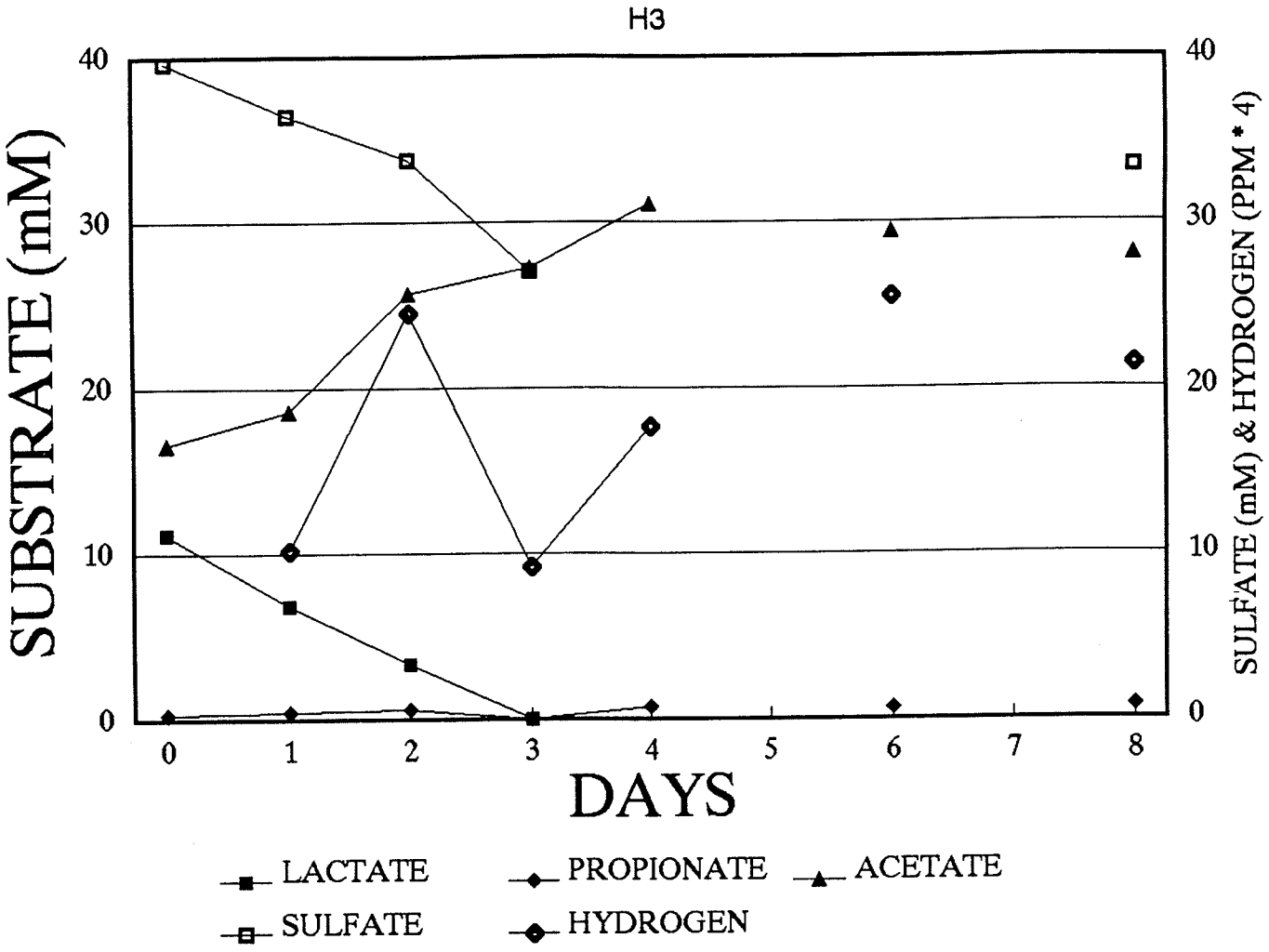
8.1 960 7.3

SO4 values on Day 3 & 8



8.3/8.2 1000

8.1 1240 3.0



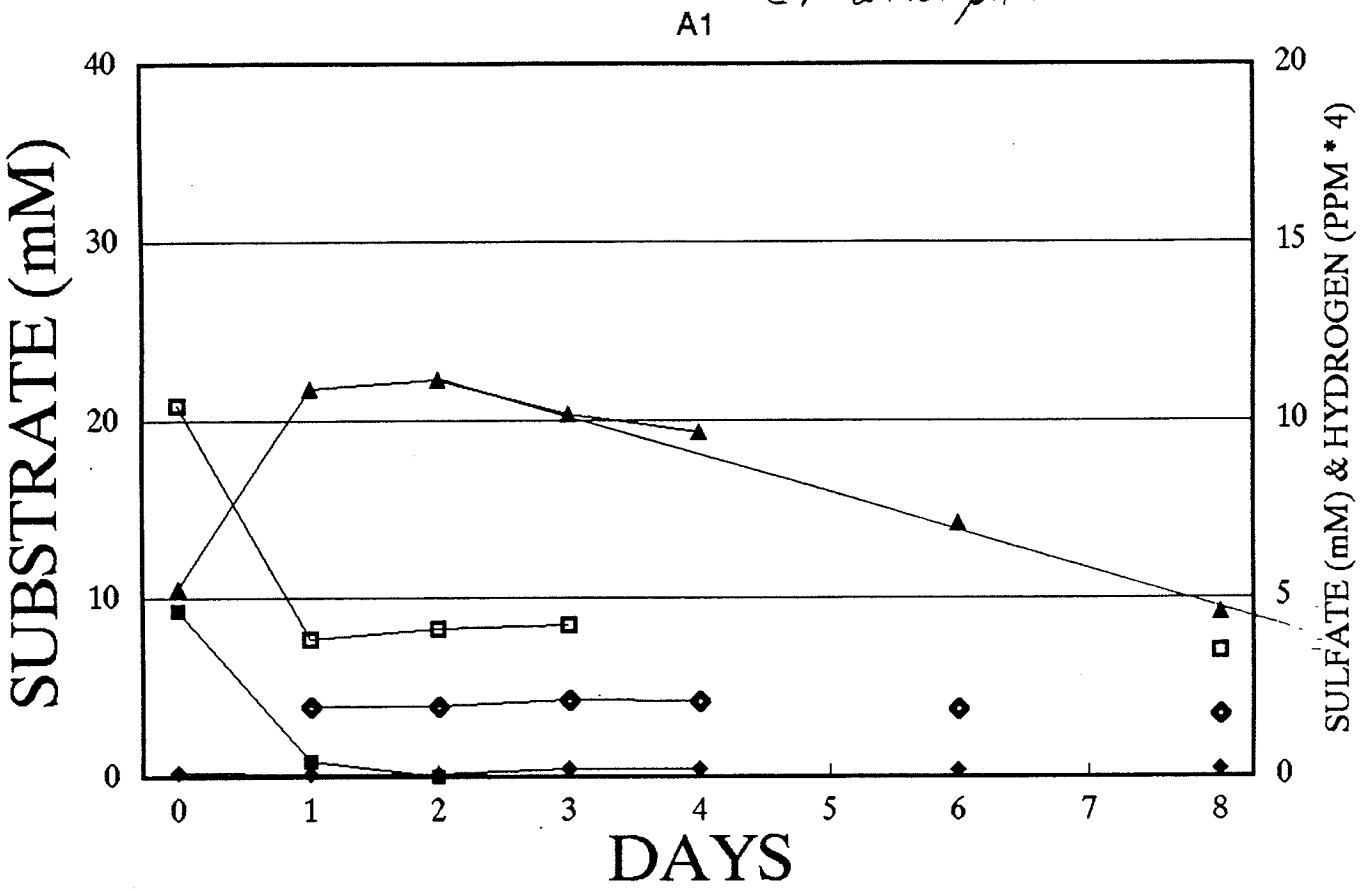
8.3/82 2040

8.1 2080 0.9

App. C
PHASE 4

PHASE 4

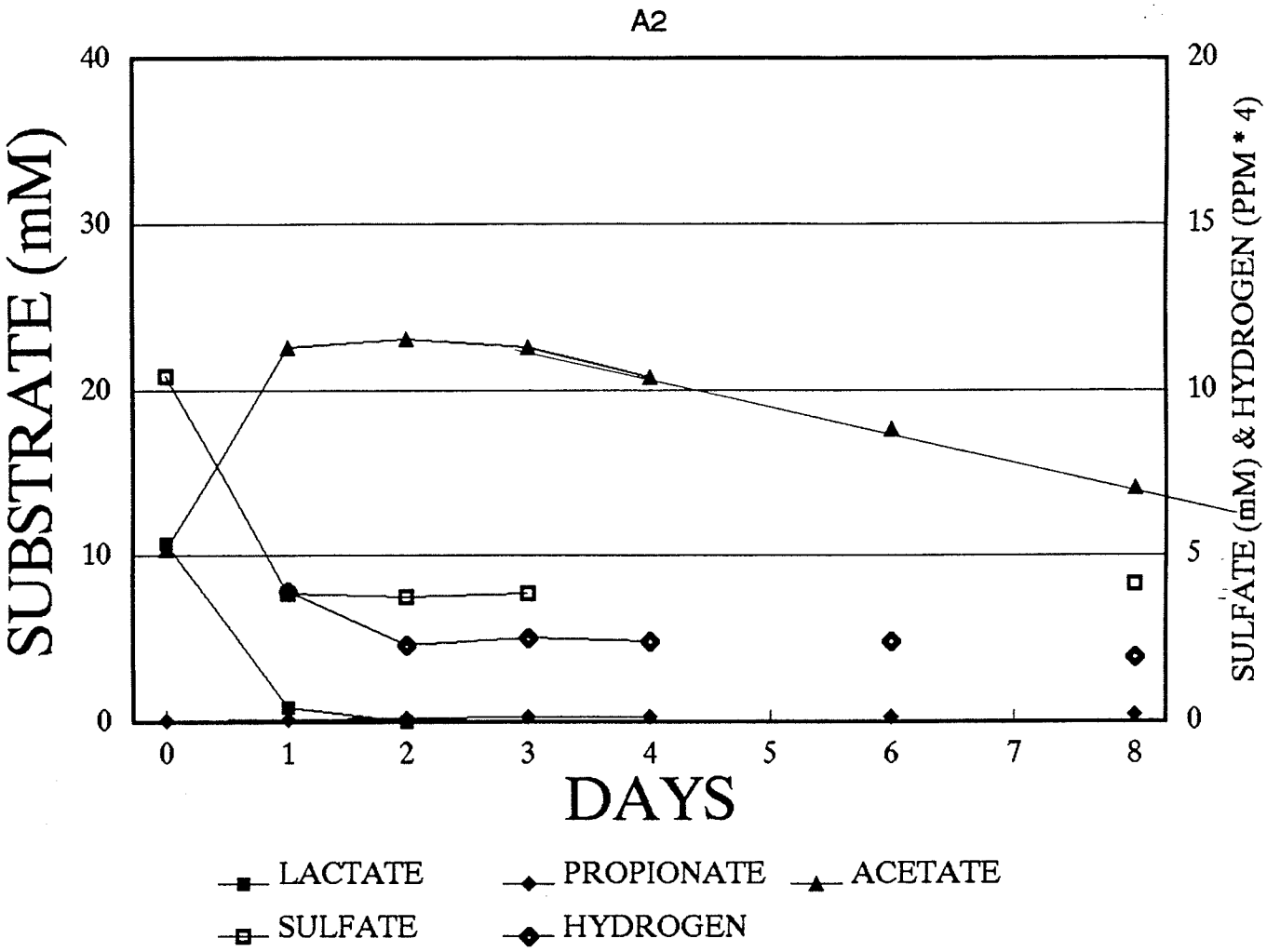
C7 - control pH7



Day 0 / Day 1 ≡ (0/1)

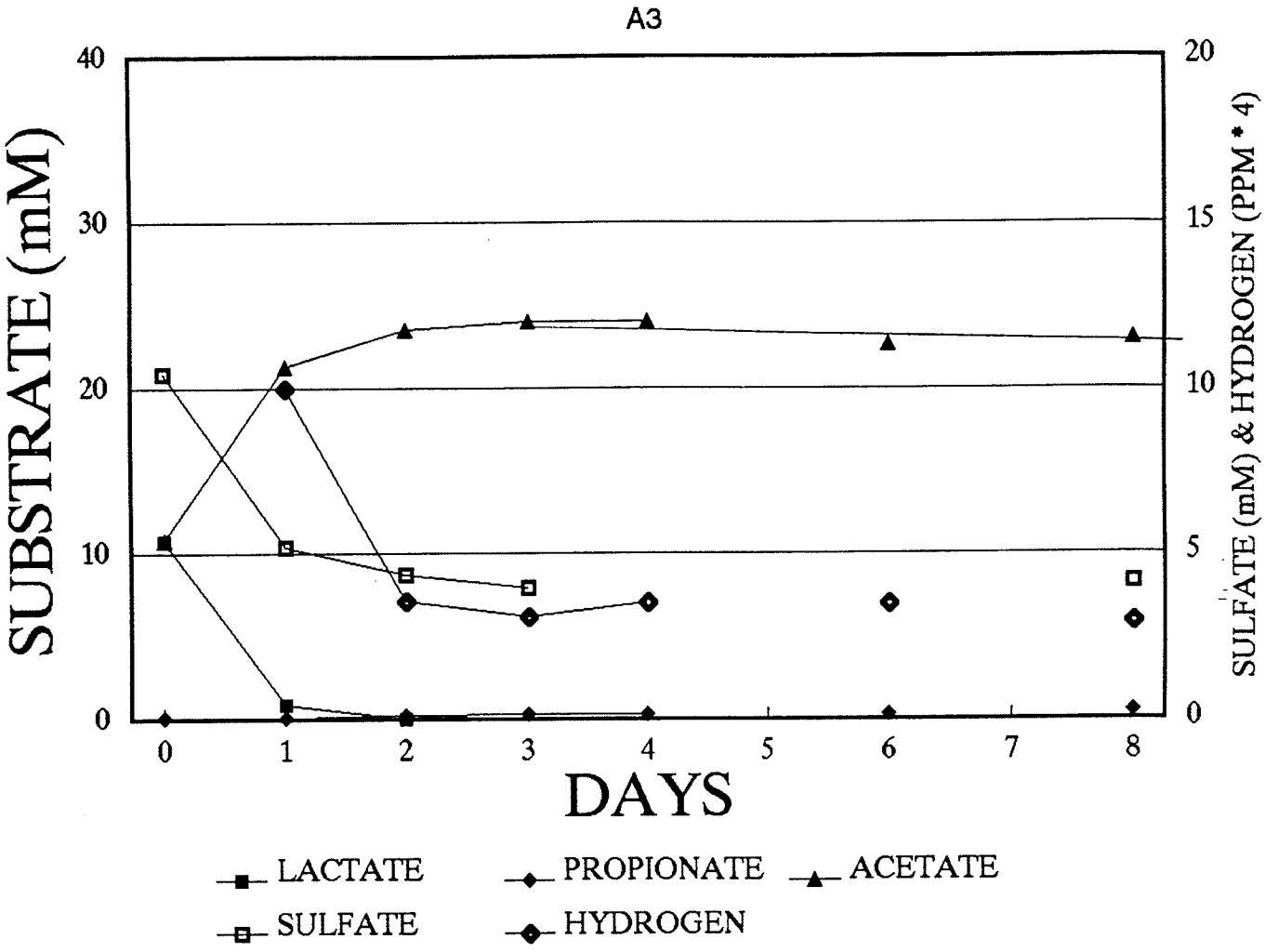
	pH (0/1)	S^{2-}	H_2S	% CH_4	mL Gas	mL CH_4
ST.	6.9/7.0	400				
ENIS	7.1	440			214	
HVG.						

- H_2S removal immediate in all reactors, not due to S^{2-}
- no H_2 build-up after all S^{2-} removed.



	pH (0/1)	TS	H_2S	% CH_4	mL CH_4
ST.	7.17	500			
EN.	7.1	560		16	
AVG					

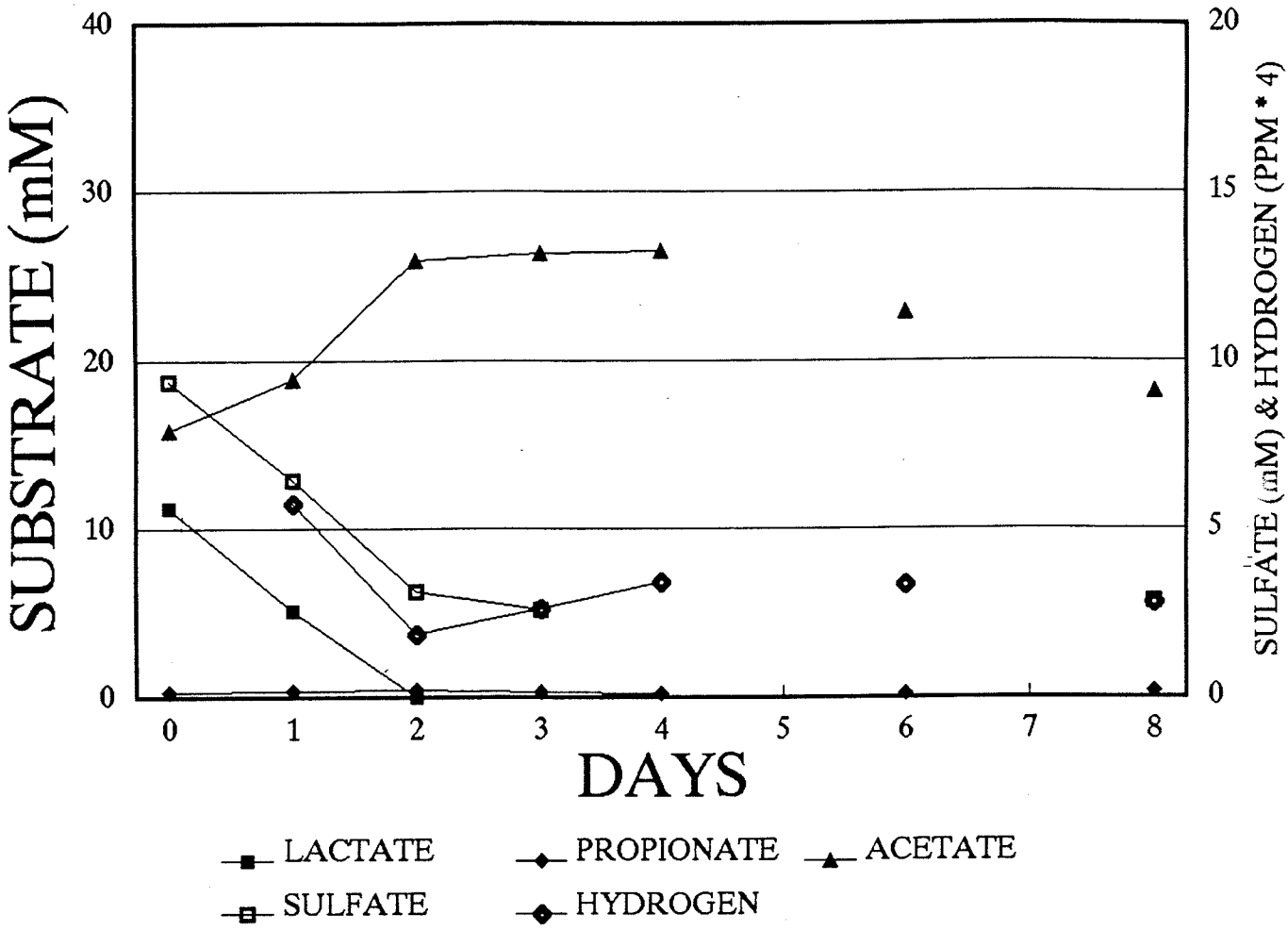
- slight H_2 build-up after 1d.



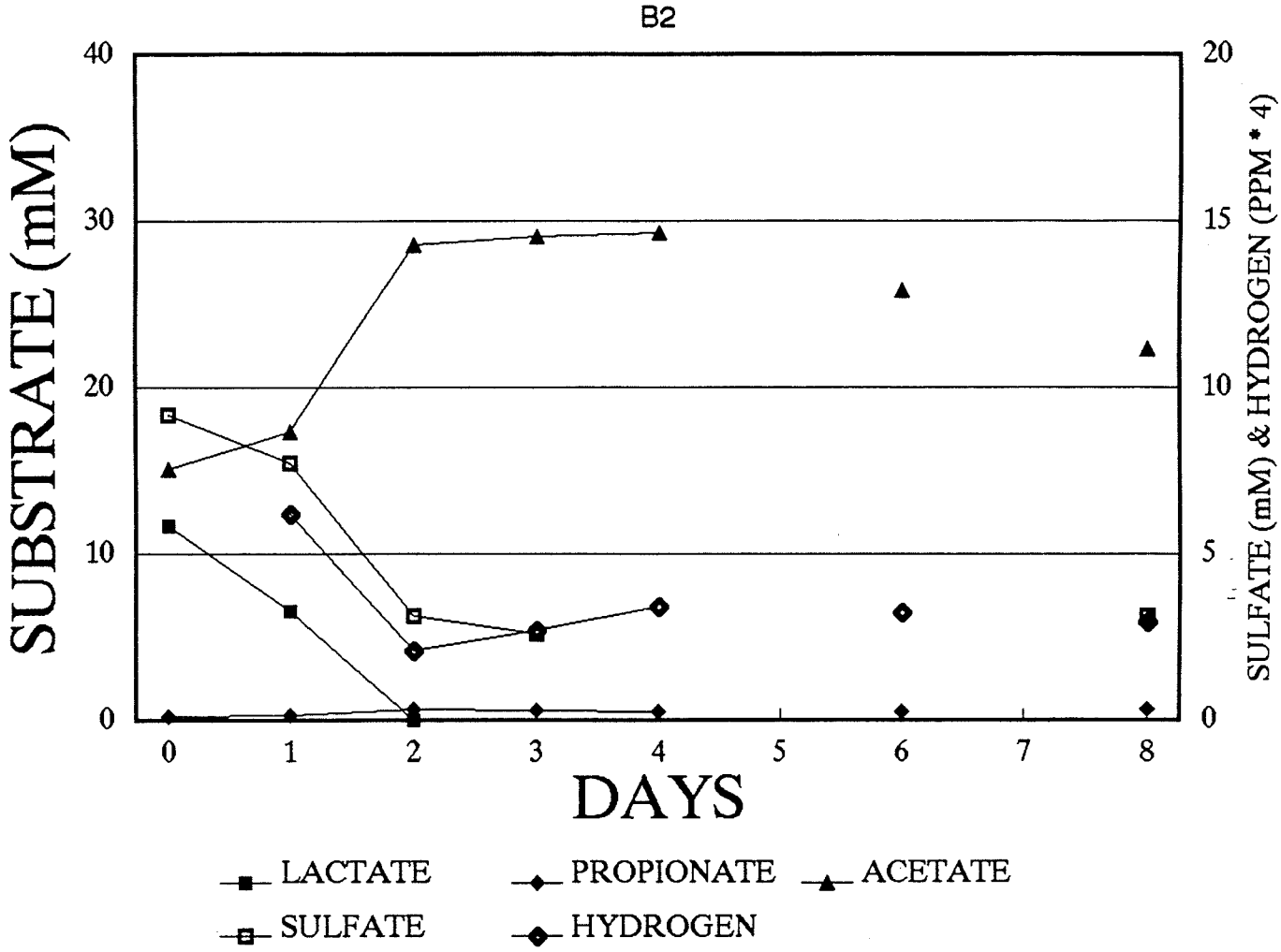
	pH (0/1)	TS	H_2S	%CH_4	mL CH_4
ST	7.1/7.1	640			
Ed.	7.2	700		0.3	
Avg					

- large H_2 build-up after 1 d.

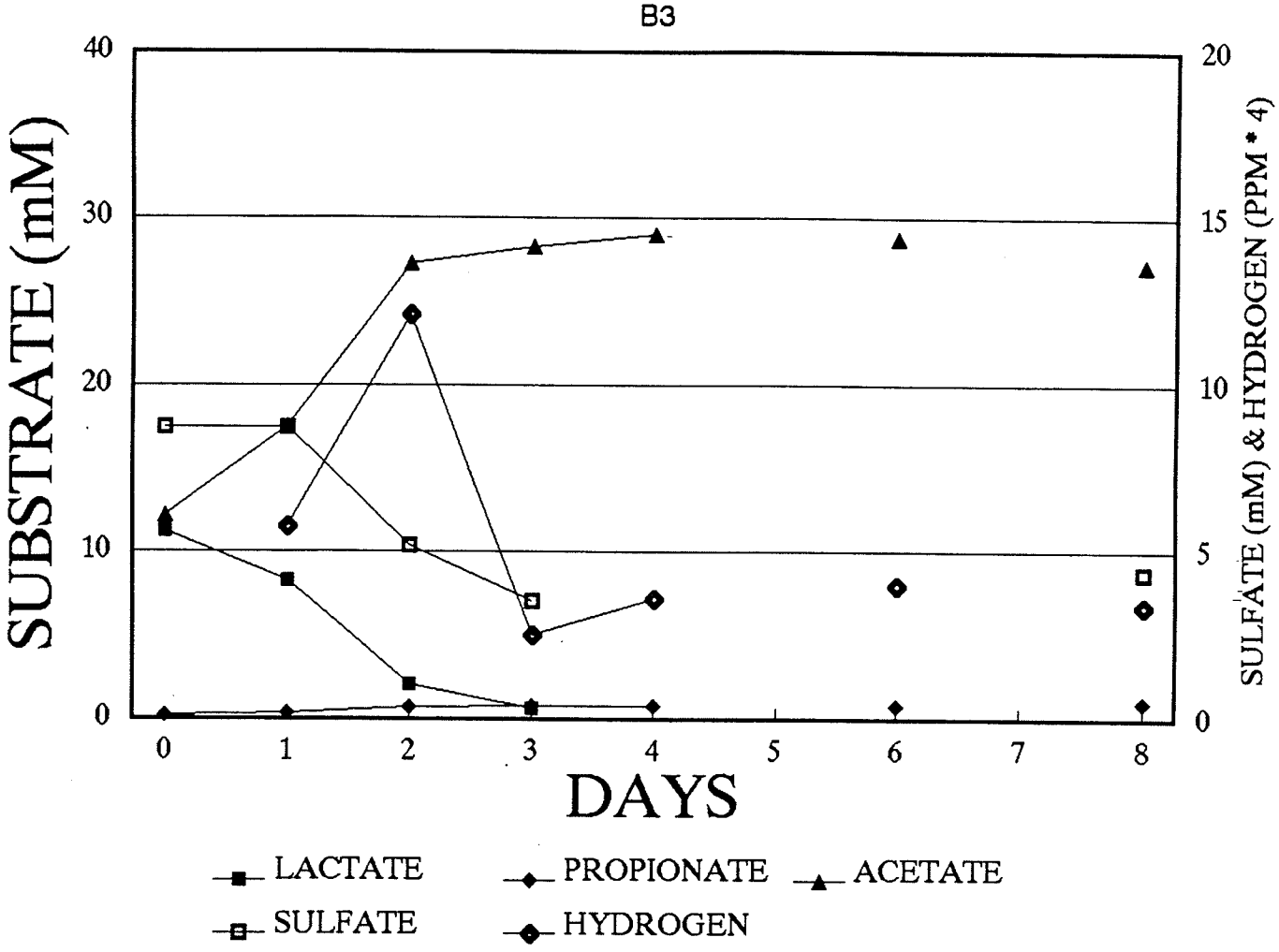
B1 pH 8↓7



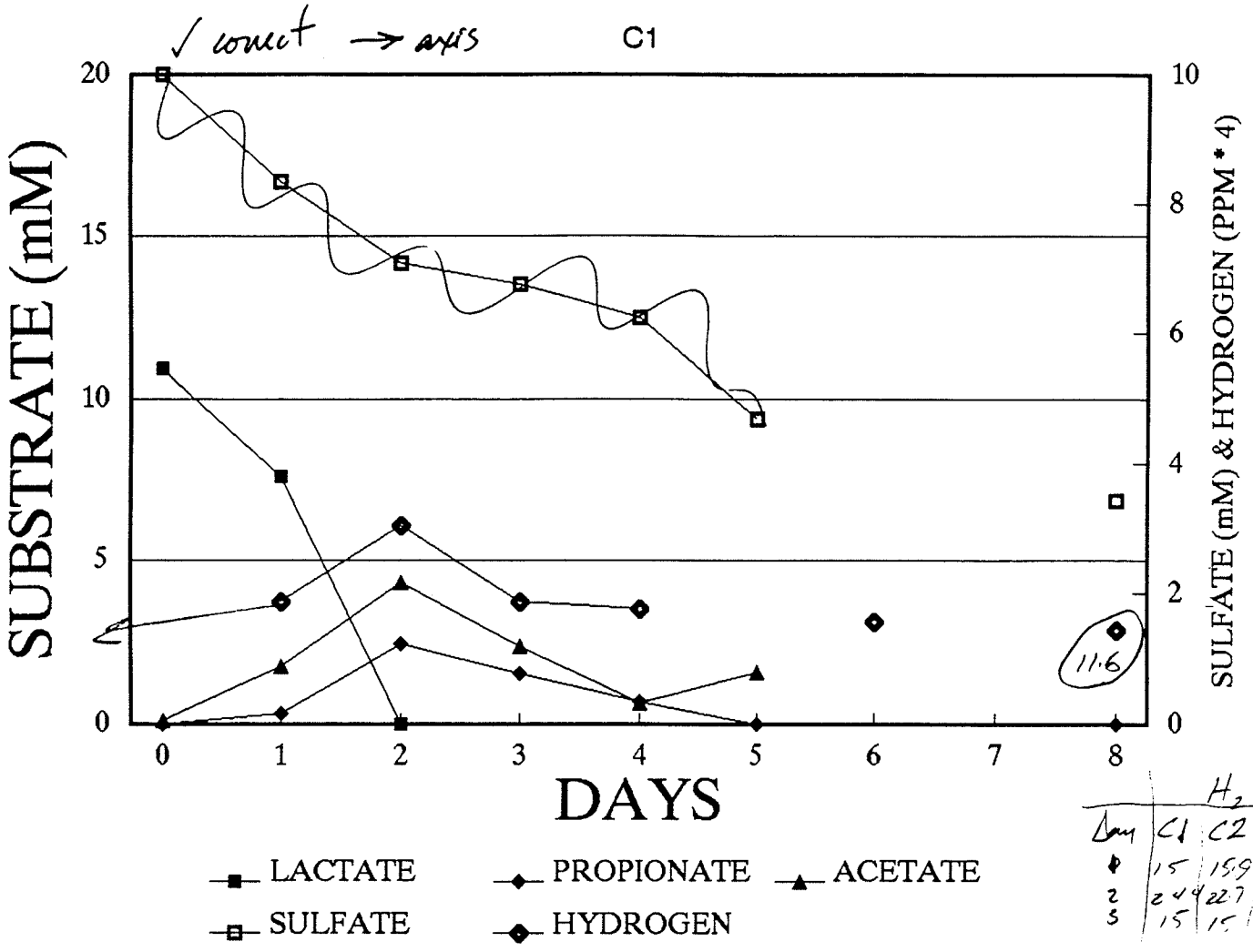
	pH (0/1)	TS	H_2S	% CH_4	mL CH_4
ST.	7.2/7.2	140			
EN.	7.4	440		17.1	
Avg.					



	H (0/1)	TB	H_2S	% CH_4	mL CH_4
ST.	7.2/7.2	240			
EN.	7.3	480		10.6	
AVG.					



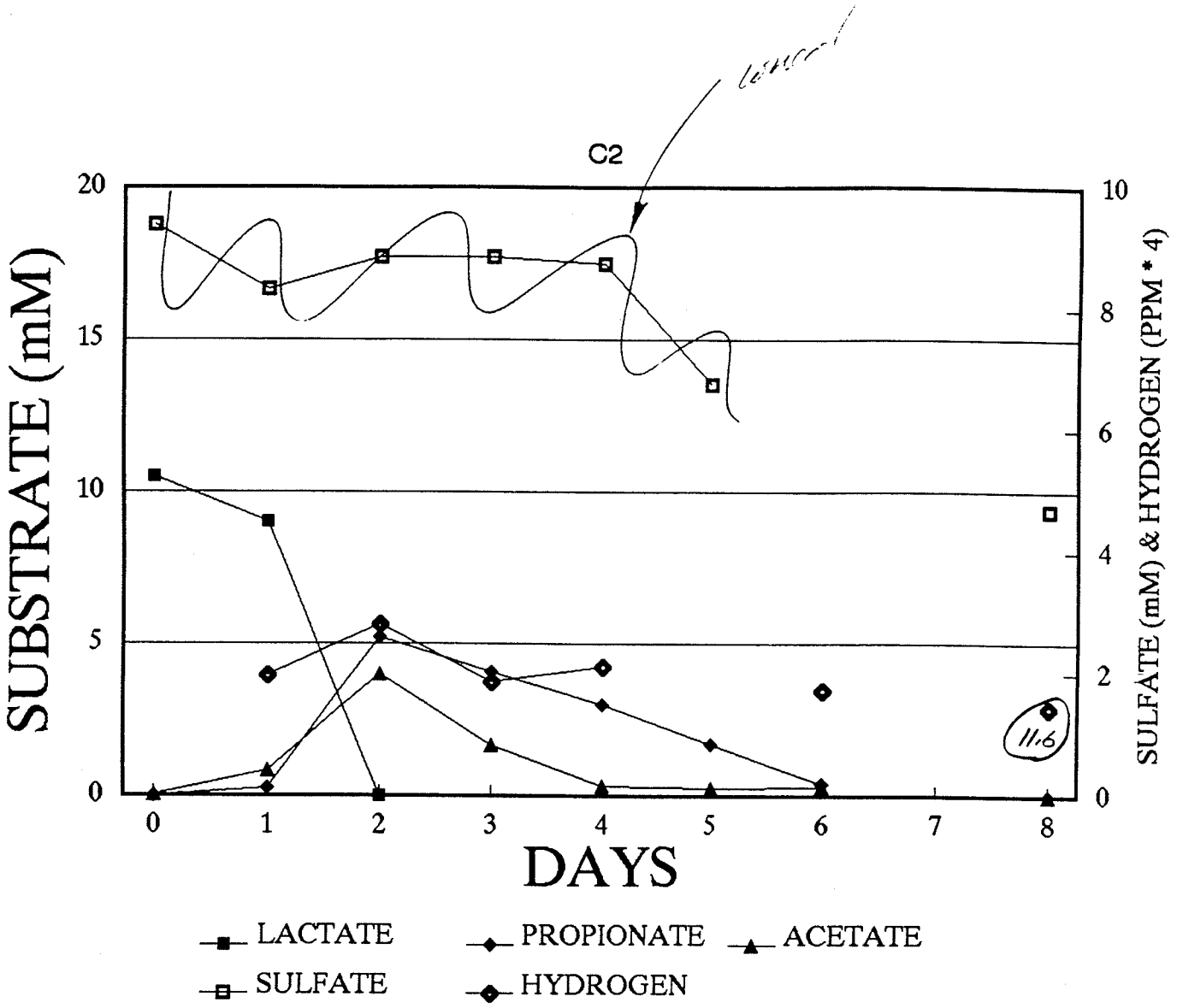
	pH (0/1)	B	H_2S	% CH_4	mL CH_4
ST.	7.2/7.2	480			
END	7.4	640			1.9
AVG.					



Day	H_2 C1	C2	C3
1	15	15.9	
2	24	22.7	
3	15	15	24

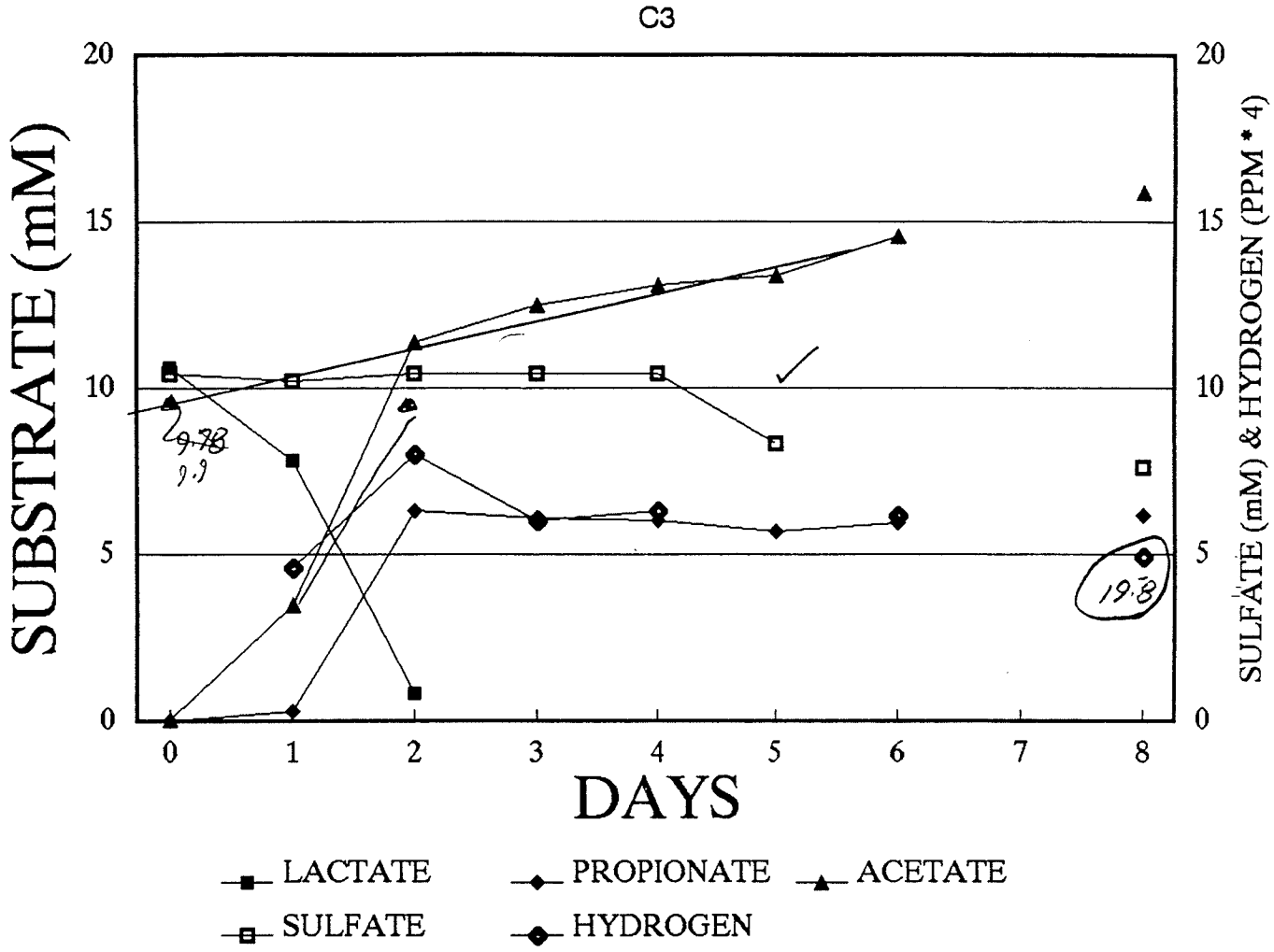
	pH (o/i)	TS	H_2S	%CH4	mL CH4
ST.	7.1/7.2	120			
END	7.2	300		36.5	
AVG					

F 4.2



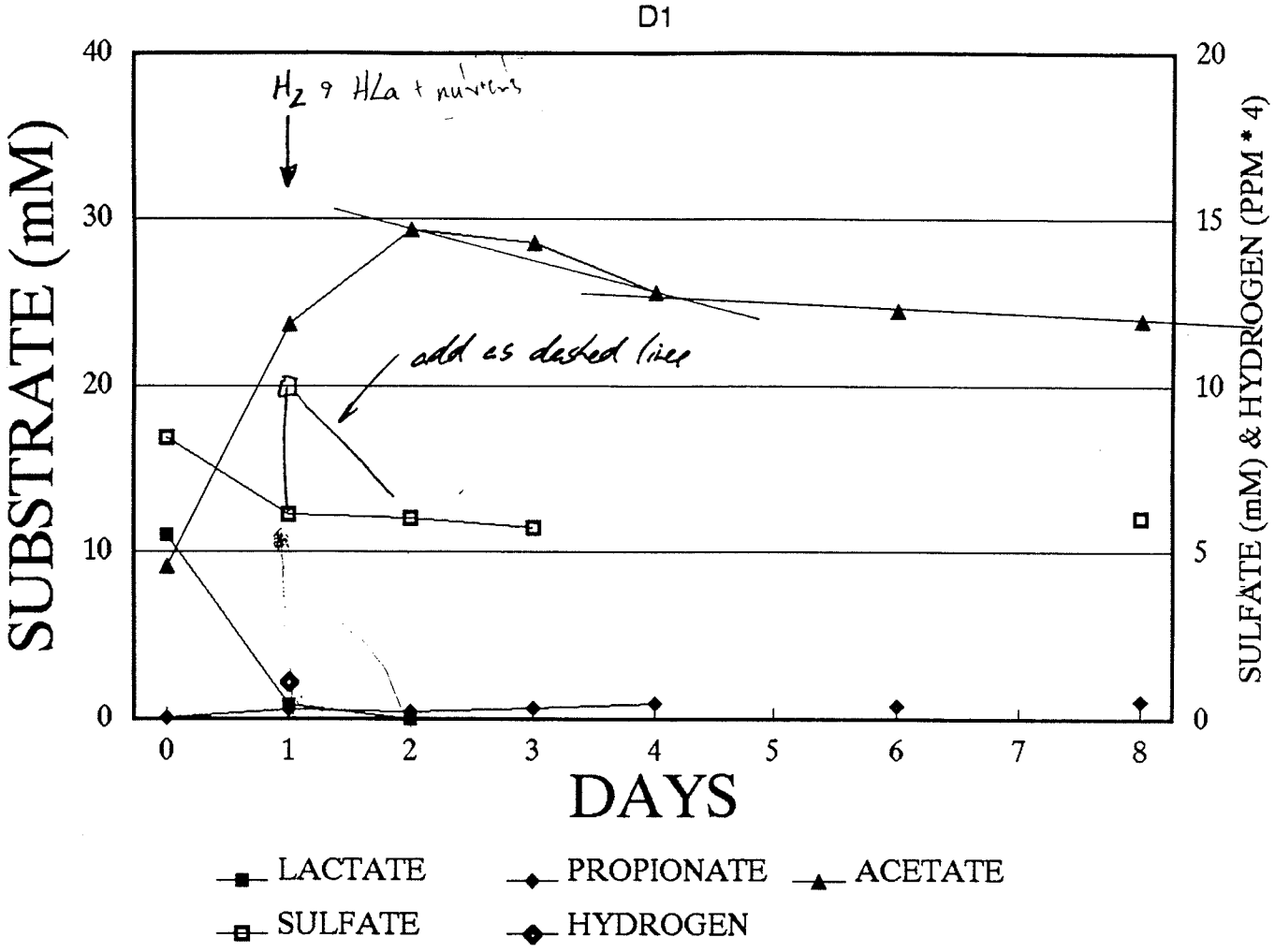
	pH	B	H_2S	% CH_4	mL CH_4
	0/1				
ST.	7.1/7.2	240			
END	7.2	300		25.7	
AVG.					

F 4.22



	pH (o/i)	TS	H_2S	% CH_4	mL CH_4
ST.	7.1/7.2	680			
END	7.2	480		13.3	
AVG					

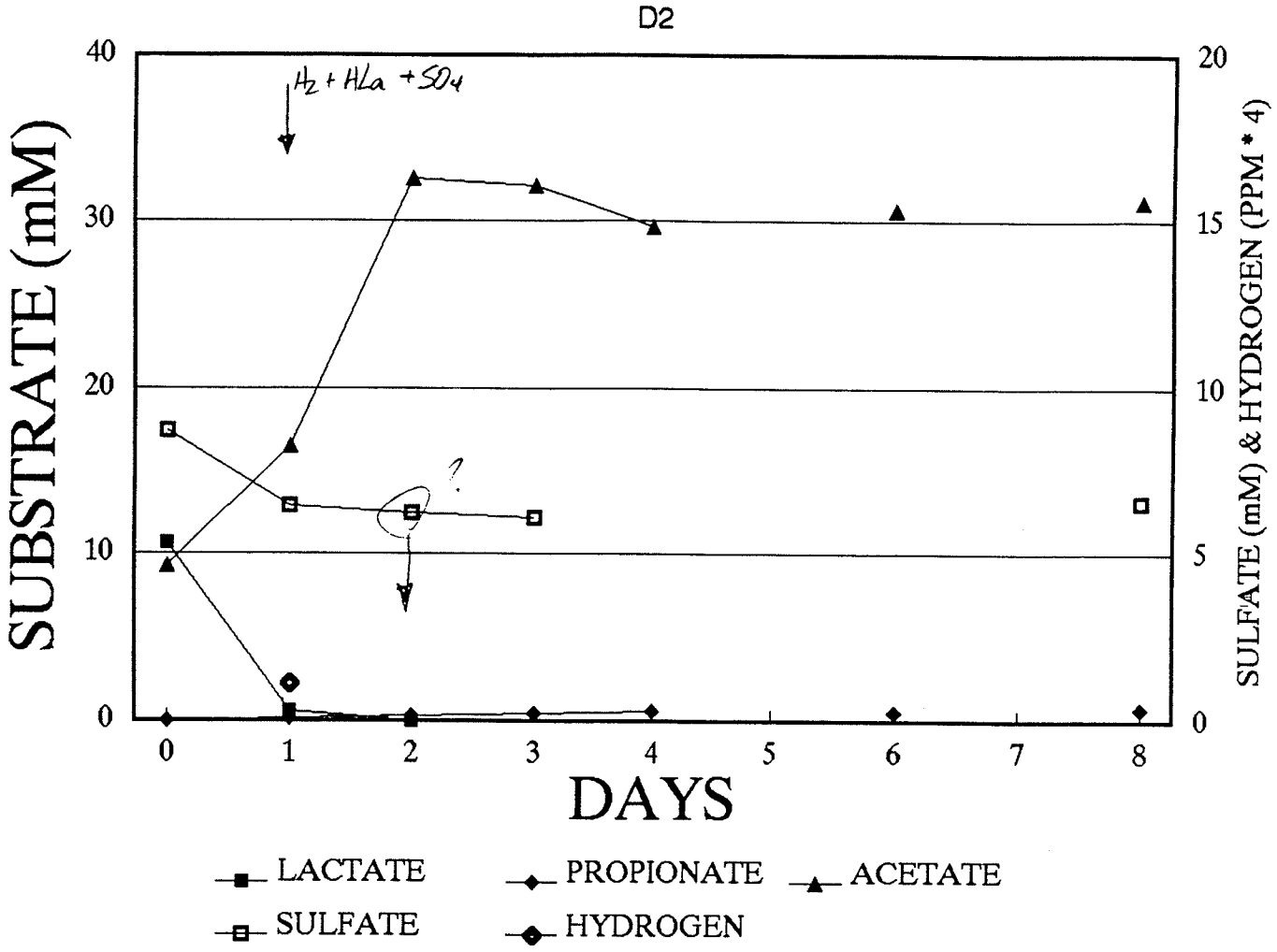
F 4-20



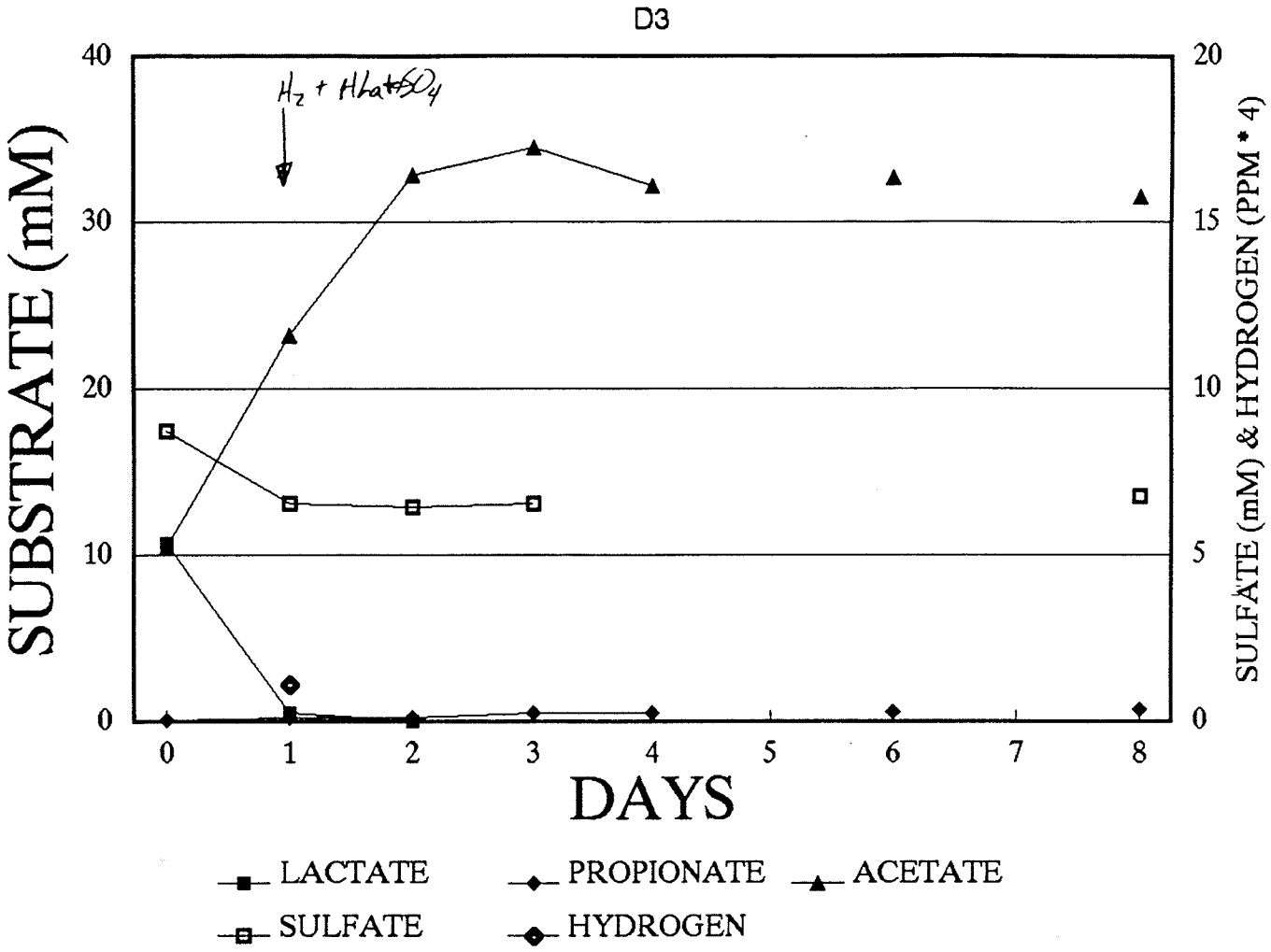
	pH (o/i)	B	H_2S	% CH_4	mL CH_4
ST.	7.1/7.1	400			
END	7.3	680		20.8	
AVG.					

- H_2 did not affect HLa degradation.

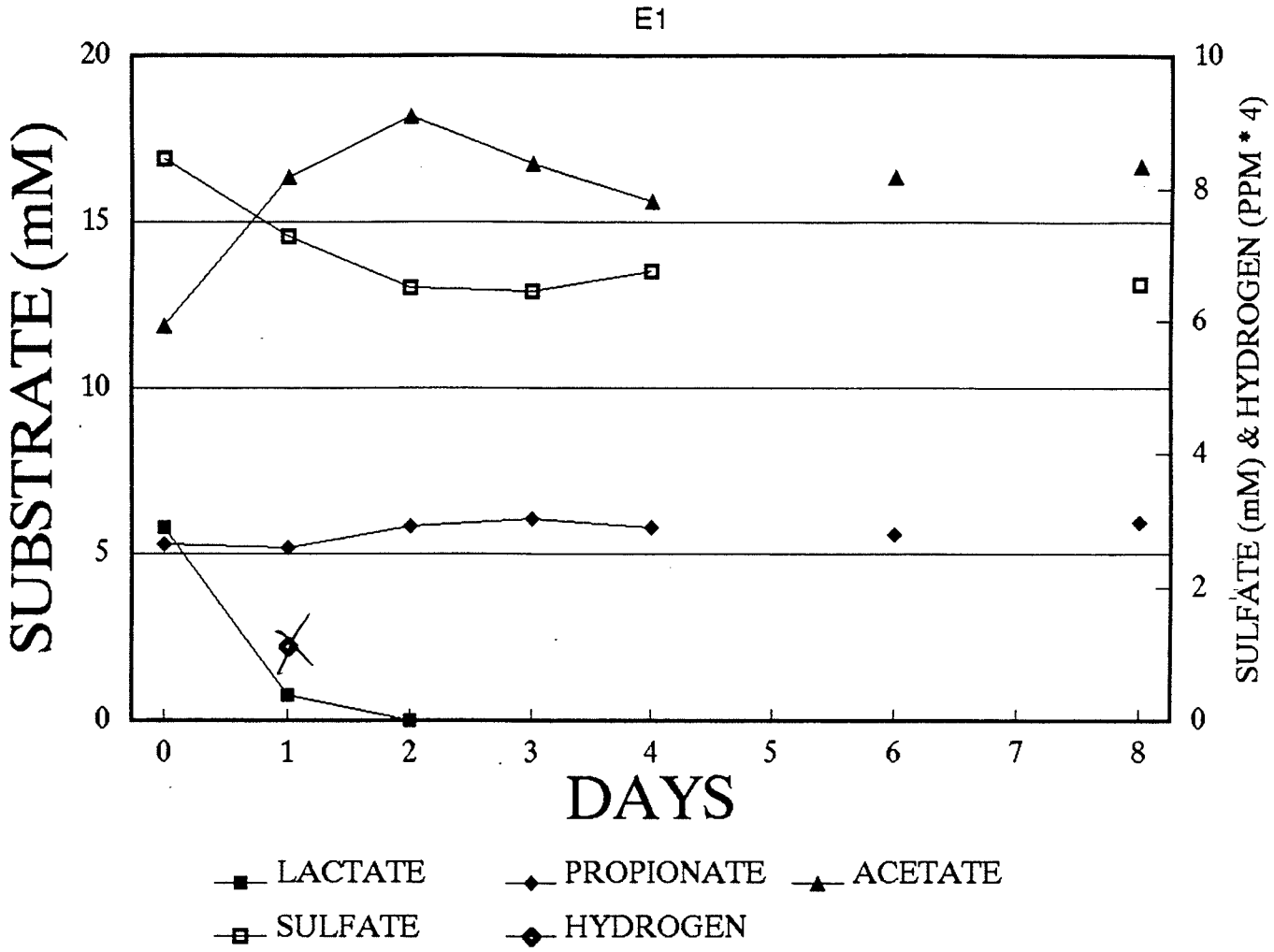
F 4.25



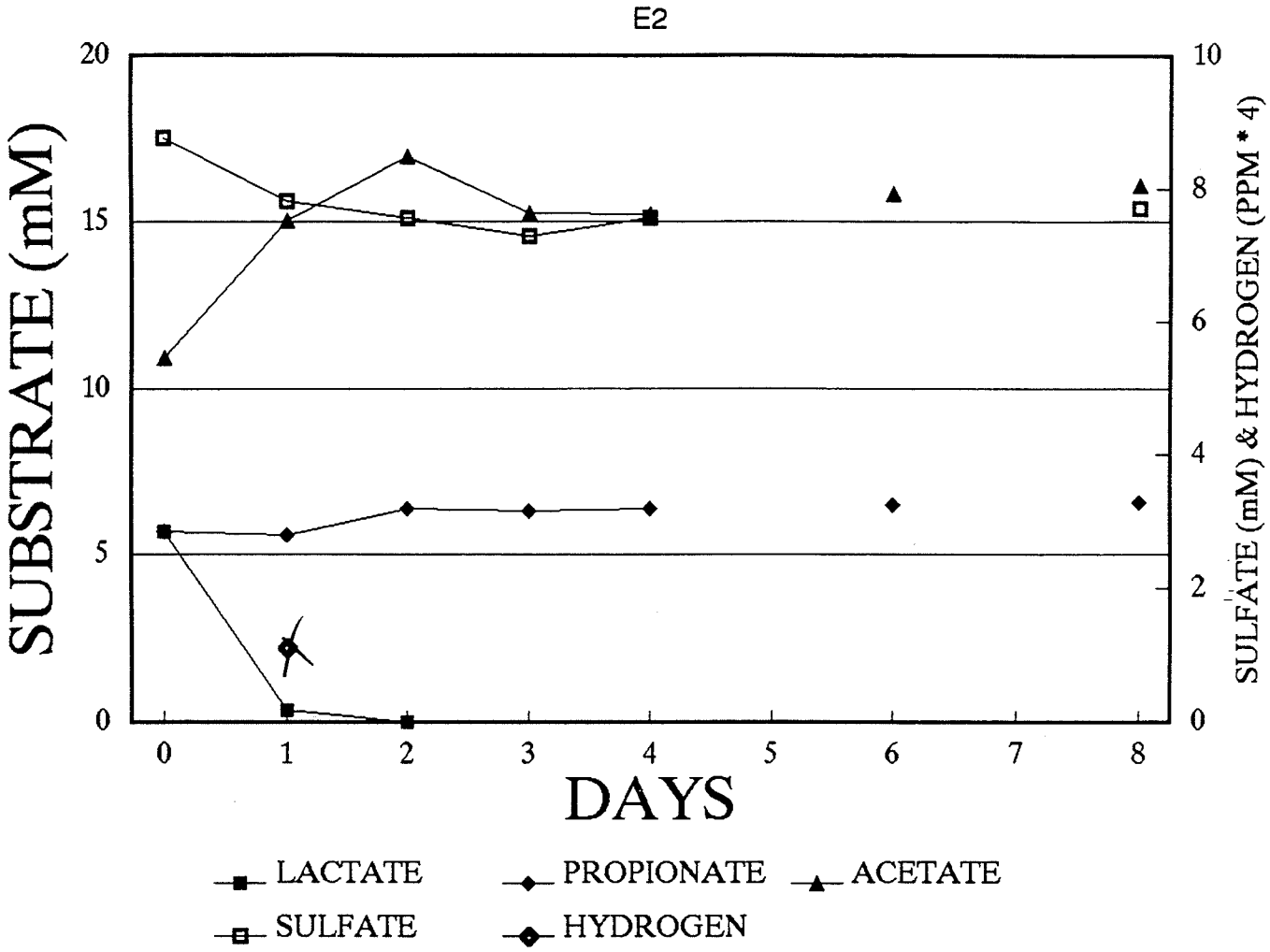
	pH (0/1)	TS	H_2S	odour	colour
ST	7.1/7.1	520			
END	7.2	800		6.8	
ADL					



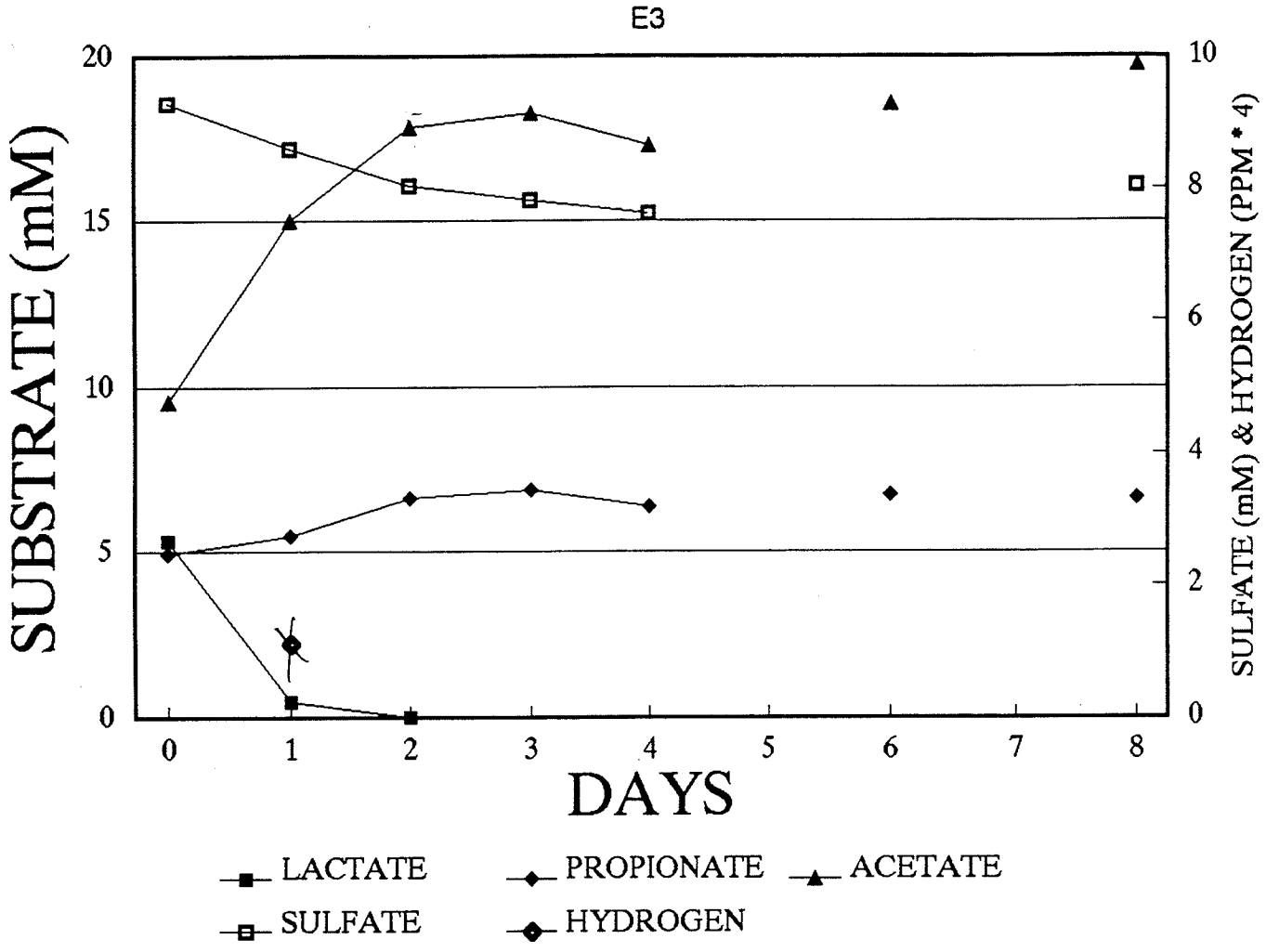
	pH (0/1)	TS	H_2S	%CH_4	-L CH_4
ST.	7.1/7.1	560			
END	7.3	960		trace	
AVG.					



pH (0/1)	TB	H_2S	% CH_4	mL CH_4
70/72	440			
72	560		14.9	

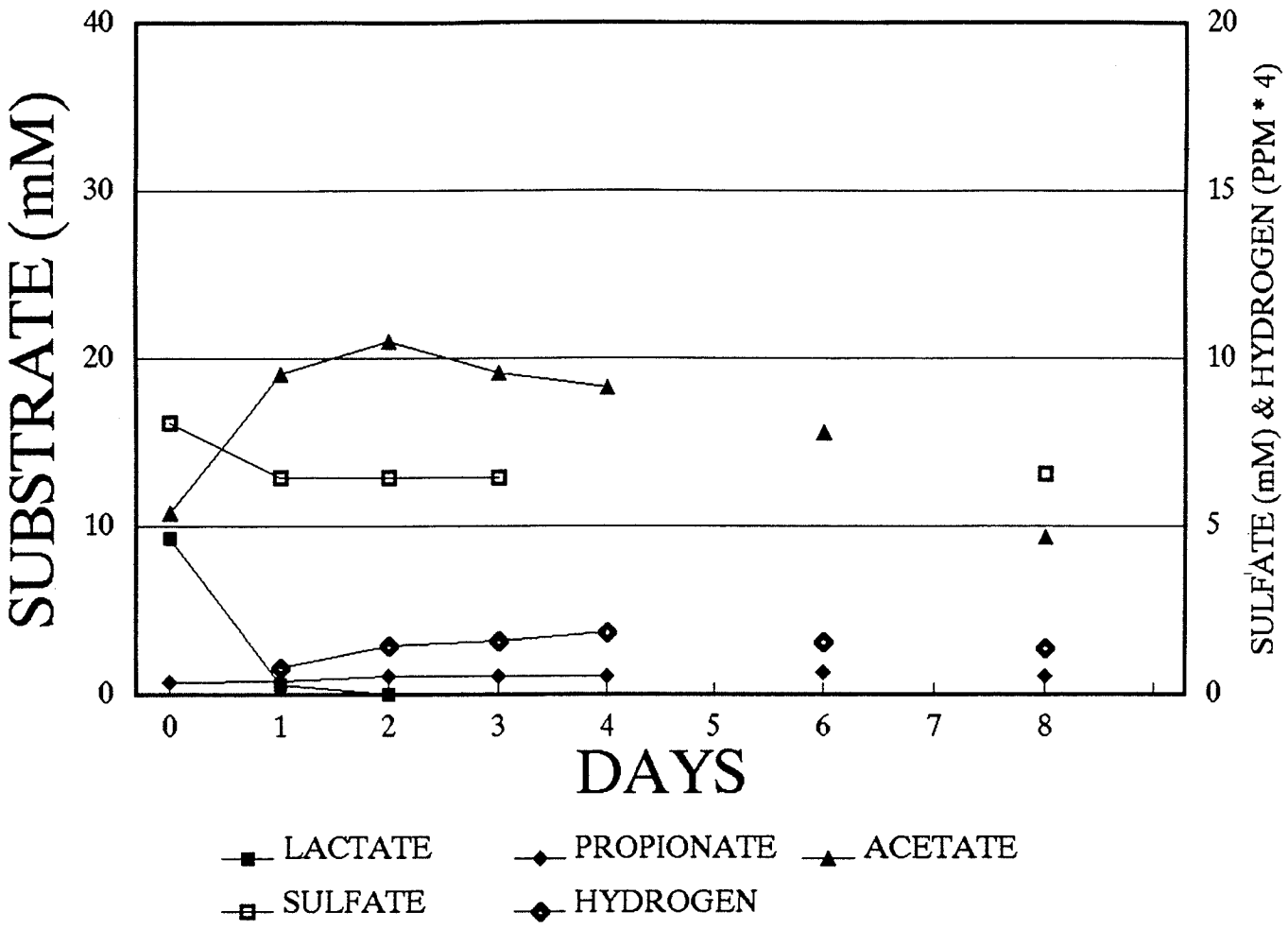


pH (0/1)	B	H_2S	%/CH_4	mL CH_4
7.0/7.2	480			
7.4	800		10.6	



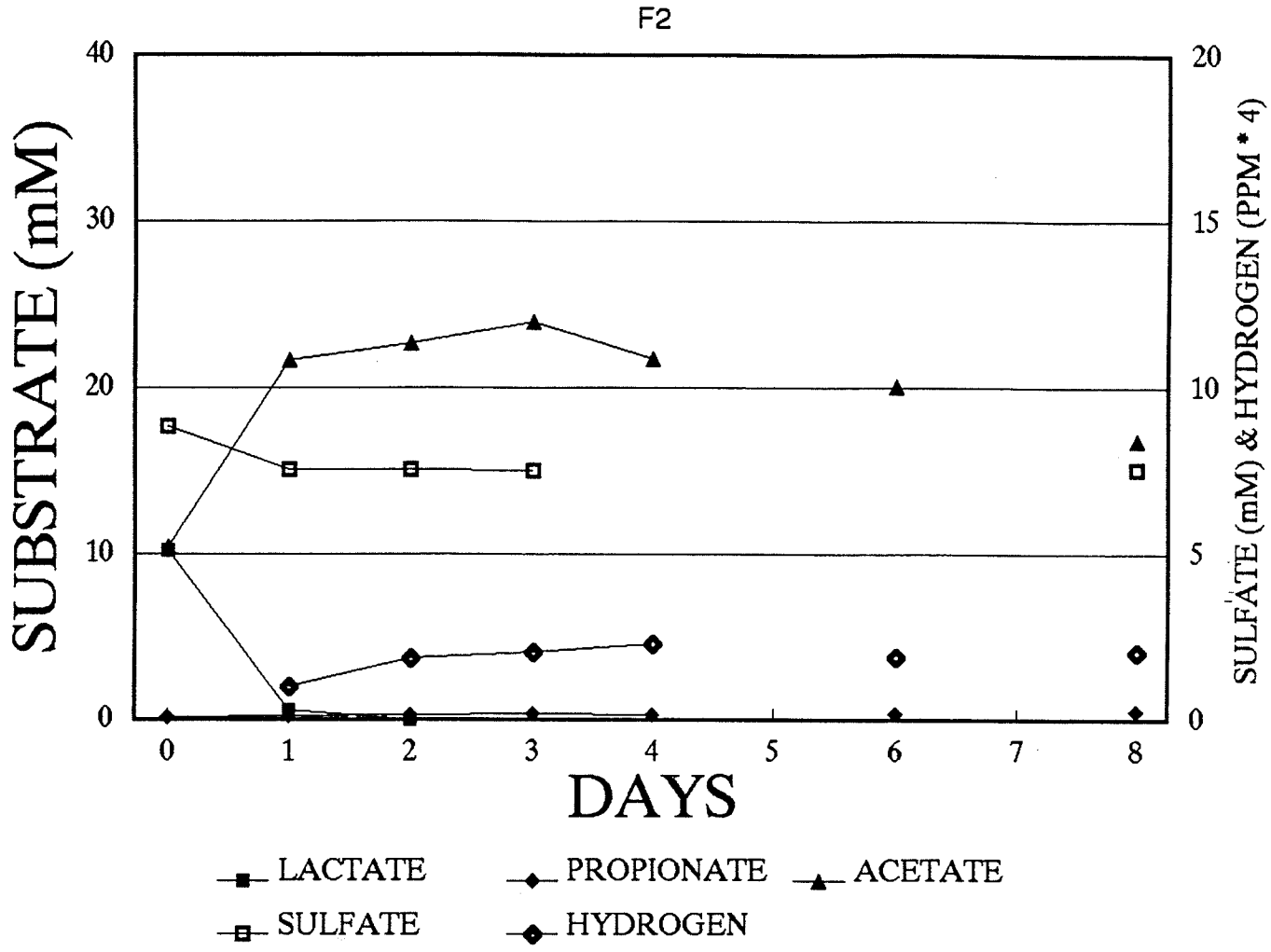
pH (o/i)	B	H_2S	o/o CH_4	i.L CH_4
7.1/7.2	720			
7.3	80 84		trace	

F1 Racine La

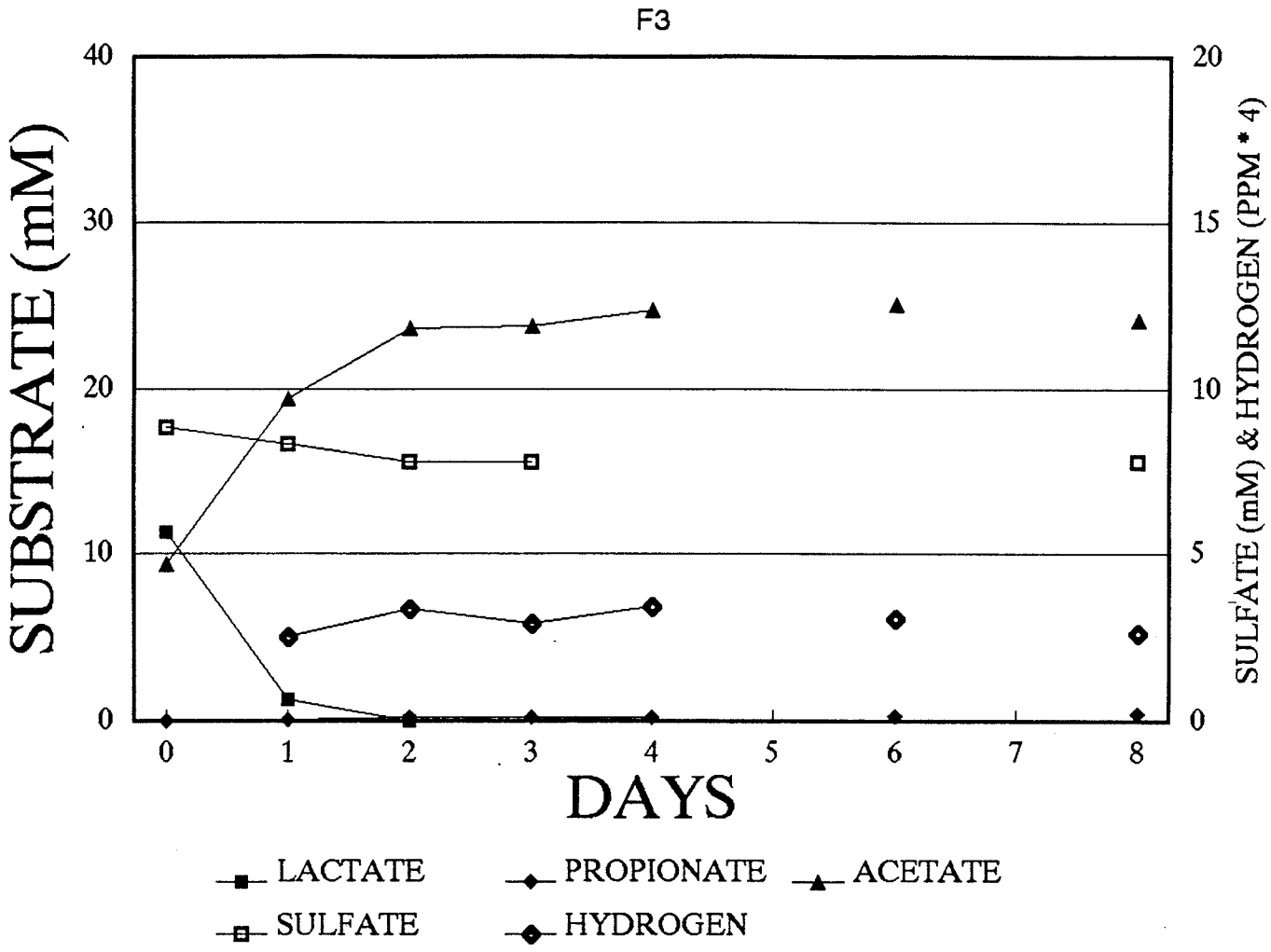


pH (0/1)	B	H_2S	% CH_4	mL CH_4
7.0/7.0	360			
7.2	480			208

F 4.27



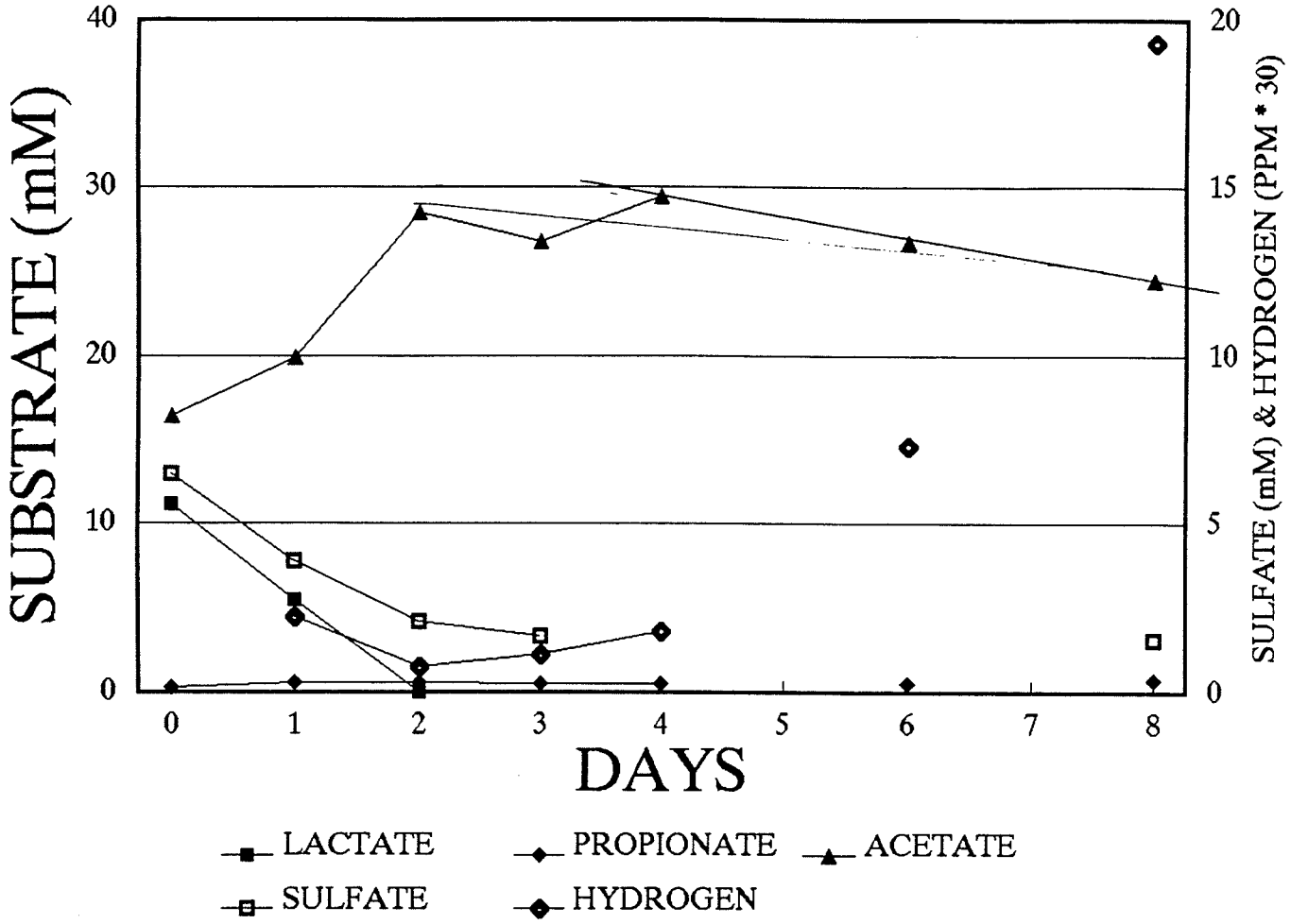
pH (o/i)	TS	H_2S	o/o CH_4	mL CH_4
7.1/7.1	440			
7.2	740		12.2	



7.1/7.1 600

7.2 840 0

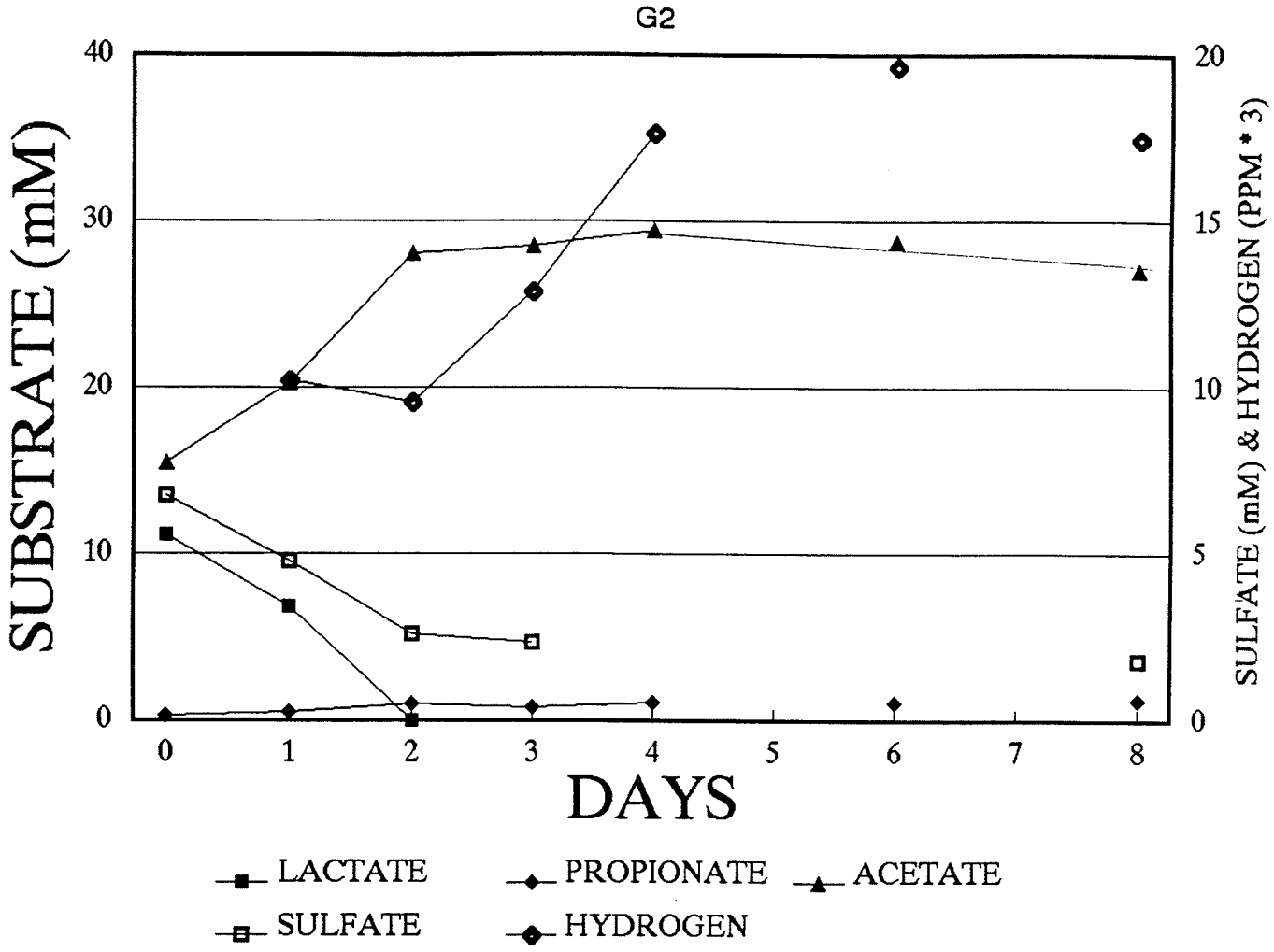
G1 C8 - control pH 8



8.2/8.1 400

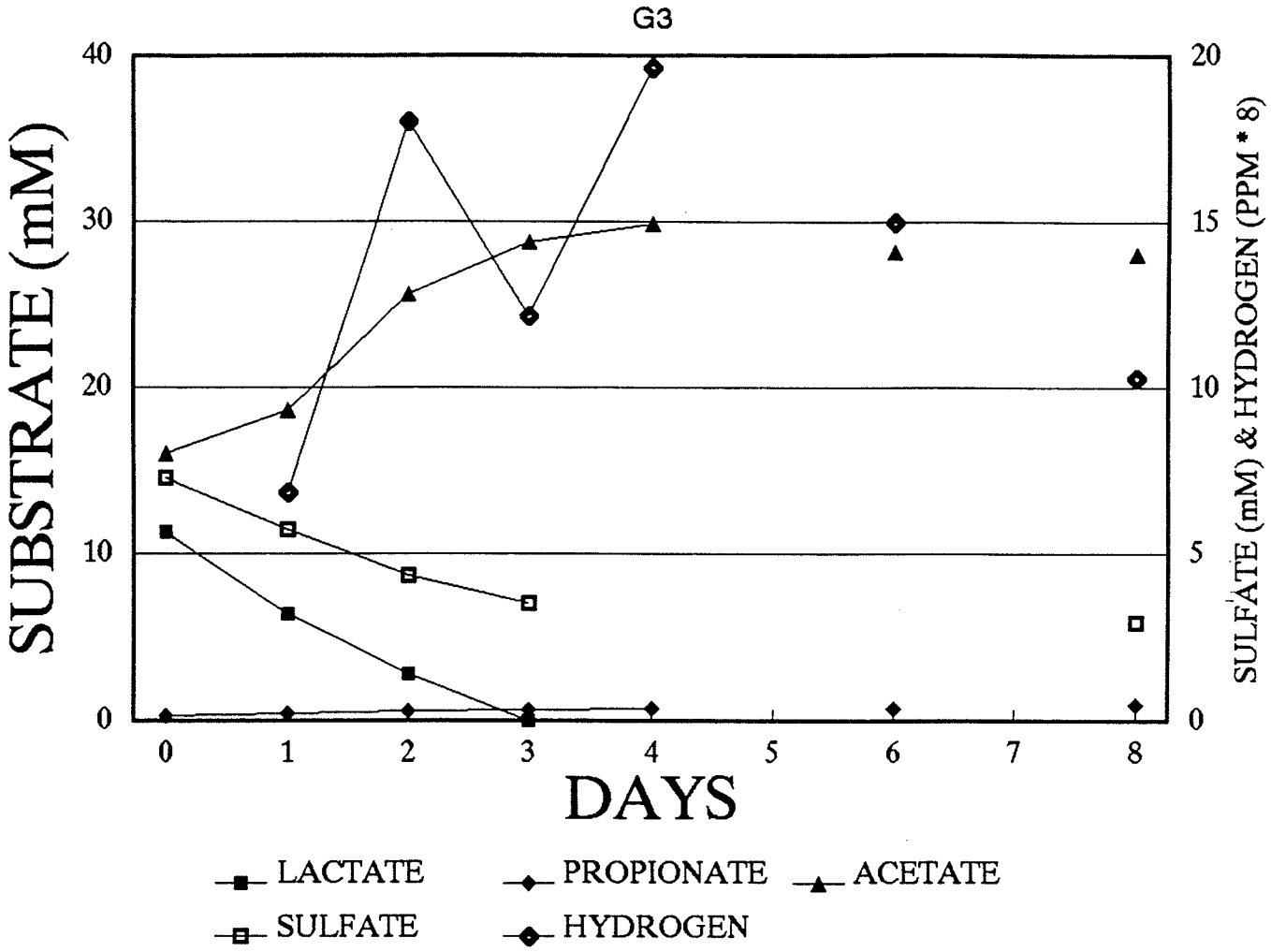
8.0 680 12.2

- H_2S removed in 2 days
- H_2 build-up after several days.



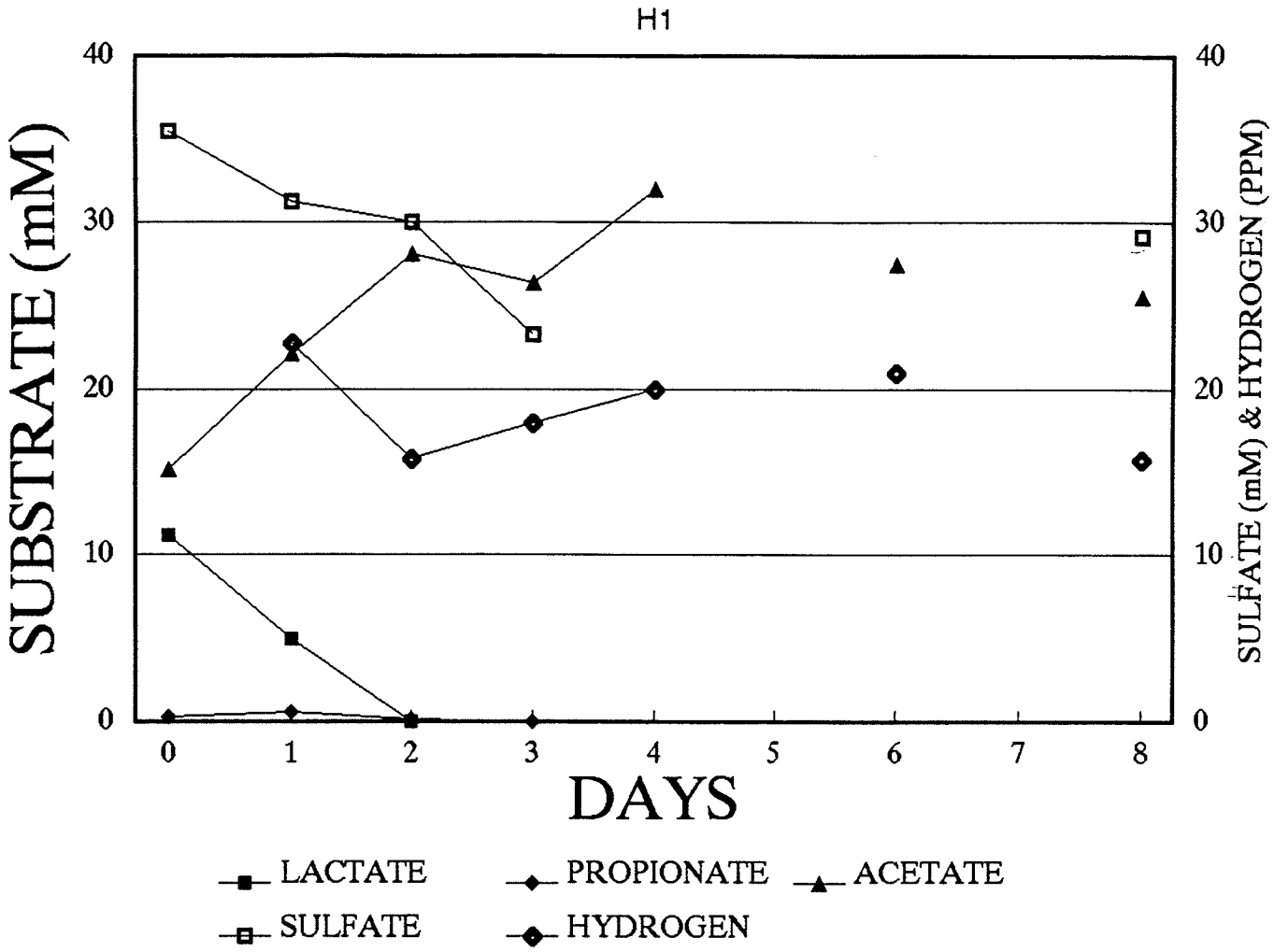
8.3/8.1 1000

8.1 1160 4.6



8.3/8.1 2000

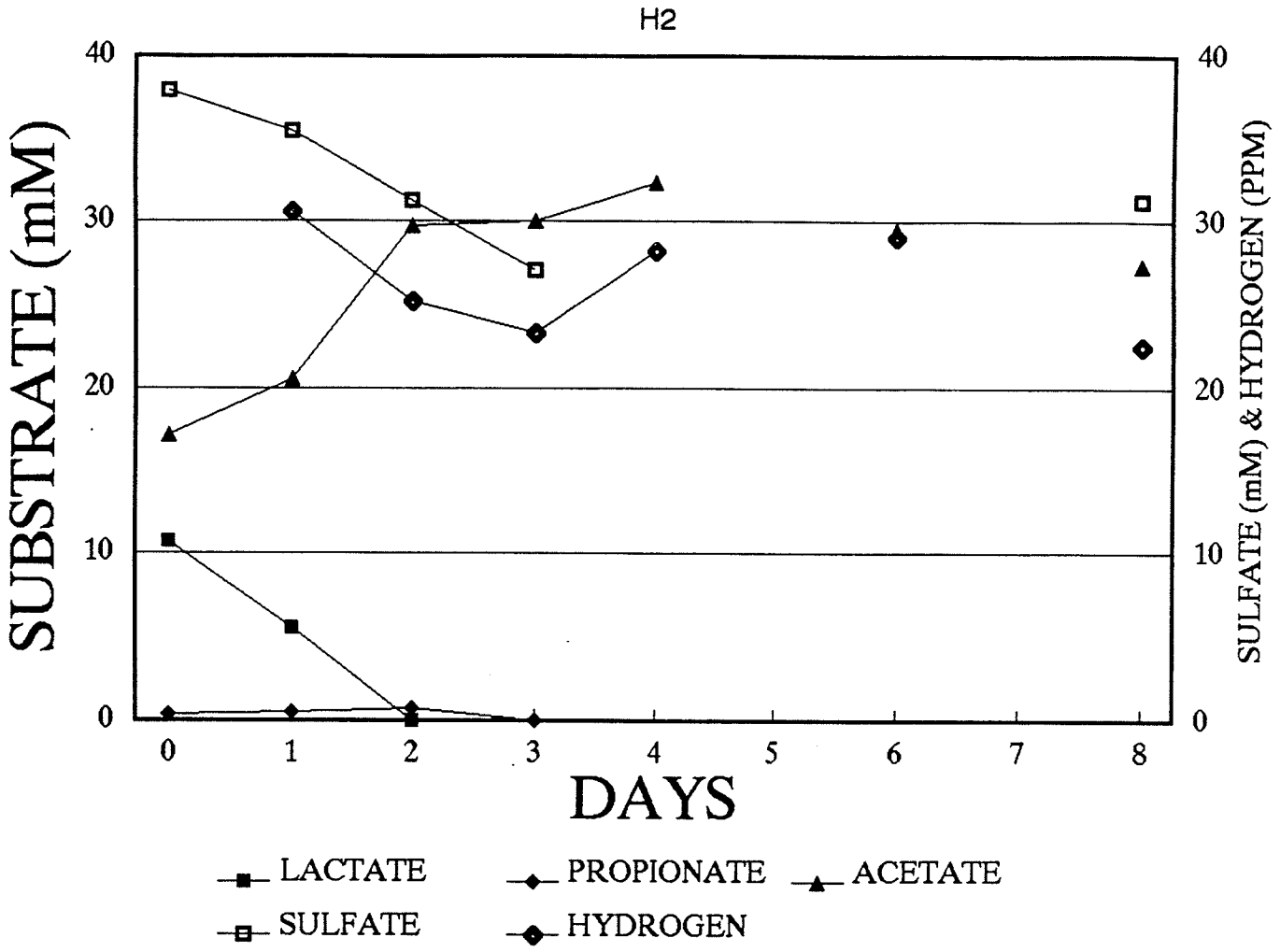
8.1 2000 1.4



83/8.1 400

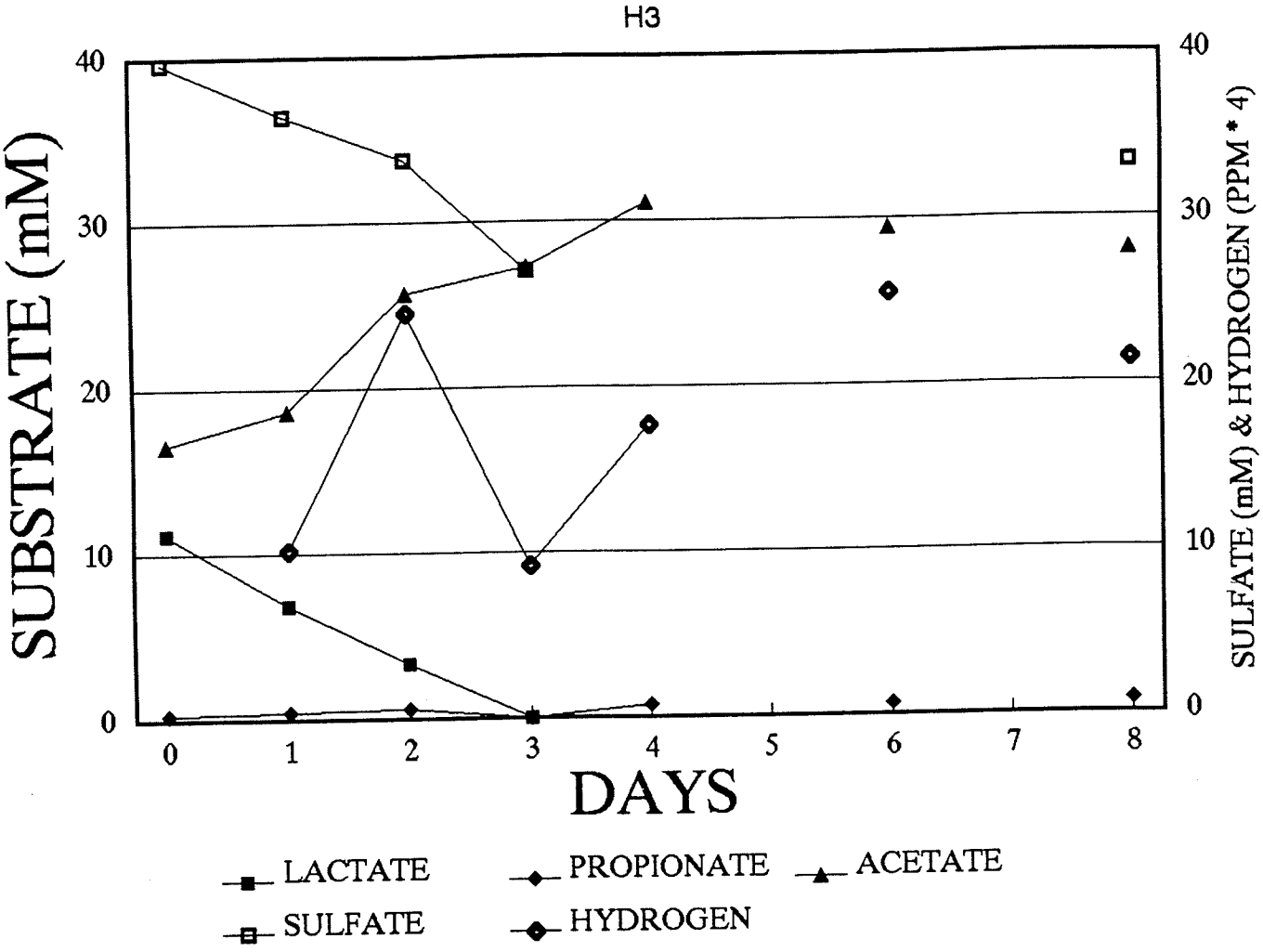
8.1 960 7.3

SO4 values on day 3 & 8



8.3/8.2 1000

8.1 1240 3.0



8.3/82 2040

8.1 2080 0.9