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COMPARISON OF ALTERNATIVE HYDROGEN DONORS
FOR ANAEROBIC REDUCTIVE DECHLORINATION OF
TETRACHLOROTHENE

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SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING
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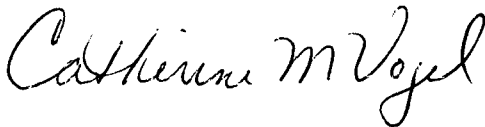
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13. ABSTRACT (Maximum 200 words) Selective delivery of H ₂ to a tetrachloroethene (PCE) dechlorinator was investigated and modeled. H ₂ was delivered using donors lactate, ethanol (EtOH), butyrate, or propionate [substrates whose fermentation to H ₂ is exergonic under H ₂ partial pressures (ceilings) of less than 1, 0.1, 10 ^{-3.5} , and 10 ^{-4.4} atm, respectively]. Recent studies of H ₂ use in reductive dechlorination show that dechlorinators have an affinity for H ₂ at least an order of magnitude greater than that of hydrogenotrophic methanogens. Slowly fermented substrates producing lower H ₂ levels may be more effective and persistent "selective" stimulators of dechlorination than rapidly fermented substrates producing higher H ₂ levels. Separate, semicontinuously operated cultures were enriched with each of the donors. Long-term, all donors equally stimulated the dechlorination of PCE to vinyl chloride and ethene; however, stimulation of methanogenesis differed (EtOH > lactate > butyrate > propionate). During short-term tests, patterns of donor fermentation and H ₂ production and consumption were significantly different for different donors. When fed amounts stoichiometrically sufficient to completely dechlorinate PCE, half the H ₂ released during EtOH fermentation was used methanogenically with the remainder channeled to incomplete dechlorination; however, only 1% of the H ₂ released during propionate fermentation was used methanogenically and the remainder was used for complete dechlorination. A comprehensive model using Michaelis-Menten-type kinetics incorporating H ₂ thresholds and thermodynamic limitations on donor fermentations was formulated. Mixed-culture behavior under a variety of conditions was fit well by the model.				
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TETRACHLOROETHENE

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Donna Elaine Fennell

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COMPARISON OF ALTERNATIVE HYDROGEN DONORS FOR
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Donna Elaine Fennell, Ph.D.

Cornell University 1998

Selective delivery of H_2 to a tetrachloroethene dechlorinator was investigated and modeled. H_2 was delivered using the donors lactate, ethanol, butyrate or propionate—substrates whose fermentation to H_2 is exergonic under H_2 partial pressures (ceilings) of less than 1, 0.1, $10^{-3.5}$, and $10^{-4.4}$ atm, respectively. The H_2 ceiling also governs the persistence of the donor. The lower the H_2 ceiling, the more slowly the fermentation proceeds because the rate is limited by biological H_2 removal, which is also slower at lower H_2 levels. Thus, while ethanol is rapidly fermented to short-lived bursts of high-level H_2 , propionate is slowly fermented to steadily-supplied low-level H_2 .

Recent studies of H_2 use in reductive dechlorination reported that dechlorinators have an affinity for H_2 at least an order of magnitude greater than that of hydrogenotrophic methanogens. Ability to use H_2 at appreciable rates at low levels may provide a competitive advantage to dechlorinators. Slowly fermented substrates producing lower H_2 levels—kinetically accessible to dechlorinators, but too low for significant use by methanogenic competitors—may be more effective and persistent “selective” stimulators of dechlorination than rapidly fermented substrates

producing higher H_2 levels—accessible to both dechlorinators and methanogens.

Separate, semi-continuously operated cultures were enriched with one of each of the donors. Long-term, all donors stimulated nearly equally the dechlorination of tetrachloroethene to vinyl chloride and ethene; however, stimulation of methanogenesis differed—ethanol>lactate>butyrate>propionate. During short-term tests, patterns of donor fermentation and H_2 production and consumption *were* significantly different for different donors. When fed amounts stoichiometrically sufficient to completely dechlorinate tetrachloroethene, half the H_2 released during ethanol fermentation was used methanogenically with the remainder channeled to incomplete dechlorination; however, only one percent of the H_2 released during propionate fermentation was used methanogenically and the remainder was used for complete dechlorination. The lack of observed differences in dechlorination with different H_2 donors during long-term studies was caused by routine addition of a nutritional supplement that also contained butyrate and propionate.

A comprehensive model using Michaelis-Menten-type kinetics incorporating H_2 thresholds and thermodynamic limitations on donor fermentations was formulated. Mixed-culture behavior under a variety of conditions was fit well by the model.

BIOGRAPHICAL SKETCH

Donna Elaine Fennell was born to Jim Bill Fennell and June Rose Hicks Fennell on September 11, 1961. She grew up in the beautiful mountains of northwest Arkansas. After graduating from Booneville, Arkansas High School in 1979, she attended the University of Arkansas at Fayetteville and in 1985 she graduated with honors with a Bachelor of Science degree in Agricultural Engineering. While at the University of Arkansas, Donna was involved in research projects involving alternative waste management and on-site domestic wastewater treatment. It was there that she first became excited about a career in environmental engineering.

In May 1985, Donna enrolled as a Master of Science student in Agricultural Engineering at Cornell University under the guidance of William J. Jewell. Her research project was part of a large biomass to energy feasibility study. She received her M.S. degree from Cornell University in May, 1988. After completing her M.S., Donna was a staff researcher in Professor Jewell's lab where she worked on reactor-based bioremediation of chlorinated solvents.

In 1993, she entered the School of Civil and Environmental Engineering as a Ph.D. candidate under the guidance of James M. Gossett. There, she studied anaerobic reductive dechlorination of tetrachloroethene. She will be continuing as a Post Doc with Professor Gossett working on a site examination protocol for chlorinated solvent bioremediation. She hopes to pursue a career involving environmental research and teaching.

PREFACE

This report was submitted as a dissertation to the Graduate School at Cornell University, Ithaca, NY, in partial fulfillment for the degree of Doctor of Philosophy. The dissertation covers work performed by Ms. Donna Elaine Fennell. The effort was partially funded by the Armstrong Laboratory Environmental Quality Directorate (AL/EQ) (now known as the Air Force Research Laboratory, Materials and Manufacturing Directorate, Airbase and Environmental Division, AFRL/MLQ).

This dissertation is being published in its original format because of its interest to the worldwide scientific and engineering community. It covers work performed between August 1993 and January 1998. The AL/EQ Project Manager was Ms. Catherine M. Vogel.

To
June Rose Hicks Fennell,
Beulah McGougan Hicks,
and Vada Lurena Garner Fennell

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LIST OF ABBREVIATIONS

AFCEE	Air Force Center for Environmental Excellence
BES	2-bromoethanesulfonate
CH ₄	methane
dt	simulation time step
ETH	ethene
1,1-DCE	1,1-dichloroethene
<i>cis</i> -1,2-DCE	<i>cis</i> -1,2-dichloroethene
<i>c</i> DCE	<i>cis</i> -1,2-dichloroethene
<i>trans</i> -1,2-DCE	<i>trans</i> -1,2-dichloroethene
DNAPL	dense non-aqueous phase liquid
FID	flame ionization detector
FYE	fermented yeast extract
GC	gas chromatography
H ₂	hydrogen
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
NAPL	non-aqueous phase liquid
PCE	tetrachloroethene, perchloroethylene
PON	particulate organic nitrogen
RGD	reduction gas detector
SFYE	surrogate fermented yeast extract
TCD	thermal conductivity detector
TCE	trichloroethene
TKN	total Kjeldahl nitrogen
VC	vinyl chloride
VFAs	volatile fatty acids
VSS	volatile suspended solids

LIST OF SYMBOLS

C_g	gaseous concentration of a compound ($\mu\text{mol/L}$)
$C_{g\text{CH}_4}$	gaseous methane concentration ($\mu\text{mol/L}$)
$C_{g\text{H}_2}$	gaseous hydrogen concentration ($\mu\text{mol/L}$)
$C_{g\text{cDCE}}$	gaseous <i>cis</i> -1,2-dichloroethene concentration ($\mu\text{mol/L}$)
$C_{g\text{ETH}}$	gaseous ethene concentration ($\mu\text{mol/L}$)
$C_{g\text{PCE}}$	gaseous tetrachloroethene concentration ($\mu\text{mol/L}$)
$C_{g\text{TCE}}$	gaseous trichloroethene concentration ($\mu\text{mol/L}$)
$C_{g\text{VC}}$	gaseous vinyl chloride concentration ($\mu\text{mol/L}$)
C_w	aqueous concentration of a compound ($\mu\text{mol/L}$)
$C_{w\text{Acetate}}$	aqueous acetate concentration ($\mu\text{mol/L}$)
$C_{w\text{cDCE}}$	aqueous <i>cis</i> -1,2-dichloroethene concentration ($\mu\text{mol/L}$)
$C_{w\text{CH}_4}$	aqueous methane concentration ($\mu\text{mol/L}$)
$C_{w\text{ETH}}$	aqueous ethene concentration ($\mu\text{mol/L}$)
$C_{w\text{H}_2}$	aqueous hydrogen concentration ($\mu\text{mol/L}$)
$C_{w\text{PCE}}$	aqueous tetrachloroethene concentration ($\mu\text{mol/L}$)
$C_{w\text{TCE}}$	aqueous trichloroethene concentration ($\mu\text{mol/L}$)
$C_{w\text{VC}}$	aqueous vinyl chloride concentration ($\mu\text{mol/L}$)
f_s	fraction of substrate used for synthesis of new biomass
f_e	fraction of substrate used for energy generation
ΔG_{rxn}	Gibbs free energy for the reaction (kJ/mol)
$\Delta G_{\text{critical}}$	critical Gibbs free energy (kJ/mol)
$\Delta G^{\circ}_{25^{\circ}}$	standard free energy of reaction (kJ/mol) at 25°C
$\Delta G^{\circ}_{35^{\circ}}$	standard free energy of reaction (kJ/mol) at 35°C
$\Delta G^{\circ}_{f25^{\circ}}$	standard free energy of formation (kJ/mol) at 25°C
$\Delta G^{\circ}_{f35^{\circ}}$	standard free energy of formation (kJ/mol) at 35°C
$\Delta H^{\circ}_{f25^{\circ}}$	standard enthalpy of formation (kJ/mol) at 25°C

γ_i	activity coefficient for constituent i
H_2 Threshold _{dechlor}	threshold for H_2 use by dechlorinators ($\mu\text{mol/L}$)
H_2 Threshold _{meth}	threshold for H_2 use by hydrogenotrophic methanogens ($\mu\text{mol/L}$)
H_c	pseudo-dimensionless Henry's constant
H_{cCH_4}	pseudo-dimensionless Henry's constant for methane
H_{cDCE}	pseudo-dimensionless Henry's constant for <i>cis</i> -1,2-dichloroethene
H_{cETH}	pseudo-dimensionless Henry's constant for ethene
H_{cH_2}	pseudo-dimensionless Henry's constant for hydrogen
H_{cPCE}	pseudo-dimensionless Henry's constant for tetrachloroethene
H_{cTCE}	pseudo-dimensionless Henry's constant for trichloroethene
H_{cVC}	pseudo-dimensionless Henry's constant for vinyl chloride
I	ionic strength (eq/L)
k	maximum specific rate of degradation ($\mu\text{mol/mg VSS-hr}$)
k_{Acetate}	maximum specific rate of acetate utilization ($\mu\text{mol/ mg VSS-hr}$)
k_{apparent}	apparent maximum specific rate of donor degradation ($\mu\text{mol/mg VSS-hr}$)
k_{Butyrate}	maximum specific rate of butyrate utilization ($\mu\text{mol/ mg VSS-hr}$)
k_c	salting-out coefficient
k_{cDCE}	maximum specific rate of <i>cis</i> -1,2-DCE utilization ($\mu\text{mol/ mg VSS-hr}$)
k_d	decay coefficient for the organism group (hr^{-1})
k_{Donor}	the maximum specific rate of donor utilization ($\mu\text{mol/ mg VSS-hr}$)

k_{Ethanol}	maximum specific rate of ethanol utilization ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)
$k_{(\text{H}_2)\text{meth}}$	maximum rate of H_2 utilization by methanogens ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)
k_{Lactate}	maximum specific rate of lactate utilization ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)
k_{PCE}	maximum specific rate of PCE utilization ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)
$k_{\text{Propionate}}$	maximum specific rate of propionate utilization ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)
k_{TCE}	maximum specific rate of TCE utilization ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)
k_{VC}	maximum specific rate of VC utilization ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)
K_{la}	mass transfer coefficient (hr^{-1})
K_{S}	half-velocity coefficient ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{Acetate})}$	half-velocity coefficient for acetate ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{Butyrate})}$	half-velocity coefficient for butyrate ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{cDCE})}$	half-velocity coefficient for <i>cis</i> -1,2-dichloroethene ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{Donor})}$	half-velocity coefficient for donor ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{Ethanol})}$	half-velocity coefficient for ethanol ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{H}_2)\text{dechlor}}$	half-velocity coefficient for hydrogen use by dechlorinators ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{H}_2)\text{meth}}$	half-velocity coefficient for hydrogen use by hydrogenotrophic methanogens ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{Lactate})}$	half-velocity coefficient for lactate ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{Propionate})}$	half-velocity coefficient for propionate ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{PCE})}$	half-velocity coefficient for PCE ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{TCE})}$	half-velocity coefficient for TCE ($\mu\text{mol}/\text{L}$)
$K_{\text{S}(\text{VC})}$	half-velocity coefficient for VC ($\mu\text{mol}/\text{L}$)
M_{g}	compound in gaseous phase ($\mu\text{mol}/\text{bottle}$)

Mg _{cDCE}	gaseous <i>cis</i> -1,2-dichloroethene (μmol/bottle)
Mg _{ETH}	gaseous ethene (μmol/bottle)
Mg _{H2}	gaseous hydrogen (μmol/bottle)
Mg _{PCE}	gaseous tetrachloroethene (μmol/bottle)
Mg _{TCE}	gaseous trichloroethene (μmol/bottle)
Mg _{VC}	gaseous vinyl chloride (μmol/bottle)
M _w	compound in aqueous phase (μmol/bottle)
M _{wcDCE}	aqueous <i>cis</i> -1,2-dichloroethene (μmol/bottle)
M _{wETH}	aqueous ethene (μmol/bottle)
M _{wH2}	aqueous hydrogen (μmol/bottle)
M _{wPCE}	aqueous tetrachloroethene (μmol/bottle)
M _{wTCE}	aqueous trichloroethene (μmol/bottle)
M _{wVC}	aqueous vinyl chloride (μmol/bottle)
M _t	total compound in bottle (μmol/bottle)
M _{tAcetate}	total acetate in bottle (μmol/bottle)
M _{tButyrate}	total butyrate in bottle (μmol/bottle)
M _{tcDCE}	total <i>cis</i> -1,2-dichloroethene in bottle (μmol/bottle)
M _{tCH4}	total methane in bottle (μmol/bottle)
M _{tCH4 from H2}	total methane formed via hydrogenotrophic methanogens in bottle (μmol/bottle)
M _{tCH4 from Acetate}	total methane formed via acetotrophic methanogens in bottle (μmol/bottle)
M _{tETH}	total ethene in bottle (μmol/bottle)
M _{tEthanol}	total ethanol in bottle (μmol/bottle)
M _{tH2}	total hydrogen in the bottle (μmol/bottle)
M _{tLactate}	total lactate in bottle (μmol/bottle)
M _{tPCE}	total tetrachloroethene in bottle (μmol/bottle)
M _{tPropionate}	total propionate in bottle (μmol/bottle)
M _{tTCE}	total trichloroethene in bottle (μmol/bottle)
M _{tVC}	total vinyl chloride in bottle (μmol/bottle)

Φ	thermodynamic factor ("thermo factor", STELLA model nomenclature) ranging from one (when there is no thermodynamic limitation on donor fermentation) to zero when $\Delta G_{\text{rxn}} \geq \Delta G_{\text{critical}}$
R	universal gas constant
S	substrate (donor) concentration ($\mu\text{mol/L}$)
S^*	hypothetical value of substrate (donor) concentration ($\mu\text{mol/L}$) that, under the instantaneous culture conditions, would result in $\Delta G_{\text{rxn}} = \Delta G_{\text{critical}}$
S_e	effluent substrate concentration ($\mu\text{mol/L}$)
S_o	influent substrate concentration ($\mu\text{mol/L}$)
T	temperature ($^{\circ}\text{K}$)
θ_c	solids retention time (d)
V_w	volume of the liquid portion of the bottle (L)
V_g	volume of the gas portion of the bottle (L)
X	biomass of the specific organism group contained in the bottle (mg VSS)
$X_{\text{Acetotroph}}$	acetotrophic methanogenic biomass contained in the bottle (mg VSS)
$X_{\text{Butyrate Fermenter}}$	butyrate fermenter biomass contained in the bottle (mg VSS)
X_{Dechlor}	dechlorinator biomass contained in the bottle (mg VSS)
X_{Donor}	donor fermenter biomass contained in the bottle (mg VSS)
X_{est}	estimated biomass concentration of a specific organism group contained in the bottle (mg VSS/L)
$X_{\text{Ethanol Fermenter}}$	ethanol fermenter biomass contained in the bottle (mg VSS)
$X_{\text{Hydrogenotroph}}$	hydrogenotrophic methanogen biomass contained in the bottle (mg VSS)
$X_{\text{Lactate Fermenter}}$	lactate fermenter (lactate to acetate) biomass contained in the bottle (mg VSS)

$X_{\text{Propionate Fermenter}}$

propionate fermenter biomass contained in the bottle
(mg VSS)

Y organism yield (mg VSS/ μmol substrate used)

Y_{energy} organism yield (mg VSS/mol substrate used for energy
production)

Z charge on the ion

CHAPTER ONE

INTRODUCTION

1.A. Context

Widespread contamination of groundwater is an unfortunate result of industrial and military development. It is estimated that in the United States there are at least 300,000 to 400,000 hazardous waste sites where groundwater may be contaminated. While the bulk of these sites consist of small, leaking, underground fuel storage tanks, a significant number, about 35,000, are seriously contaminated abandoned or closed hazardous waste sites, sites where active cleanup is already ongoing, military sites, and Department of Energy sites [161].

The chlorinated ethenes are commonly detected groundwater contaminants [262], with trichloroethene (TCE) and tetrachloroethene, (perchloroethylene, PCE) ranking as the number 1 and number 3, respectively, most-frequently detected pollutants at hazardous wastes sites [161]. This is of great concern because PCE and TCE are suspected carcinogens, and of their daughter compounds—*cis*-1,2-, *trans*-1,2-, and 1,1-dichloroethene (DCE), and chloroethene (vinyl chloride, VC)—VC is a known carcinogen [111, 147, 246, 247]. Maximum contaminant levels are 5 ppb for PCE and TCE, 7 ppb for 1,1 DCE, and 2 ppb for VC under the Safe Drinking Water Act [77].

PCE and TCE are excellent solvents that are widely used in degreasing and dry-cleaning applications. They are nonflammable, thus safer in that respect for workers to handle than some alternatives. The use of TCE in the military and PCE primarily in the domestic dry-cleaning industry and in CFC production increased rapidly in the 1960s [37]. Their

use leveled out in the 1980s, but spills and improper disposal of both of these DNAPLs (dense non-aqueous phase liquids) at hazardous waste disposal sites, domestic dry-cleaning establishments, military bases, and industrial complexes have resulted in many contaminant plumes. Currently, the use of chloroethenes is decreasing through regulatory changes intended to phase out their use because of their carcinogenic or potentially carcinogenic nature [269].

Finding practical methods to remove chlorinated solvents and other contaminants from groundwater has become a major task for environmental professionals. Pumping contaminated water to the surface for treatment—pump-and-treat—was the remediation method of choice in the 1980's and early 1990's [89]. However, the heterogeneity of the subsurface, the presence of difficult-to-locate and sparingly soluble NAPLs (non-aqueous phase liquids), the migration of pollutants to inaccessible regions, sorption of contaminants to subsurface materials, and difficulty in characterizing the subsurface have been cited as reasons why the restoration of groundwater to drinking water standards—regardless of the process employed—is proving to be a very difficult, complex, time-consuming, and expensive task [161]. After evaluating remediation results at 77 sites where pump-and-treat methods were employed, the conclusion of the National Research Council was that as the complexity of a site increases, the likelihood of successful pump-and-treat remediation decreases drastically. Now it is recognized that only simple, homogeneous sites are likely to be completely and economically restored by pump-and-treat technology. At most sites, enormous volumes of water which contain low levels of contaminants must be extracted and treated with either chemical-physical or biological methods. Treatment times are estimated to

range from decades to perhaps hundreds, or even thousands of years. Even if the plume appears to have been remediated, if DNAPL remains in the aquifer, as soon as the pumps are turned off, re-contamination of the groundwater is observed. The recognition of the very high cost, prohibitively long duration, and the often-times minimal effect on the contamination problem has resulted in a new reluctance to apply pump-and-treat to many sites except for very simple ones where a high probability of success exists; for prevention of plume migration to sensitive receptors; and for removing free product at well-defined "hot spots" [1].

Since the recognition of the serious problems associated with pump-and-treat, more attention is now focused on *in situ* remediation of pollutants, including the chlorinated solvents. If microorganisms are able to convert harmful compounds to innocuous ones, or in some cases to completely mineralize them, biological treatment has a significant advantage over physical-chemical treatment schemes, which usually transfer the pollutant from one phase to another (i.e. from water to air). Furthermore, because of the inefficiency and difficulty associated with aerobic chlorinated solvent treatment, and the pre-existence of anaerobic zones within contaminated plumes, *anaerobic* biological reductive dechlorination of chlorinated solvents has been recognized as a promising alternative for chlorinated solvent remediation both through natural attenuation and as an enhanced *in situ* bioremediation scheme. While the technology is still in the early stages of development, early reports of its application are promising and its potentially competitive cost makes it an attractive alternative.

The recognition of reductive dechlorination as a potentially useful bioremediation alternative has come about at nearly the same time that

several new dehalorespiring organisms, which gain energy when using PCE, TCE, or DCE as an electron acceptor, have been isolated. These organisms, isolated from diverse environments, exhibit high rates of dechlorination. The combination of these discoveries and the problematic historical experience with previously applied technologies have increased the interest in anaerobic reductive dechlorination as a viable treatment alternative that deserves more widespread trial application and follow-up research. A draft protocol for evaluating natural attenuation of chlorinated solvents in groundwater has already been developed by the U.S. Air Force Center for Environmental Excellence (AFCEE) [264].

Except for one published report, [54], PCE is generally thought to be recalcitrant under aerobic conditions. However, under anaerobic conditions, PCE is reductively dechlorinated to its lesser or non-chlorinated daughter compounds TCE, DCE isomers, VC, and ETH by a number of organisms. The anaerobic process becomes slower and less efficient as the number of chlorines decreases. TCE, DCE, and VC are degradable aerobically via co-metabolic activities, however, efficiency of aerobic treatment generally decreases with an increased number of chlorines. Thus reductive dechlorination is a very attractive alternative for PCE, TCE, and DCE remediation. While VC is believed to be the most harmful compound of the series, its production under anaerobic conditions is not now thought to be catastrophic since it is known to be readily degraded under aerobic conditions by isolates that use it as a primary substrate [97], environmentally-occurring organisms [53], methanotrophic cultures [162], ethane- and ethene-degrading cultures [81, 82], and iron-reducing organisms [31]. At many sites, if dechlorination can be pushed to VC in the anaerobic zone, then the residual VC is readily degraded as the plume

converts to aerobic conditions. If however, DCE is the final product of the anaerobic zone, it may persist in these aerobic zones.

Reductive dechlorination requires an external electron donor. One of the important donors in terms of organism use and environmental importance is molecular hydrogen, H_2 . H_2 is produced in large quantities through direct fermentation of substrates such as complex organic compounds and volatile fatty acids such as butyrate; or as a trace intermediate as in the conversion of methanol to acetate, or even in the conversion of methanol or acetate to CH_4 .

If there are either very low or very high concentrations of PCE present, supplying donor is not problematic. When very low concentrations of PCE are present, only a tiny fraction of the electron donor added must be channeled to dechlorination. Relatively large additions of any type of donor, even one that is used directly by methanogens, would likely produce enough trace, scavengeable H_2 to fuel complete dechlorination of trace amounts of PCE to ETH [80]. When high PCE concentrations—approaching the maximum solubility of PCE—are present, hydrogenotrophic methanogens are inhibited and the H_2 produced from the transformation of the added donor is primarily channeled to dechlorination; thus, a smaller donor to PCE ratio—and even a methanogenic donor such as methanol—can be used to sustain dechlorination because methanogenic competition for the donor is inhibited [61].

A problem is encountered when an “intermediate” PCE concentration is present that, while requiring a significant amount of donor for complete dechlorination, is not at a level inhibitory to the competing methanogens. In this scenario, a methanogenic substrate such

as methanol is unsuitable since it is rapidly converted to CH_4 , and the trace amount of H_2 produced is subject to competition both by dechlorinators and hydrogenotrophic methanogens. Thus, methanol addition stimulates the growth of high concentrations of the methanogenic competition and a higher relative donor application is required to supply the trace H_2 to achieve the same amount of dechlorination. Subsequently, more donor must be added, which stimulates the growth of even more of the competition, and so on, until dechlorinators are completely marginalized. This has been termed a "spiral to failure" [224]. Competition for H_2 at the "intermediate" PCE levels is thus an important consideration for enhanced stimulation of dechlorination.

This study has focused on a method to more effectively deliver the electron donor, H_2 , to give a selective advantage to hydrogenotrophic dechlorinating organisms over hydrogenotrophic methanogens at these problematic "intermediate" PCE concentrations and to formulate a fundamentally-based, comprehensive model to describe the process.

1.B. Objectives and Experimental Strategy

Of the PCE- and TCE-utilizing dehalorespiring organisms currently known, four use H_2 as an electron donor, and two of these use only H_2 as an electron donor. H_2 is an important environmental compound—it is produced through the action of many fermentative organisms and it is consumed by (among others) methanogens, sulfate-reducing bacteria, and ferric iron-reducing bacteria in natural environments. Currently, investigators are using H_2 levels at contaminated sites as one of a number of parameters to characterize contaminated plume zones [140] as methanogenic, sulfate-reducing, or iron-reducing. Each of those types of

microbial activities has a different threshold or minimum level of H_2 at which it can operate. Therefore, under H_2 -limiting conditions the H_2 is poised at a different concentration depending upon which microbial process predominates. At sites contaminated with chlorinated solvents, hydrogenotrophic dechlorinators must also compete for this limited donor.

Stover [224] examined the effect of different electron donors in a mixed dechlorinating culture and found that dechlorinators were active at H_2 levels that were apparently too low to support methanogenesis. This difference in affinity was later quantified by Smatlak *et al.* through a comparison of half-velocity coefficients for H_2 use by the dechlorinator and the methanogens in a mixed culture [210]. A ten-fold lower half-velocity coefficient (100 nM) was observed for H_2 use by dechlorinators (presumably "*Dehalococcoides ethenogenes*" strain 195, the dechlorinator present in the mixed culture) than was observed for the hydrogenotrophic methanogens in the culture (960 nM) [210]. In a study of a mixed methanogenic culture that contained an unknown dechlorinator(s) Ballapragada *et al.* also reported low half-velocity coefficients for H_2 use by dechlorination of 9 to 21 nM [13].

Thermodynamic considerations indicate that the high affinity for H_2 may be generally true for all dechlorinators. At 25°C, pH = 7, {PCE} and {TCE} = 0.1 mM, {Cl⁻} = 1 mM, and { H_2 } (aq) = 0.001mM, the H_2 -PCE couple, for example, offers an organism -168 kJ/mol H_2 utilized {using ΔG°_f (kJ/mol) at 25°C for H_2 (aq) = 17.57 [225]; H^+ (aq) = 0 [225]; Cl⁻(aq) = -131.3 [225]; PCE (aq) = 27.59 [63]; TCE (aq) = 25.41 [63]}. Thus, even at very low H_2 concentrations, this bioreaction is energy yielding. It is likely that the organisms would evolve with the ability to use H_2 at these low, but still

energetically favorable concentrations—i.e., they would likely have a high affinity/low half-velocity coefficient for H₂ use.

The primary objectives of this research were as follows:

- (1) Investigate whether the high affinity for H₂ exhibited by dechlorinators could be exploited through the mechanism of H₂ addition in order to give a selective competitive advantage to dechlorinators over methanogens in a mixed culture;
- (2) Determine whether slowly fermented substrates that produce low H₂ levels (such as propionic and butyric acid) would be superior to rapidly fermented ones that can theoretically generate higher H₂ levels (such as ethanol and lactic acid); and
- (3) Describe and quantify the relationships between H₂-producing organisms, and the hydrogenotrophic dechlorinators and competing hydrogenotrophic methanogens in a mixed dechlorinating community via a comprehensive model.

The strategy followed to accomplish these objectives was to develop enrichment cultures on four organic electron donors—butyric acid, ethanol, lactic acid, and propionic acid—that may be microbially oxidized directly to acetate and H₂ via the fermentation reactions shown in Table 1.1.

Table 1.1. Fermentation reactions for hydrogen donors examined during this study.

Fermentation of Donors to Acetate and H ₂	
Butyrate ⁻ + 2 H ₂ O	→ 2 Acetate ⁻ + H ⁺ + 2 H ₂
Ethanol + H ₂ O	→ Acetate ⁻ + H ⁺ + 2 H ₂
Lactate ⁻ + 2 H ₂ O	→ Acetate ⁻ + HCO ₃ ⁻ + H ⁺ + 2 H ₂
Propionate ⁻ + 3 H ₂ O	→ Acetate ⁻ + HCO ₃ ⁻ + H ⁺ + 3 H ₂

The relationship between the H_2 partial pressure and the Gibbs free energy of reaction for each substrate is shown in Figure 1.1. Because of the thermodynamics of these bioreactions, each of these donors would be expected to produce a different H_2 level. Fermentations of lactic acid, ethanol, butyric acid and propionic acid are exergonic under H_2 partial pressures (ceilings) of less than about 1, 0.1, $10^{-3.5}$ or $10^{-4.4}$ atm, respectively (Figure 1.1). These H_2 ceilings also govern the persistence of the donor. The lower the H_2 ceiling, the more slowly the substrate ferments because its fermentation is limited by the removal of the end product, H_2 , by hydrogenotrophs; and, the microbial use of H_2 is slower at lower H_2 levels. Slowly fermented substrates producing lower H_2 levels that are accessible only to dechlorinators may be more effective and persistent "selective" stimulators of dechlorination than the rapidly fermented substrates that produce higher H_2 levels that persist only for a short time and that are freely accessible to both dechlorinators and their competitors, hydrogenotrophic methanogens. Amendment with the different donors allowed the examination of the effect of different biologically-produced H_2 levels on the competition for H_2 by dechlorinators and methanogens.

The following experimental strategy was used:

- (1) Maintain semi-continuously operated enrichment cultures with each of the H_2 donors (ethanol, lactic acid, butyric acid, and propionic acid) for long-term studies and examine the relative proportion of H_2 flowing to the two alternative H_2 sinks—dechlorination and hydrogenotrophic methanogenesis.
- (2) Carry out short-term time-intensive studies with individual enrichment cultures to quantify dynamic patterns of donor

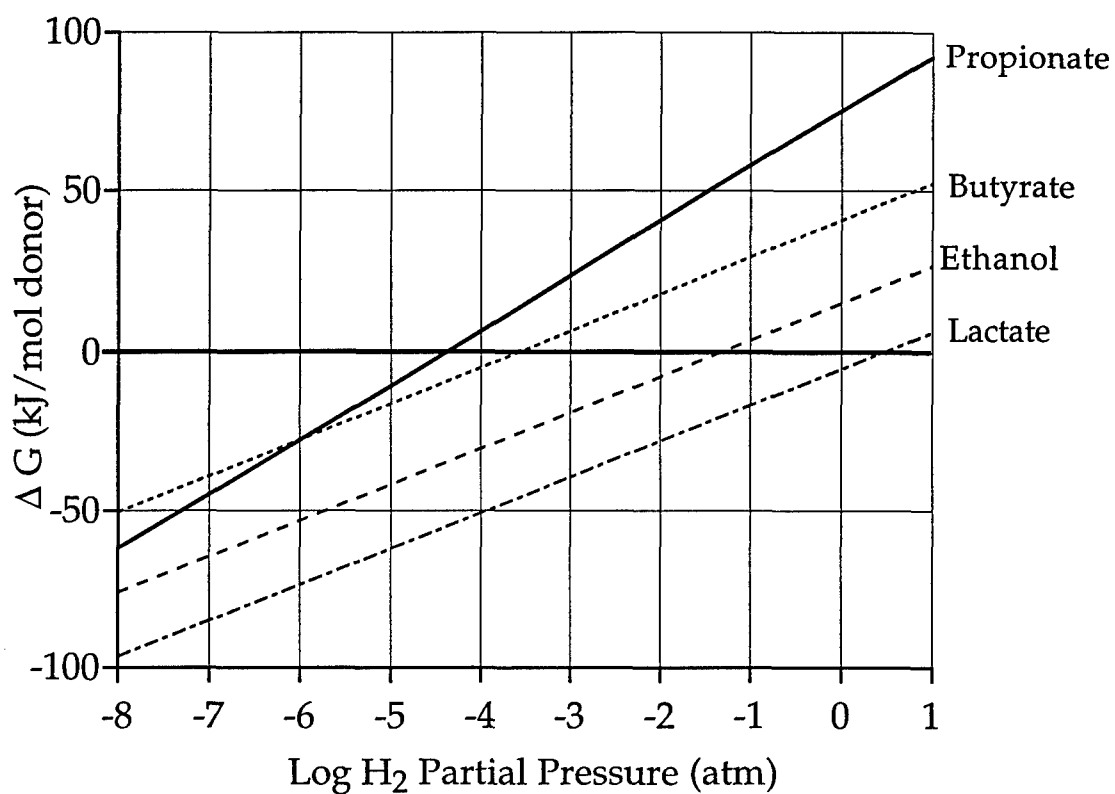


Figure 1.1. Effect of hydrogen partial pressure on the free energy available from the fermentation of propionate, butyrate, ethanol, and lactate. Calculations were based on standard free energies and reactions in Thauer *et al.* [233] with: temperature = 25°C; pH = 7; bicarbonate = 70 mM; butyrate, ethanol, lactate, and propionate = 0.5 mM; and acetate = 5 mM.

fermentation, H_2 production, and H_2 consumption by dechlorination and methanogenesis.

- (3) Examine the effect of donor mixtures and the influence of the added electron-donating capacity from fermented yeast extract, a nutritional supplement.
- (4) Develop source cultures using a promising H_2 donor—butyric acid—to show long-term stability of dechlorinators in a viable methanogenic mixed culture operated at noninhibitory intermediate PCE concentrations.
- (5) Develop a comprehensive model based on Michaelis-Menten-type kinetics but incorporating H_2 thresholds for dechlorination and methanogenesis and thermodynamic limitations on H_2 donor fermentation, to describe a mixed culture of donor degraders, methanogens, and dechlorinators.
- (6) Construct a computer application of the model using STELLA II®. Use experimental data to calibrate and test the model and run both short-term and long-term simulations to determine whether the model adequately simulates experimental conditions.

If anaerobic reductive dechlorination is to become a widely used bioremediation technology, in addition to understanding the dechlorinators, it is also important that we understand the fate of applied donor and the extent to which it is channeled to desirable dechlorination or is wasted on stimulating the growth of competing organisms. A fundamentals-based model will provide a justifiably more complex and highly developed description of the microbial interactions in chlorinated solvent reductive dechlorination that is currently lacking in most

groundwater models that are used for analyzing bioremediation schemes and data from naturally attenuated sites.

CHAPTER TWO

BACKGROUND

Early studies by McCarty and co-workers using mixed cultures and bioreactors provided evidence that PCE and TCE are reductively dechlorinated to the DCE isomers [28, 29, 30] and VC under anaerobic, methanogenic conditions [250, 251]. At the same time, Parsons *et al.* reported that PCE was dechlorinated to TCE, *cis*-1,2-DCE, *trans*-1,2-DCE, and VC in sediment microcosms [171, 172]. Radiotracer studies of sediment samples by Kleopfer *et al.* also confirmed that TCE was reductively dechlorinated to 1,2-DCE [117]. Kinetics of TCE depletion in microcosms was examined by Barrio-Lage *et al.* [15], and efforts were made to stimulate this activity by additions of various chemicals [16]. Investigation of actual contaminated sites by Milde *et al.* confirmed the production of VC in sediments originally contaminated only with PCE and TCE [158].

The first evidence that reductive dechlorination proceeded completely to the non-chlorinated, environmentally benign compound, ethene (ETH) was provided by Freedman and Gossett [80]. de Bruin *et al.* later reported that ETH was transformed to ethane in dehalogenating cultures [33] under anaerobic conditions. Dechlorination of PCE to TCE and *cis*-1,2-DCE by sulfate-reducing cultures was shown in another study [12].

Reductive dechlorination proceeds according to Figure 2.1. An external electron donor—represented by 2H in the diagram—is required. Many electron donors have been observed to stimulate dechlorination in mixed culture studies, including sucrose [38, 44], glucose [29, 80], acetate

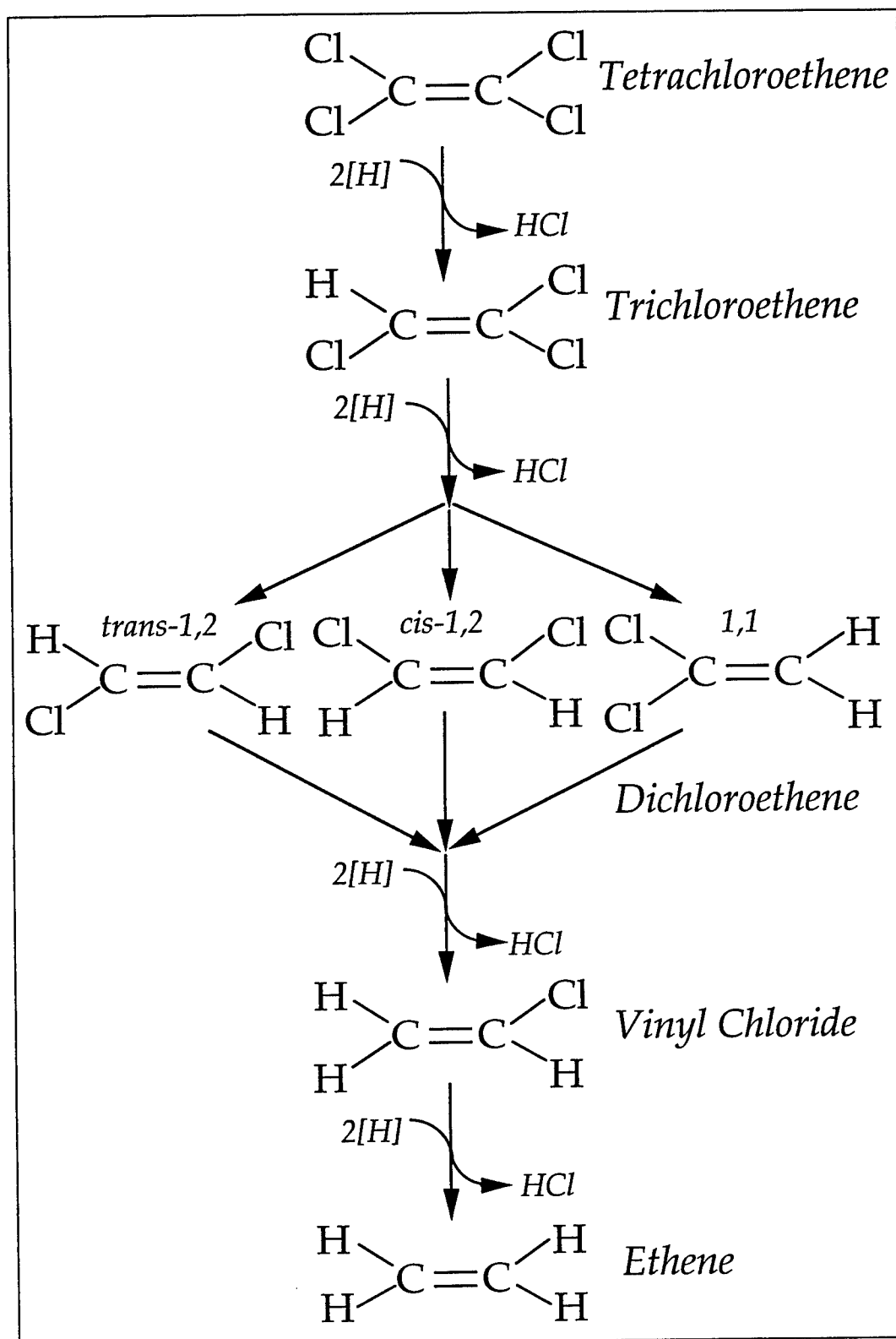


Figure 2.1. Pathway for anaerobic reductive dechlorination of tetrachloroethene to ethene.

[29, 80, 251], dichloromethane [79], toluene [135, 203], benzoate [196], butyrate [87, 88], lactate, ethanol [87, 88, 224], methanol [80], H_2 [61], and the natural organic carbon present in aquifer material [143].

Observations of dechlorination by mixed cultures prompted examination of pure cultures to determine which organisms were responsible for dechlorination; and specific enzymes, coenzymes and cofactors were examined separately for dehaloreductive activities.

2.A. Co-metabolic Reductive Dechlorination of Chloroethenes

Co-metabolism is defined as “the metabolism of a non-growth substrate in which no apparent benefit is accrued by the metabolizing organism” [254]. In some cases, the reaction may be incomplete and end products are usually metabolized by other organisms. Co-metabolism may occur through the action of enzymes with broad specificity as in the classic case of the methanotrophic enzyme, methane monooxygenase, which works on TCE, among other compounds [138]; or through reaction with the metal centers of various coenzymes or factors. Wackett has criticized the prolific use of the term co-metabolism [254] and has suggested that more specific descriptions should be offered, when they are available, to describe these types of reactions. Despite their low rates and inefficiency, these reactions may be important environmentally, since from site to site, it is not known whether dehalorespiring organisms will be present or whether fortuitous dehalogenation will be the dominant mechanism.

2.A.1. Microbially-Mediated Fortuitous Dehalogenation Under Anaerobic Conditions

Methanobacterium thermoautotrophicum dehalogenated small amounts of 1,1-dichloroethane with release of ETH; and *Desulfobacterium autotrophicum* dechlorinated tetrachloromethane to tri- and dichloromethane and 1,1,1-trichloroethane to 1,1-dichloroethane [69]. *Acetobacterium woodii* and *Clostridium thermoaceticum* dechlorinated tetrachloromethane; and radiolabeling demonstrated that tetrachloromethane was transformed to dichloromethane and CO₂ by *A. woodii*. Other organisms tested, which did not contain the acetyl-coenzyme A pathway, did not carry out dechlorination. The authors speculated that the corrinoid enzymes involved in the acetyl-coenzyme A pathway were responsible for the dechlorination [70].

Methanobacterium thermoautotrophicum ΔH, *Methanococcus deltae* ΔLH, and *Methanococcus thermolithotrophicus* produced ethane, ETH, and acetylene from bromoethane, dibromo- or dichloroethane, and 1,2-dibromoethylene, respectively [24].

Dechlorination of PCE to TCE by the acetotrophic methanogens *Methanosarcina* sp. strain DCM and *Methanosarcina mazei*; and by a pyruvate-grown 3-chlorobenzoate dechlorinator, strain DCB-1 (later named *Desulfomonile tiedjei*) was observed [75]. When fed methanol, acetate, methylamine, or trimethylamine, *Methanosarcina* sp. strain DCM dechlorinated PCE to TCE. The reaction was linked directly to CH₄ formation—when there was no CH₄ production, there was no dechlorination. The authors proposed that electrons transferred during methanogenesis were diverted to PCE via an electron-transfer agent

involved in CH_4 formation, such as methyl-coenzyme-M reductase—of which factor F_{430} (a nickel-containing porphyrin unique to methanogens), is the prosthetic group [73]. In support of this idea, when 2-bromoethanesulfonate (BES), an inhibitor of methanogenesis, was added to an anaerobic sludge dechlorinating PCE to TCE, dechlorination was significantly inhibited [74].

Castro *et al.* [39] reported slow dehalogenation of chloroform, 1,1,2-trichloroethane, fluorotrichloromethane, carbon tetrachloride, 1,1,1-trichloroethane and 1,2-dichloroethane by *Methanobacterium thermoautotrophicum* at 60°C; however VC was not dehalogenated.

Eight different strains of homoacetogenic bacteria from the genera *Acetobacterium*, *Clostridium*, and *Sporomusa* were tested for dechlorination [232]. While cell-free extracts of *A. woodii*, *C. formicoaceticum*, the methanogen *Methanolobus tindarius* and *S. ovata* transformed PCE to TCE in the presence of Ti(III) or CO as electron donors, only *S. ovata* reductively dechlorinated PCE to TCE during concomitant acetogenesis from methanol and CO_2 . In *S. ovata*, corrinoids were shown to be involved in dechlorination, and the authors presented data suggesting that enzymes involved in the Wood pathway were involved. Since cell extracts of all the organisms tested showed dechlorination, but only one of the actively growing cultures dechlorinated, the authors speculated that in the environment, much of the dechlorinating activity observed might be caused by reactions of lysed cell contents.

Resting cells of four different strains of methanogens dechlorinated 1,2-dichloroethane to ETH or to chloroethane and ethane. Activity increased when methanogenesis was stimulated [105].

Rates of dehalogenation during co-metabolic dechlorination are slow and the reactions in many cases are incomplete. That these reactions may occur in the environment, however, should not be dismissed. While factor F_{430} is unique to methanogens, other enzymes with active metal centers are widely distributed in the microbial world. The extent to which true dehalorespiring organisms are distributed in the environment is not currently known. In some cases co-metabolic reactions may be important, especially if they are the only activity at a site.

2.A.2. Studies with Enzymes and Cofactors

Reports of microbial co-metabolic dehalogenation and the implication of the involvement of specific enzymes and cofactors prompted more detailed studies of the dehalogenating activity of specific enzymes.

The corrinoids aquocobalamin or methylcobalamin catalyzed the reductive dechlorination of tetrachloromethane with titanium (III) citrate or dithiothreitol as electron donors [121]. Factor F_{430} was also identified as a mediator of reductive dechlorination of tetrachloromethane [120].

Gantzer and Wackett [84] tested several bacterial transition-metal co-enzymes for reductive dechlorination of PCE. Vitamin B_{12} (containing Co), and coenzyme F_{430} (containing Ni) catalyzed the dechlorination of PCE to ETH while hematin (containing Fe) dechlorinated PCE to VC, albeit at much slower rates.

Purified CO dehydrogenase enzyme complex from *M. thermophila*, an acetotrophic methanogen, dechlorinated TCE to *cis*-1,2-DCE, *trans*-1,2-DCE, VC, and ETH when reduced with CO or Ti(III) citrate. A cobalt-containing iron-sulfur corrinoid was involved [112].

Crude and boiled extracts of *Methanosarcina barkeri* also possessed dechlorinating activity with Ti(III) acting as the electron donor. The authors concluded that both corrinoids and factor F₄₃₀ were involved in the dechlorination by the cell extracts [107]. Furthermore, it was shown that methyl-coenzyme-M reductase was responsible for dechlorination in cell-free extracts of *Methanobacterium thermoautotrophicum* [103].

Fortuitous reductive dehalogenation by the monooxygenase heme protein, cytochrome P450_{CAM}, was reported [134].

Burris *et al.* [36] examined the use of vitamin B₁₂ in aqueous and immobilized forms and reported dechlorination of PCE and TCE to *cis*-1,2-DCE, ETH, acetylene; smaller amounts of 1,1-DCE, *trans*-1,2-DCE, and VC; and trace amounts of chloroacetylene with Ti(III) citrate as a reductant. In addition to hydrogenolysis (reductive dechlorination), a reductive β -elimination reaction pathway was also proposed since chloroacetylene and acetylene were detected. Vitamin B₁₂ bound to agarose—which would allow the re-use of the catalyst in engineered bioremediation or waste treatment systems—showed activity similar to that of the aqueous vitamin B₁₂.

Glod *et al.* [91] also examined aqueous corrinoid-mediated reduction of PCE and TCE and observed the formation of only *cis*-1,2-DCE, *trans*-1,2-DCE, ETH, and acetylene. A different pathway—formation of radicals, including a highly unstable 1,1-dichlorovinyl radical, which through subsequent elimination of a chloride radical yields chloroacetylene and then acetylene—was proposed. The authors speculated that the unstable nature of the 1,1-dichlorovinyl radical as compared to the 1,2-dichlorovinyl radicals may be one reason why 1,1-DCE formation is rarely observed in microbially-mediated reductive dechlorination.

2.B. Dehalorespiring Organisms

The focus on microbially-mediated dechlorination is now directed toward isolation of organisms that use dehalogenation as a respiratory process—dehalorespiring organisms. Dehalorespiring organisms using PCE or TCE as an electron acceptor along with an electron donor for energy generation exhibit much higher rates of dechlorination than do microorganisms or cell extracts carrying out fortuitous reactions [104, 108, 229]. The dehalorespiring organisms thus-far isolated exhibit much biodiversity, which may be indicative that many more exist. In this section, the currently known dehalorespiring organisms (both PCE dechlorinators and aryl-dechlorinating organisms) are described. With the exception of "*Dehalococcoides ethenogenes*" strain 195, which dechlorinates PCE completely to ETH, none of the other PCE-dechlorinating organisms described to date can carry out the dechlorination of PCE past DCE. Mixed cultures containing organisms that dechlorinate PCE to *cis*-1,2-DCE, however, have also exhibited dechlorination to ETH or ethane [33, 85]. This suggests that other organisms exist that can carry the dechlorination to ETH; however, to date, no such organisms (other than *D. ethenogenes*) have been identified.

2.B.1. *Desulfomonile tiedjei*

Studies arising from the observation of the degradation of halogenated aromatic compounds in anaerobic environments [226] led to the isolation of *Desulfomonile tiedjei* (previously known as strain DCB-1) which reductively dechlorinates 3-chlorobenzoate using H₂, pyruvate, or formate (probably first converted to H₂) as electron donors [56] and couples this dechlorination to growth [56, 62, 159, 205]. In the mixed culture from

which the organism was isolated, 3-chlorobenzoate was dechlorinated by *D. tiedjei* to benzoate which was then degraded to acetate, H_2 , and CO_2 and eventually to CH_4 by other members of the consortium [64, 205]. Initially the organism was cultivated successfully only with relatively large volumetric additions of ruminal fluid, and its metabolic capabilities were difficult to ascertain [220, 221]. After specific nutritional deficiencies were found and alleviated, the substrate range of the organism was found to be much broader than at first thought, and the organism was classified as a new genus of sulfate-reducing bacteria. *D. tiedjei* reduces thiosulfate and sulfate coupled with the oxidation of H_2/CO_2 , formate, pyruvate, benzoate, and several other more complex compounds; and, it can grow fermentatively on pyruvate [57]. It can additionally grow supported only on pyruvate plus CO_2 , acetate plus CO_2 , or acetate plus butyrate or lactate, and it fixes CO_2 [221].

While thiosulfate and sulfite directly inhibited dechlorination, sulfate did not. However, in the presence of sulfate, H_2 was rapidly consumed by the organism to a level that made dechlorination unfavorable. It has been speculated that this may be why little reductive dehalogenation of aryl compounds has been observed in sulfate-reducing environments— H_2 is pulled to levels that are too low to support energetically favorable dechlorination [56]. Townsend and Suflita [245] reported that under growth conditions, sulfur oxyanions serve as preferred electron acceptors (over dehalogenation), that these reactive sulfur species may interact with the enzymes involved in dehalogenation, and that the inhibition is not necessarily caused by competition for reducing equivalents.

D. tiedjei dechlorinated PCE to TCE, and *cis*- and *trans*-1,2-DCE at a rate of 528 nmol PCE to TCE/mg protein-day [47]; however, PCE dechlorination by *D. tiedjei* is probably co-metabolic since it is dependent upon induction of the same enzyme system that dechlorinates 3-chlorobenzoate, and both dechlorination activities share many common characteristics [168, 244, 245].

2.B.2. *Dehalospirillum multivorans*

Dehalospirillum multivorans, was isolated from activated sludge that had not previously been exposed to chlorinated ethenes [195]. *D. multivorans* dechlorinates PCE to *cis*-1,2-DCE using H₂ as an electron donor and acetate as a carbon source, but has a much more widely varied biochemical repertoire; it additionally uses organic substrates such as pyruvate, lactate, ethanol, formate, and glycerol as electron donors and also uses nitrate and fumarate as electron acceptors [163, 195, 196]. The dehalogenase of *D. multivorans* mediates both PCE and TCE dechlorination [164] and contains vitamin B₁₂ and an Fe-S cluster [165] as prosthetic groups.

2.B.3. *Dehalobacter restrictus*

A mixed-culture, packed-bed bioreactor that dechlorinated PCE to ETH and then to ethane was reported [33]. The reactor was packed with a mixture of sediment from the Rhine river and ground granular sludge from an upflow anaerobic sludge blanket (UASB) reactor treating waste from a sugar beet processing plant. Complete dechlorination to ETH and ethane occurred only when a mixture of the two sources was used. A microscopically pure dechlorinating culture was isolated that reductively

dechlorinates PCE via TCE to *cis*-1,2-DCE and couples the dechlorination to growth [106]. The organism, *Dehalobacter restrictus*, only grows using H₂ as an electron donor and PCE or TCE as electron acceptor [108]. Preliminary studies were performed with a second enrichment culture that dechlorinated *cis*-1,2-DCE and VC to ETH, but a pure culture was not isolated [33].

2.B.4. Strain MS-1 and *Enterobacter agglomerans*

Strain MS-1, and a closely related organism, *Enterobacter agglomerans*, are facultative aerobic bacteria that dechlorinate PCE to TCE then to *cis*-1,2-DCE [204]. Strain MS-1 was isolated from a PCE-contaminated site in Victoria, Texas. It grows on yeast extract and many different carbohydrates, fatty acids, amino acids, alcohols, purines, and pyrimidines; either fermentatively, or with O₂ or nitrate as an electron acceptor. PCE was only used as an electron acceptor after nitrate and O₂ were depleted, and dechlorination did not occur when high concentrations (e.g. glucose at 1 g/L) of fermentable substrates were available. After fermentation, nonfermentable fermentation products such as acetate were then used as donors for dechlorination. Strain MS-1 is closely related to *E. agglomerans* which was also found to dechlorinate PCE to *cis*-1,2-DCE.

2.B.5. *Desulfitobacterium* sp. strain PCE1

Desulfitobacterium sp. strain PCE1 is a strictly anaerobic bacterium that grows with either lactate, pyruvate, butyrate, formate, succinate, or ethanol as electron donors and PCE, 2-chlorophenol, 2,4,6-trichlorophenol, 3-chloro-4-hydroxy-phenylacetate, sulfite, thiosulfite, or fumarate as electron acceptors [85]. It also grows fermentatively with pyruvate as the

sole substrate. It dechlorinates PCE to TCE and only small amounts of *cis*- and *trans*-1,2-DCE. The culture was isolated from an enrichment that dechlorinated PCE completely to ETH under some circumstances, depending upon the electron donor added.

2.B.6. Strain TT4B

A Gram negative rod, strain TT4B, grows with acetate or pyruvate as electron donors and PCE, TCE, fumarate, or ferric nitrilotriacetate as electron acceptors. PCE and TCE are dechlorinated to *cis*-1,2-DCE [122, 123].

2.B.7. Other Aryl Compound Dechlorinators

Strain 2CP-1 grows with acetate as an electron donor and 2-chlorophenol or 2,6-dichlorophenol as an electron acceptor, and produces phenol as an end product of the dechlorination. Dechlorination is inducible and only the *ortho* position is dechlorinated. Fumarate also serves as an electron donor and yeast extract also supports growth. This organism also grows under aerobic conditions with acetate or yeast extract and it groups with the myxobacteria [46].

A chlorophenolic compound dechlorinator, Strain DCB-2, thought to be related to the *Clostridium* was isolated [145]. The organism grows fermentatively on pyruvate and uses H₂ produced from the fermentation of pyruvate for dechlorination.

Desulfitobacterium dehalogens dechlorinates pentachlorophenol and other chlorophenolic compounds at the *ortho* positions using pyruvate or H₂ as the electron donor [248, 249].

2.C. “*Dehalococcoides ethenogenes*” strain 195

The dechlorinator present in the cultures used in this study was recently isolated by Maymó-Gatell *et al.* and tentatively named “*Dehalococcoides ethenogenes*” strain 195 [151]. The organism was originally obtained from cultures that were developed from anaerobic digester sludge. The following section describes research performed with these cultures and lays some background for the current work.

2.C.1. Culture Development

Dechlorinating cultures were initially developed by Freedman and Gossett using dilutions from an anaerobic laboratory reactor that was started using digester sludge from the old Ithaca Wastewater Treatment Plant [80]. The cultures were methanogenic, and relatively low doses of PCE and TCE were added—3 to 4.5 μmol PCE added/L and 5.5 to 7 μmol TCE added/L. Electron donor—methanol, glucose, formate, or acetate—was added at a concentration of 50 mg/L, which was high compared to the amount of chloroethenes added. For example, for methanol, about 390 times the amount required for dechlorination on an equivalent basis was added.

Freedman and Gossett showed conclusively that $[^{14}\text{C}]\text{PCE}$ was dechlorinated primarily to $[^{14}\text{C}]\text{VC}$ and $[^{14}\text{C}]\text{ETH}$. $[^{14}\text{C}]\text{CO}_2$ and $[^{14}\text{C}]\text{CH}_4$ were not significant end products. The dechlorination of PCE was by the reductive pathway shown in Figure 2.1. This was the first study that showed complete dechlorination of PCE to ETH. The culture was found to dechlorinate most favorably and completely with methanol as an electron donor; however, glucose, acetate, formate and H_2 also supported dechlorination [80]. Only a tiny fraction of the reducing equivalents were

channeled to dechlorination in these cultures, with the remainder going to methanogenesis, but this was enough to carry out complete dechlorination of the relatively low concentrations of TCE and PCE added.

The addition of 2-bromoethanesulfonate (BES), thought at that time to be a selective inhibitor of methyl-coenzyme M reductase, the enzyme which catalyzes the final step in methanogenesis, completely stopped the dechlorination of TCE and the formation of CH_4 . However, repeated dosages of BES were required to completely shut-down the dechlorination of PCE, possibly because the culture could degrade BES [80]. The ability of BES to inhibit dechlorination led to the tentative conclusion that a methanogen was involved in the dechlorination.

DiStefano *et al.* [59, 60] converted the mixed anaerobic cultures of Freedman and Gossett to a non-methanogenic, primarily dechlorinating culture by incrementally increasing the PCE concentration from $3.5 \mu\text{M}$ to $550 \mu\text{M}$. By increasing the PCE loading and adding methanol at a 2:1 ratio to PCE added on an equivalent basis—an electron donor amount much closer to the amount of acceptor added—a higher percentage of electron equivalents was channeled to dechlorination. A higher concentration and proportion of dechlorinators was thus cultivated, and isolation of the dechlorinator was possible. This culture is referred to as the high-PCE/methanol culture. In the high-PCE/methanol culture, CH_4 production by acetotrophic and hydrogenotrophic methanogens declined when the PCE dosage reached $250 \mu\text{M}$, then ceased when the dosage was increased to $550 \mu\text{M}$. PCE continued to be dechlorinated after methanogenesis ceased. Routinely, after four days of incubation, less than 1 percent of the added PCE remained as VC, the balance was ETH. An electron balance showed that 31 percent of the added methanol equivalents

was channeled to dechlorination and 69 percent was channeled to acetate production.

Further studies with the high-PCE/methanol culture confirmed that H_2 was the electron donor used directly by the dechlorinators; however, to sustain or transfer the culture with H_2 as the sole donor, filtered culture supernatant from the methanol-fed culture was required, presumably to supply nutritional factors [61]. Evidence that supported the hypothesis that the dechlorinating organisms are H_2 utilizers was provided from studies with microbial inhibitors. Methanol-fed dechlorinating cultures and H_2 -fed dechlorinating cultures were amended with vancomycin—a eubacterial peptidoglycan synthesis inhibitor. In the methanol-fed cultures, acetogenesis and dechlorination were inhibited. When PCE was withheld from the bottles, methanogenesis was observed even in the presence of vancomycin. In the H_2 -fed cultures with vancomycin added, PCE dechlorination continued; however, acetogenesis was inhibited. Methanogenesis was more significant in the vancomycin-amended bottles than in bottles which received no vancomycin. BES inhibited dechlorination in both methanol- and H_2 -fed cultures while acetogenesis was not affected. DiStefano *et al.* proposed the roles shown in Figure 2.2. for the organisms in the mixed dechlorinating culture.

A key to the success of the culture was the apparent inhibition of methanogens—competitors for reducing equivalents—by the high concentration of PCE. Methanol was primarily converted to acetate and the acetogenic reaction provided a pool of H_2 [49, 98]. The dechlorinators apparently scavenged this H_2 pool as a direct source of electrons to reductively dechlorinate PCE and its daughter compounds. Additionally, it

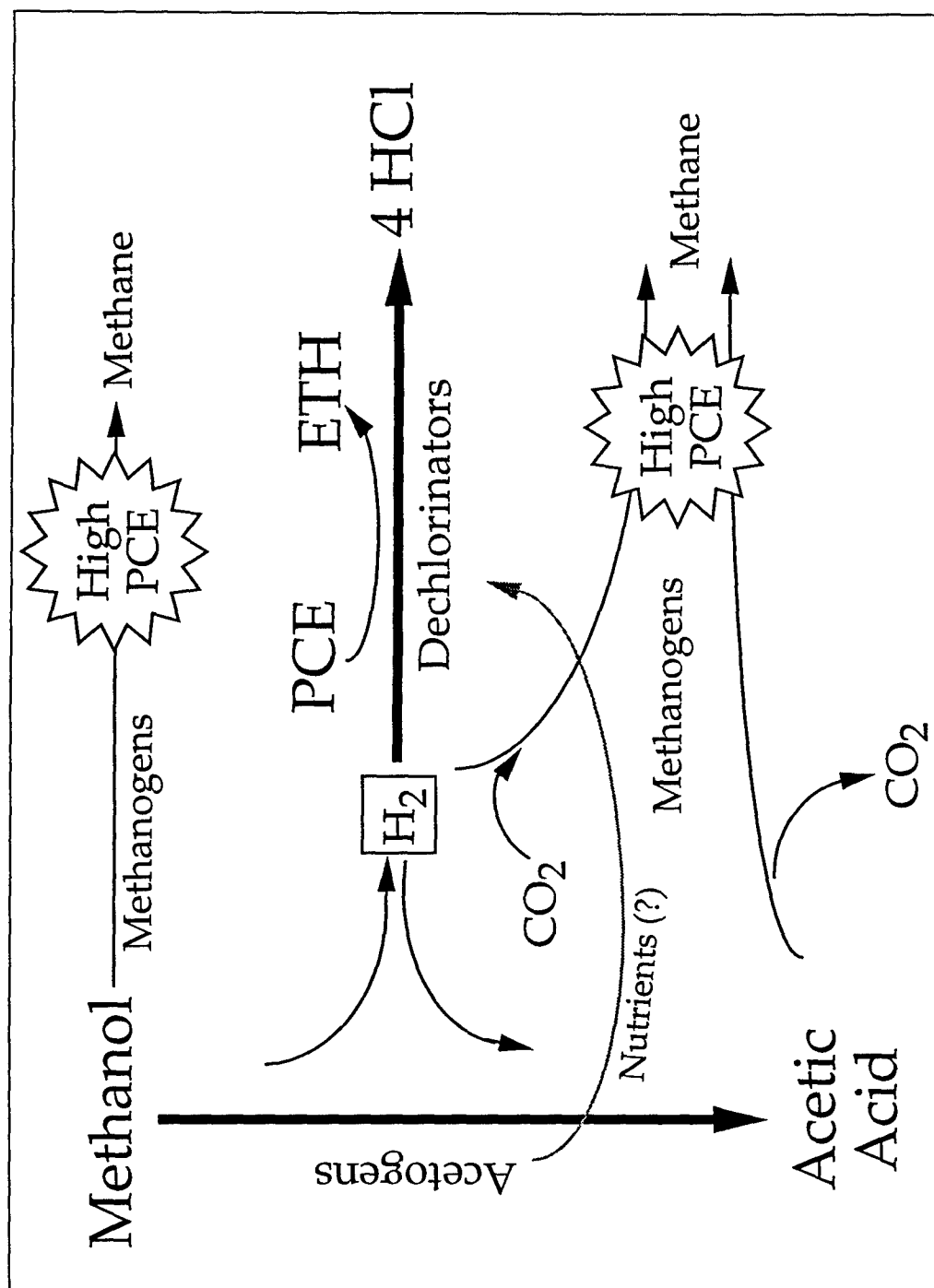


Figure 2.2. Hypothetical roles of acetogens, dechlorinators, and methanogens in the high-PCE/methanol-enriched culture.

now appears that essential nutrients were supplied to the dechlorinators by the methanol-using acetogens [152].

The rate of PCE dechlorination to VC by the high-PCE culture operated with H_2 as an electron donor was 2.7 to 4.6 μmol per mg volatile suspended solids per day [229]—much higher than those reported for organisms carrying out fortuitous dechlorination [75] or for co-enzymes [84].

2.C.2. Isolation of "*Dehalococcoides ethenogenes*" strain 195

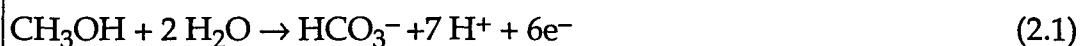
Maymó-Gatell *et al.* performed MPN analysis on the high-PCE/methanol culture and highly dilute cultures grown with PCE and H_2 were used as starting material to isolate the dechlorinator [152]. The dechlorinator uses acetate as a carbon source and it requires vitamin B_{12} at a rather high concentration—0.05 mg/L. The exact identity of other required nutrients was not determined and culture transfer continued to depend upon the addition of the complex nutrient sources yeast extract, anaerobic digester sludge supernatant, or extracts of cells from cultures grown with more complex electron donors [152]. The dechlorinating organism was eventually isolated by Maymó-Gatell *et al.* in a microscopically pure form and was given the tentative name "*Dehalococcoides ethenogenes*" strain 195 [151]. The organism gains energy for growth from H_2 use coupled with the reduction of PCE, TCE, *cis*-1,2-DCE, 1,1 DCE, and dichloroethane—but not from *trans*-1,2-DCE or VC (though it dechlorinates them) [150].

From an engineering standpoint, how to supply H_2 to hydrogenotrophic dechlorinators such as *D. ethenogenes* is a primary concern. H_2 is sparingly soluble in water and its rapid use by organisms

would ensure its depletion at aquifer injection wells. It would be very difficult to supply uniform H_2 concentrations throughout a contaminated aquifer by injecting water saturated with H_2 . It is more likely that highly active biological zones would develop at the injection points which would rapidly remove the H_2 . Its gaseous and explosive nature also make it difficult to handle on site. There are other concerns as well. The ability of the high-PCE/methanol culture to support dechlorination without the addition of vitamin B_{12} has led to the conclusion that the acetogens (known to contain corrinoids) supplied B_{12} for *D. ethenogenes*. Other co-contaminants in the culture apparently supplied yet other unidentified nutritional factors. Since the direct addition of H_2 eventually resulted in failure in the absence of the addition of complex nutrient sources, and since the use of gaseous H_2 would be difficult from an engineering standpoint, addition of other electron donors that are converted to H_2 and/or support a more complex population to provide growth factors—may be a more realistic solution.

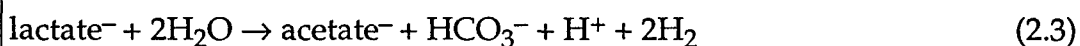
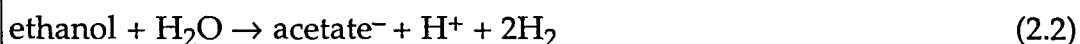
2.C.3. Competition Between Dechlorinators and Methanogens

Complete dechlorination in Freedman and Gossett's cultures relied on the addition of large ratios of electron donor to PCE or TCE on an equivalent basis. Only a tiny fraction of the donor was channeled to dechlorination, but since the amount of chloroethene added was small, dechlorination was complete. In the high-PCE/methanol cultures of DiStefano *et al.*, large amounts of PCE could be completely dechlorinated with only a 2:1 excess of methanol on an equivalent basis (6 eq/mol based on its oxidation to CO_2 , Equation 2.1) because the primary competitors for the reducing equivalents—the methanogens—were inhibited by high PCE.



Development of cultures that could sustain dechlorination at PCE concentrations that are non-inhibitory, but substantial in relation to the amount of donor added was more problematic.

Stover compared the use of methanol to the use of non-methanogenic electron donors that are fermented to H_2 [224]. Using the high-PCE/methanol culture as inoculum, enrichment cultures were developed using methanol, ethanol, or lactate as electron donors at a 2:1 ratio to PCE on an equivalent basis. The equivalents available from ethanol and lactate were defined based on the amount of H_2 (which supplies 2 equivalents per mol) formed during their fermentations as shown in Equations 2.2 and 2.3. Ethanol and lactate both provide 4 eq/mol.



The PCE concentration administered was 110 μM (18 mg PCE/L, nominal concentration), a level somewhat more representative of those encountered at contaminated sites and within the problematic “intermediate”, noninhibitory range.

As expected, methanol was a poor donor at these PCE concentrations since it was rapidly converted to CH_4 by methanogens (see Figure 2.3) and the pool of H_2 available from methanogenic methanol use may be smaller than that from acetogenic use [124, 141]. Furthermore, any H_2 that was evolved was also subject to use by hydrogenotrophic methanogens. Thus, at such intermediate, noninhibitory PCE concentrations, when methanol

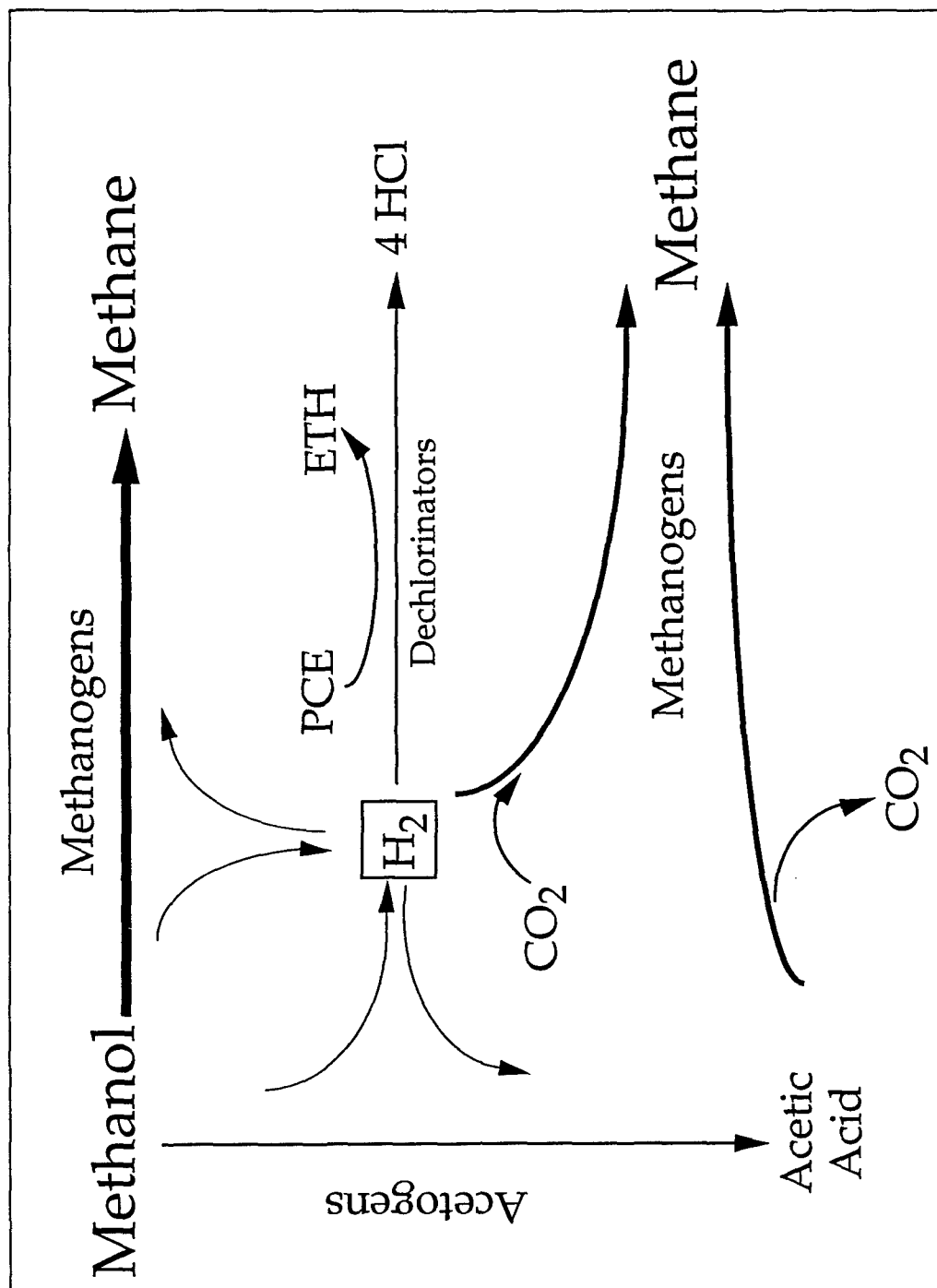


Figure 2.3. Hypothetical roles of acetogens, dechlorinators, and methanogens in the low-PCE/methanol-enriched culture.

was fed, a “spiral to failure” was observed where methanol simply supported the formation of more and more methanol- and H₂-using methanogens, which eventually took over the culture and left few reducing equivalents for dechlorinators to scavenge. Dechlorination eventually failed in these systems.

The non-methanogenic donors, ethanol and lactate supported dechlorination of PCE to VC and ETH for the 50-day study. Lactate and ethanol were degraded fairly rapidly and formed a significant pool of H₂ of 1×10^{-3} to 2×10^{-3} atm within 2 to 3 hours after addition. Stover observed that when H₂ was high, both dechlorination and methanogenesis occurred rapidly; but when H₂ levels were below about 4×10^{-4} atm, no methanogenesis occurred, whereas dechlorination continued. This suggested that dechlorinators could use H₂ at lower levels than could the methanogens, and thus, had a higher affinity and/or lower threshold for H₂ use. The H₂ evolved from non-methanogenic substrates such as ethanol and lactate (used by Stover and this study) or by butyrate or propionate (this study) could be used both for methanogenesis and dechlorination (Figure 2.4).

The apparently different affinities for H₂ for methanogens and the dechlorinator were quantified by Smatlak *et al.* through measurement of H₂ half-velocity coefficients, $K_{S(H_2)dechlor}$ for the dechlorinator, and $K_{S(H_2)meth}$ for the methanogens, in a mixed culture [208, 210]. $K_{S(H_2)dechlor}$ for H₂ use by dechlorinators averaged 100 nM while, the $K_{S(H_2)meth}$ for H₂-using methanogens averaged 960 nM. Ballapragada *et al.* reported $K_{S(H_2)dechlor}$ values for H₂ use by dechlorinators of 9 to 21 nM in a mixed culture containing methanogens and dechlorinator(s) [13].

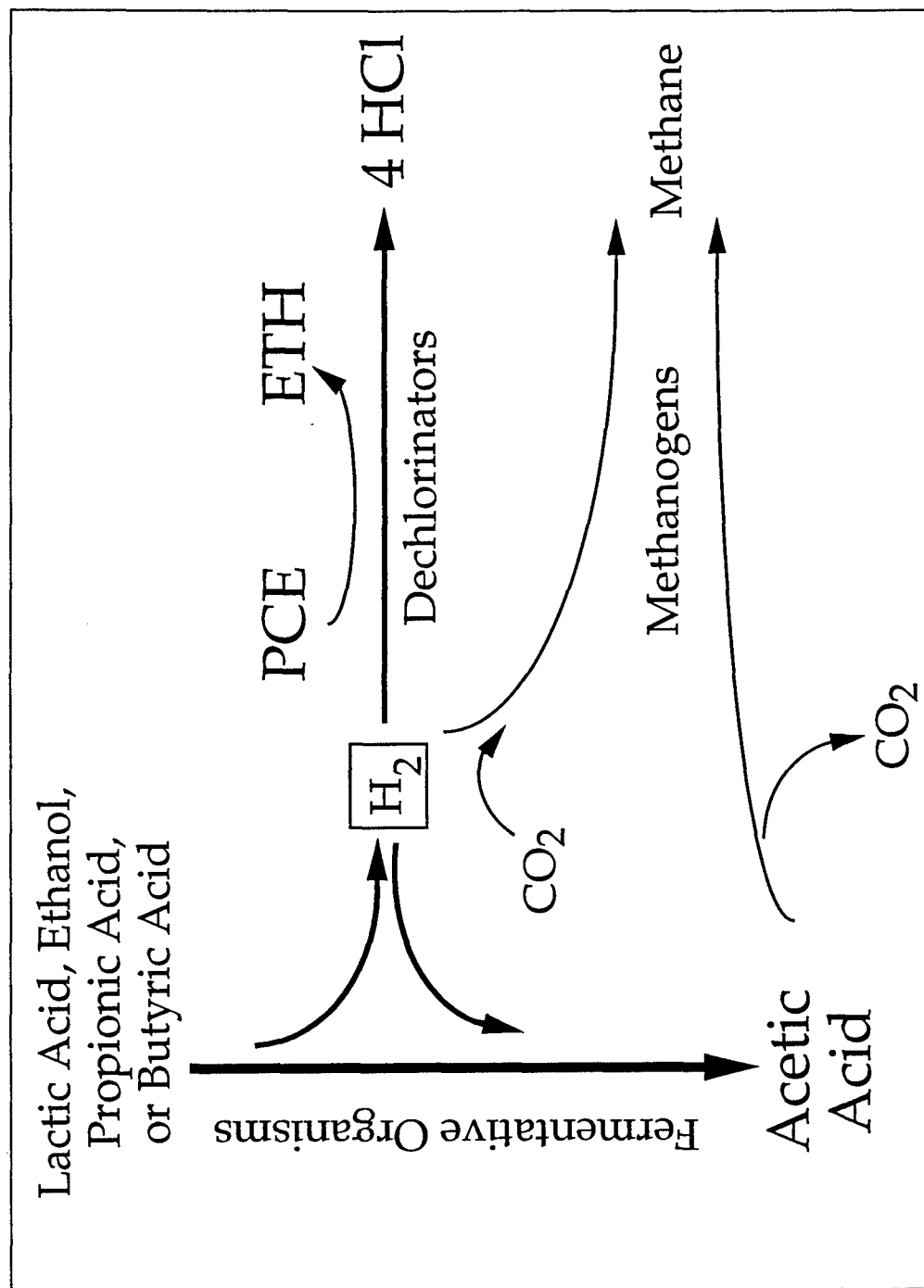


Figure 2.4. Hypothetical roles of fermentative organisms, dechlorinators, and methanogens in low-PCE enrichments with lactic acid, ethanol, propionic acid, or butyric acid as the hydrogen donor

Thermodynamic considerations suggest that a low $K_{S(H_2)dechlor}$ could be universal for hydrogenotrophic dehalogenators. So much energy is available from the H_2 -PCE couple (-168 kJ/mol H_2), that organisms can gain energy for growth even when the H_2 concentration is very low—meaning that they will have a low threshold for H_2 . It seems thus likely that such organisms would evolve kinetics of H_2 use that are favorable at these low concentrations—i.e., that they would also have a relatively low $K_{S(H_2)dechlor}$ value for H_2 use.

The order-of-magnitude difference in K_S values between dechlorinators and methanogens suggests that a competitive advantage could be had by dechlorinators if the H_2 could be supplied in such a way as to be energetically and kinetically unfavorable for methanogenic use while being accessible to dechlorinators.

2.D. Application of Reductive Dechlorination in Bioreactors and Bioremediation

Application of reductive dechlorination of PCE and TCE has been studied in laboratory reactors and aquifer microcosms and is now being seriously considered as an engineered full-scale bioremediation scheme. Furthermore, natural attenuation—the degradation of PCE and its daughter products through the activities of organisms on site, without engineered intervention—has also been recognized as a promising option for some sites where the plume remains on site and does not immediately threaten sensitive receptors such as municipal or private water supplies. A review of some of the applications is given here.

2.D.1. Bioreactor Application of Reductive Dechlorination

Bioreactor studies identify the feasibility of reductive dechlorination as an above-ground or end-of-pipe treatment option, but also help identify what problems might be encountered in *in situ* applications. Many of the bioreactor studies feature two-stage anaerobic reductive dechlorination of highly chlorinated compounds coupled with aerobic (sometimes co-metabolic) treatment of the lesser chlorinated reduction products. This combination of anaerobic-aerobic activity is precisely what is now thought to be the primary remedial activity at some naturally attenuated sites.

Fathepure and Vogel [76] used a two-stage anaerobic-aerobic reactor system to treat hexachlorobenzene, PCE, and chloroform. Acetate was reported to be the best electron donor for the anaerobic stage while the aerobic stage was operated with glucose. Of the radiolabeled TCE in the influent, 96 percent was converted to CO₂ and non-volatile intermediates.

Kästner [116] reported dechlorination of PCE to TCE and then to *cis*-1,2-DCE in a mixed aerobic culture upon transition from aerobic to anaerobic conditions and a concomitant release of sulfide which caused the redox potential to decrease from 0 to -150 mV. After the culture made the transition to anaerobic conditions, no further dechlorination occurred, thus, the author suggested that an aerobic or facultative anaerobic organism was involved in the dechlorination.

Sucrose-fed anaerobic, methanogenic attached-film expanded-bed reactors containing media that was initially developed for wastewater treatment were used for reductive dechlorination of PCE at 20 and 15°C [38]. PCE and TCE were dechlorinated primarily to VC and *cis* 1,2-DCE with trace amounts of ETH. A related study at 35°C [44] also reported that *cis*-1,2-DCE

was the major intermediate while VC and ETH were detected in trace amounts. The authors proposed that a follow-up aerobic treatment step would be needed to remove DCE and VC.

Jewell *et al.* reported that an anaerobic, methanogenic expanded-bed reactor fed sucrose as the primary electron donor, operated in sequence with a methanotrophic expanded-bed reactor did completely remove PCE and its reduction products from an influent stream. Effluent from the anaerobic stage contained primarily VC with lower concentrations of *cis*-1,2-DCE. While all evolved VC was readily removed in the second stage, shorter HRTs or non-optimal electron donor (anaerobic stage) or CH₄ (methanotrophic stage) concentrations sometimes resulted in residual *cis*-1,2-DCE in the effluent from the system. The authors proposed that *cis*-1,2-DCE would be the “weak link” in such anaerobic-aerobic systems [113]. This has been a commonly reported problem in other such reactor studies and at some naturally attenuated sites

Gerritse *et al.* [86] coupled an upflow anoxic reactor inoculated with a dechlorinating culture, with a downflow, oxic, methanotrophic reactor for chloroethene removal. Complete removal of the chloroethenes by the two-stage system was observed using pyruvate, formate, or lactose as electron donor for the dechlorinating stage. The methanotrophic stage was the “bottleneck” for the system. It was sensitive to exposure to PCE and did not operate well at reduced HRTs.

Enzien *et al.* [72] reported reductive dechlorination of TCE and PCE to primarily *cis*-1,2-DCE in a sediment column operated under aerobic conditions. CH₄ and methanol were added to the column. The authors believe that anaerobic microsites developed and facilitated the dechlorination activities despite the bulk aerobic conditions.

Wild *et al.* [265] reported dechlorination of TCE to ETH in a glucose-fed packed-bed reactor containing an anaerobic, primarily acetogenic dechlorinating culture. Although developed on TCE, the reactor also readily transformed PCE to ETH.

PCE transformation in a laboratory-developed UASB with ethanol as the primary electron donor was reported. The primary end product of the dechlorination was *trans*-1,2 DCE [43].

Bioaugmentation of a UASB with *D. tiedjei* was successful and 3-chlorobenzoate degrading capacity was imparted to a UASB that previously had no 3-chlorobenzoate degrading capacity [3]. Antibody probing revealed that *D. tiedjei* was able to incorporate itself into a pre-established complex granular microbial environment. Bioaugmentation was also successful with DCB-2, a pentachlorophenol degrader [42]. These studies show that successful bioaugmentation is possible even into complex, robust communities like granular activated sludge.

A benzoate-degrading batch culture using biomass from an anoxic fixed-bed reactor was amended with bromoethane-sulfonic acid, BES, an inhibitor of methanogenesis [196]. Since benzoate was degraded by the consortium to acetate, H_2 , and CO_2 and then to CH_4 , inhibiting CH_4 production caused inhibitory amounts of H_2 to accumulate, thus inhibiting benzoate degradation. When PCE was added along with BES, benzoate was degraded and PCE was dechlorinated to DCE even in the absence of methanogenesis. In this case, PCE served as an alternative electron acceptor and was reductively dechlorinated to DCE by an unidentified member of the consortium. When PCE only was added, it was dechlorinated to the same degree in the presence of methanogenesis.

Different electron donors—lactate, formate, methanol, ethanol, H_2/CO_2 , and acetate—were examined to determine differences in outcome of TCE dechlorination in fixed-bed reactors operated at 14°C [194]. TCE was dechlorinated only to *cis*-1,2-DCE. No VC, ETH, or ethane was detected. A major objective was to determine which substrate allowed for the fastest start-up time and most complete TCE dechlorination. This study was a prelude to a not-yet-reported-study that was to look at results of coupling these first-stage anaerobic dechlorinating reactors to methanotrophic reactors for continued removal of the dechlorination products. Thus, significant CH_4 production from the donors—to fuel the second stage—was also desirous. All substrates tested supported dechlorination, but lactate and formate yielded the quickest start-up times. Electron donor was added at roughly 45 to 210 times the amount that would be needed for complete dechlorination of the TCE to DCE. Lactate plus methanol was proposed as perhaps the best combination of substrates, although all the substrates apparently supported this dechlorination step and the addition of excess donor should have resulted in excess CH_4 formation. Addition of methanol—resulting in extra production of CH_4 —was proposed to be needed to successfully run the methanotrophic second-stage.

Complete dechlorination of PCE to ETH was reported using acetate as the primary electron donor in both laboratory- and field-scale packed-bed reactors seeded with sludge from industrial waste treatment [99].

Use of anaerobic bioventing has been proposed and studied by Sayles *et al.* [184]. Bioventing is typically an aerobic application during which air is blown in to the unsaturated zone to stimulate hydrocarbon degradation. Anaerobic bioventing was demonstrated at laboratory scale by blowing anoxic gas (1% H_2 , 1% CO_2 , 5% He, balance N_2) through an unsaturated soil

column. PCE, delivered at 10 ppmv in the feed gas, was dechlorinated to *trans*-1,2-DCE and VC. CH₄ was also formed. The authors proposed a two-stage bioventing process that could be implemented by introducing air—after the PCE is dechlorinated to lesser-chlorinated compounds and CH₄ is formed—to promote methanotrophic, co-metabolic degradation of the remaining DCE and VC.

2.D.2. Aquifer Sediment Microcosm Studies

Microcosm studies are performed in conjunction with field-site activities to elucidate what microbial activities are occurring at the site, and to gauge the likely success or failure of proposed remediation schemes.

PCE dechlorination was examined in field-contaminated oligotrophic soil from a chloroethene- and aviation-gasoline-contaminated site at a Coast Guard Air Station at Traverse City, MI. Microcosms were amended with various electron donors in an attempt to stimulate dechlorination. Different electron donors—lactate, propionate, crotonate, butyrate, and ethanol—did stimulate native organisms to dechlorinate PCE to TCE and then to *cis*-1,2- and *trans*-1,2-DCE. Methanol and acetate did not stimulate dechlorination in that same study [88]. Mixtures of fatty acids at different concentrations also stimulated dechlorination. Lactate did not persist—it was degraded very rapidly in the sediments, propionate degradation was not observed, and butyric acid was thought to be the most effective donor [87]. H₂ was unfortunately not measured in these microcosms. Toluene, a component of BTEX and a substance that is present in jet fuel—a common co-spilled contaminant at Air Force sites—was also found to stimulate PCE dechlorination in aquifer sediments from Traverse City [203].

Liang and Grbic-Galic [135] also investigated PCE dechlorination associated with toluene degradation. Toluene degradation was coupled to dechlorination in only one (Tyndall Air Force Base) of their three sets of aquifer sediments. Interestingly, the samples from Traverse City collected from a different site than those of Gibson and Sewell and a year later, showed no dechlorination and no toluene degradation. A comparison of the two studies emphasizes the uncertainty of assessing the applicability of microcosm results to the field site, especially in extrapolating results over time and space.

Field-contaminated soils amended with various electron donors (acetate, lactate, and ethanol) and nutrient amendments were examined for PCE dechlorination [173]. PCE and TCE were dechlorinated to *cis*-1,2-DCE and small amounts of VC when donor was present, both under methanogenic and sulfate-reducing conditions. In microcosms to which nitrate was added, dechlorination was not complete due to suspected depletion of electron donor. Fate and degradation of electron donors was not followed.

Wilson *et al.* [267] state that the two primary uses for microcosm studies are to 1) qualitatively illustrate the important processes that control the fate of organic contaminants, and 2) to estimate rate constants for biotransformation of contaminants that can be used in site-specific fate-and-transport models. A case study of the Tibbetts Road Superfund site in Barrington, NH was presented. First-order rate constants for TCE, benzene, and toluene were computed from the microcosms. Rate constants from microcosms were several-fold higher than field-computed rates. For example, the rate constant from microcosm studies for TCE was 3.69/yr versus *circa* 0.5/yr for the field.

An extensive set of laboratory microcosm studies coupled with data from the associated field sites was reported by Edwards and Cox [68]. Three different field sites—a former waste lagoon in California, a private landfill in New Hampshire, and a former industrial facility in Ontario—showed evidence of extensive natural attenuation. Microcosm studies using aquifer sediments from critical locations were used to confirm field findings and to help delineate exactly which processes were responsible for the degradation processes. A combination of anaerobic reductive dechlorination with either aerobic mineralization or co-metabolism of VC and DCE was responsible for the removal of chlorinated. This study is an excellent example of how monitoring of natural attenuation processes coupled with laboratory studies to help understand the sites and processes occurring, will help further the use and application of biological processes on a field scale.

2.D.3. Field Demonstrations of Reductive Dechlorination

There are now many examples of full-scale *in situ* application of biological reductive dechlorination in both engineered systems and naturally attenuated sites. Large amounts of data from monitoring wells or through the use of punch technology must be collected to fully describe these sites. The screening protocol for natural attenuation of chlorinated solvents being developed by AFCEE lists 19 types of measurements for fully describing a site, with another 4 under consideration for recommendation [264].

2.D.3.a. Enhanced Bioremediation

Beeman *et al.* [23] presented data for an *in situ* system where PCE was dechlorinated to DCE under sulfate-reducing conditions. VC was produced

as sulfate became limiting. Since DCE is less toxic than VC, the authors proposed that stopping the dechlorination at DCE is more desirable. The DCE could then be removed via aerobic conditions.

A PCE- and TCE-contaminated aquifer at a landfill near Victoria, Texas was investigated for *in situ* enhanced bioremediation [22]. Test sites were constructed with extraction, recharge, and monitoring wells. Extracted water was amended with nutrients before being recharged to the aquifer. Aerobic treatment for 203 days did not remove PCE, TCE, or DCE. Anaerobic conditions were established by adding sodium benzoate to the recharge water. PCE and TCE concentrations decreased while DCE concentration increased, then DCE decreased as VC increased. Under sulfate-reducing conditions (imposed by adding magnesium sulfate), PCE, TCE, and DCE were converted to ETH and ethane. This was the first demonstration of dechlorination of PCE to ETH under sulfate-reducing conditions. Previous studies had shown that PCE dechlorination terminated at DCE under sulfate-reducing conditions [12].

Spuij *et al.* [214] reported an engineered anaerobic-aerobic co-metabolic remediation system for PCE-contaminated soil beneath a dry-cleaning business. A spatially separated anaerobic loop and a co-metabolic aerobic bioscreen were employed. Methanol was the donor for the anaerobic loop where PCE was dechlorinated primarily to DCE. At the aerobic co-metabolic bioscreen, phenol was infiltrated as the co-substrate for DCE co-metabolism. DCE and phenol were completely degraded in the aerobic zone.

2.D.3.b. Natural Attenuation

One of the earliest examples of field-data confirming natural attenuation via reductive dechlorination came from a chemical transfer

facility in North Toronto, Ontario, Canada [146]. At this site, PCE had been spilled on the ground along with other organic solvents such as methanol, methyl ethylketone, vinyl and ethyl acetate, and butyl acrylate. PCE, TCE, *cis*-1,2-DCE, VC, ETH, and very low concentrations of ethane were detected in the groundwater at the site. Methanol and acetate were also present. The data, along with microcosm studies with sediment samples from the site, confirmed that PCE was being dechlorinated to ETH. Subsurface sediments contained low population densities of both methanogens and sulfate-reducing bacteria. The authors believed that methanol was being converted to acetate by acetogens in the sediment and that the acetogens played an important role in the dechlorination.

Moutoux *et al.* [160] described plume characteristics from four Air Force sites contaminated with chlorinated solvents. The importance of determining whether donor sources will become depleted before complete dechlorination is accomplished and recognizing when natural attenuation must be supplemented with remedial action if sensitive receptors are at risk were stressed as goals of the extensive monitoring. At three of the four sites, BTEX was thought to be the primary electron donor and extensive reductive dechlorination activity was documented. The fourth site had no co-spilled donor—the only available donor was thought to be natural organic matter—and little dechlorination was occurring in this primarily aerobic plume.

A spill of chlorinated solvents and acetone was described. Natural attenuation of PCE and TCE was occurring and acetone degradation was thought to be the primary electron-donating mechanism [177].

Johnson *et al.* [114] reported heterogeneous reductive dechlorination of TCE that occurred only within the aquifer where TCE levels were less

than 20 mg/L. Inhibition or toxicity appeared to prevent dechlorination above that concentration.

Natural attenuation was verified through additional microcosm data for a hydraulically controlled plume at a chemical manufacturing site. The site exhibited ETH and VC plumes. Reductive dehalogenation was observed in microcosms with no added donor and in those amended with lactate [259].

2.E. Hydrogen Donor Fermentation

H₂ is an environmentally critical microbial product and substrate. Numerous organisms produce it through their fermentative activities or utilize it as an electron donor. Since four of the PCE/TCE dehalorespiring organisms thus-far isolated use H₂, H₂ production and subsequent competition for its use are important issues to consider in selecting an electron donor for dechlorination. The literature concerning organisms that produce H₂ through breakdown activities and the competition for H₂, primarily between sulfate-reducing bacteria and methanogens, is extensive. Fermentations of alcohols and short-chain volatile fatty acids (VFAs) to H₂ are carried out by syntrophic, obligate proton-reducing organisms and, under sulfate-depleted conditions, by sulfate-reducing bacteria. Substrates like butyrate and propionate are oxidized to acetate and CO₂ and the electrons liberated during the oxidation are then disposed of by the reduction of protons to H₂ or perhaps by reduction of HCO₃⁻ to formate. Some uncertainty still exists about whether formate or H₂ is the most significant interspecies electron carrier. Organisms that oxidize alcohols and VFAs with concomitant production of H₂ and acetate (see Table 1.1) exist syntrophically with other organisms that utilize H₂ and acetate. They

are dependent upon their syntrophic partners to remove these end products so that conditions are thermodynamically favorable for further metabolism. Obligate syntrophic proton reducers are fastidious anaerobes and have themselves proven difficult to culture and study. An excellent review of some of these processes has been provided by Schink [189].

A review of some of the organisms that degrade the donors studied—ethanol, butyric acid, propionic acid, and lactic acid—is given in this section. Note that Appendix I contains literature values of kinetic parameters for both fermentations of butyric acid, ethanol, lactic acid, and propionic acid; and for the bioconversion of acetate and H_2 by methanogens.

2.E.1. Ethanol

The first described syntrophic combination of an H_2 -producing acetogen and an H_2 -using methanogen was that of "*Methanobacillus omelianskii*" [35] originally thought to be a single methanogen that converted ethanol to CH_4 [14], but later shown to be a coculture of the "S" organism and an H_2 -using methanogen. The "S" organism was never successfully cultured in the absence of its partner, and the culture was eventually lost.

Several syntrophic alcohol-oxidizers, which have other biochemical abilities, have been isolated by Schink and co-workers from fresh- and salt-water sediments. *Pelobacter carbinolicus*, an organism that ferments 2,3-butanediol, acetoin, and ethylene glycol to acetate and ethanol, also grows syntrophically oxidizing ethanol, propanol, or butanol to acetate and H_2 [187]. *Pelobacter acetylenicus*, an organism that ferments acetylene to ethanol and acetate was also found to oxidize ethanol to acetate and H_2 in

the presence of an H_2 -scavenging syntrophic partner [188]. This organism was cocultured with various syntrophic partners in a study of energy partitioning in syntrophic cocultures [201, 202]. Several other types of alcohol-oxidizing syntrophic organisms were isolated from sediment and sewage [71].

Bryant *et al.* [34] reported that sulfate-reducing bacteria can grow syntrophically on ethanol or lactate when sulfate has been depleted. This a very important consideration for sulfate carrying-groundwaters. If excess donor such as ethanol or lactate is added to deplete sulfate, organisms will very probably be present that will have the important ability to produce H_2 from the remaining donor.

2.E.2. Lactic Acid

Pelobacter acetylenicus, (mentioned in Section 2.E.1) ferments acetylene to ethanol and acetate and also grows by oxidizing ethanol or lactate to acetate and H_2 in the presence of an H_2 -scavenging syntrophic partner [200]. *Syntrophobacter pfennigii* [256] oxidizes propionate and lactate in combination with hydrogenotrophs and also grows on either of these substrates in the presence of sulfate. In the absence of sulfate, some species of the genus *Desulfovibrio* will grow with their normal substrates lactate and ethanol in syntrophic association with H_2 -using organisms [34, 154, 170, 200]. Again, these organisms may be important in low-sulfate anaerobic environments or where sulfate has been depleted through addition of excess donor.

A coculture of *Clostridium formicoaceticum* and *Methanosarcina mazei* was reported [271]. In this pair, lactate is converted to acetate which

is in turn used by the methanogen. No H_2 is evolved under these conditions.

2.E.3. Butyric Acid

Butyric acid is an important intermediate in the anaerobic digestion of complex wastes such as sewage, animal manures, and plant residues. Butyrate degradation has been studied extensively to better understand the relationships between its degradation, H_2 partial pressure, and pH in anaerobic digestors.

It was also initially believed that methanogens were responsible for the degradation of fatty acids such as butyric acid to CH_4 and CO_2 [216]. The first syntrophic association of a butyric acid catabolizer and a hydrogenotroph was reported by McNerney *et al.* [156]. The organism, *Syntrophomonas wolfei* [155], oxidizes the four- to eight- carbon fatty acids (butyrate, caproate, and caprylate) to acetate and H_2 and the five- and seven- carbon fatty acids (valerate and heptanoate) to acetate, propionate, and H_2 . This organism was the first to be characterized which anaerobically degrades these compounds without light or sulfate, nitrate, or other electron acceptors [156, 157]. Although the organism was further characterized, it was not possible to culture it without the presence of an H_2 utilizer, even when H_2 was scrubbed from the gas headspace. The isolation of *S. wolfei* was eventually accomplished on crotonate [17] which it oxidizes to acetate and reduces to butyrate with the accumulation of a small amount of caproate. *S. wolfei* requires cyanocobalamin for growth at a concentration of 5 $\mu g/L$ [19]—well below that required by "*D. ethenogenes*", 50 $\mu g/L$.

Two syntrophic butyrate users along with H₂ users were isolated from the 3-chlorobenzoate-enriched consortium from which *D. tiedjei* was isolated [205]. One strain, NSF 2, resembled *S. wolfei*, and the other strain, SF 1, was a Gram-positive sporeformer. This organism could also reportedly catabolize isobutyrate.

Two endo-spore forming butyric-acid degrading organisms were isolated in syntrophic association with H₂-utilizing methanogens or sulfate reducing bacteria [242]. Since the butyrate-degrading bacteria formed spores, they could withstand pasteurization and could then be cocultured with a H₂ utilizer of choice—allowing an interesting option for forming cocultures. Some of the H₂-utilizing methanogens grew attached to the butyric acid utilizer presumably to gain advantageous access to the H₂ as it was produced.

Syntrophospora bryantii is a sporeforming, obligately syntrophic, fatty-acid-degrading organism that oxidizes fatty acids of 4 to 11 carbons to acetate, propionate (odd-numbered fatty acids), and H₂ [65, 156, 222, 278]. This organism was grown in pure culture in a membrane-separated coculture apparatus with *Desulfovibrio* sp. strain E70 [223].

Syntrophomonas sapovorans ferments linear fatty acids with from 4 to 18 carbons in the presence of a H₂-using partner [182]. This organism also requires a B-vitamin mixture.

The obligate proton-reducing bacteria that degrade butyric acid have been assigned to the family *Syntrophomonadaceae* [279]. None of the butyrate fermenters have thus far been reported to have the sulfate-reducing ability that has been shown for the propionate oxidizers (described in section 2.E.5).

The relationship between butyric-acid degradation, acetate concentration, and H_2 partial pressure under thermophilic conditions has been studied in co- or tri- cultures by Ahring and Westermann. Butyric acid degradation was completely inhibited by the presence of oxygen, but resumed at a reduced rate 1 to 2 days after its removal [5]. Butyric acid degradation was also inhibited when the methanogenic syntrophic partner was inhibited by BES. Butyric acid degradation rates were higher when acetate-utilizing methanogens were present because acetate also exerts a thermodynamic limitation on the degradation of butyric acid. H_2 partial pressures of 0.75×10^{-3} atm did not inhibit butyrate consumption; increasing to 2×10^{-2} atm gradually inhibited butyrate consumption; and addition of 3×10^{-2} atm completely inhibited butyrate consumption [6]. Butyric acid fermentation was inhibited by 6.9 and 96.6 percent by the addition of 10 mM or 75 mM acetate, respectively. Inhibition by H_2 was reversible after a lag time of a few days when it was removed; inhibition by acetate was reversible without a lag period.

Labib *et al.* [128, 129, 130] performed extensive experimentation, analysis, and modeling of the effect of H_2 and acetate on the degradation of butyric acid in a continuous-flow, attached-film fluidized bed reactor at mesophilic temperature. The butyric-acid-degrading consortium was more sensitive to acetate than to H_2 . An acetate concentration of 78 mM (5000 mg COD/L) completely inhibited butyric acid degradation while H_2 -using methanogens present in the consortium were able to quickly remove excess added H_2 and allowed faster recovery of butyric acid degradation. An overload of acetate had a more severe effect because the acetotrophic methanogens were slow to reduce the acetate concentration and the inhibited butyric acid utilizers began to wash out of the reactor. A

lengthened solids retention time helped offset the inhibitory effects. Despite a butyric acid loading rate of 10 g COD/L-day (63 mmol/L-d), the mass of volatile solids accumulated to only 1.2 g VS/L. The researchers estimated the SRT in their bioreactors to be less than 10 days.

Wu *et al.* [270] have constructed syntrophic fatty-acid degrading granules from known strains of butyrate degraders and methanogens. Rates and kinetic constants for butyrate use were reported.

2.E.4. Isobutyric Acid

The isomerization of butyric acid to isobutyric acid was noted in lake sediments when H_2 was added and butyric acid degradation was inhibited [142]. It has been proposed that isobutyric acid is first isomerized to normal-butyric acid prior to degradation to acetate and H_2 [280]. In a study which supported this idea, isobutyric acid enriched cultures were observed to accumulate transient, but significant quantities of butyric acid and ^{13}C -NMR studies suggested that the isomerization was accomplished by the migration of the carboxyl group [238]. Similar accumulation of isomers were also reported by Lin and Hu [137] and they also noted a constant pool of propionate in their isomerizing enrichments, suggesting some intermediate role for propionate. No propionate accumulation was noted in other anaerobic enrichment cultures that isomerized butyrate and isobutyrate [8]. Apparent co-metabolic isomerization of butyrate to isobutyrate and vice-versa by anaerobic bacterium strain WoG13 which ferments glutarate to butyrate, isobutyrate, acetate, and CO_2 was reported by Matthies and Schink [148].

2.E.5. Propionic Acid

Propionate fermentation is even more unfavorable than butyrate fermentation and fewer propionate oxidizing-bacteria have been isolated. *Syntrophobacter wolinii* [26] was first identified by Boone and Bryant as an obligately syntrophic propionate oxidizing bacterium that could grow only when tightly coupled to a H₂-using sulfate-reducing bacterium or methanogen. Phylogenetically, this organism groups with the sulfate-reducing bacteria [96], and recent experimental findings have confirmed that *S. wolinii* can additionally grow as a sulfate-reducing bacterium with propionate; and, it can also grow fermentatively in the absence of a hydrogenotrophic partner with pyruvate as a sole substrate [255].

MPOB, a syntrophic propionate-oxidizing bacterium that is related to *S. wolinii* has been shown to couple the oxidation of propionate to acetate with the reduction of sulfate, but with very low growth rates [125].

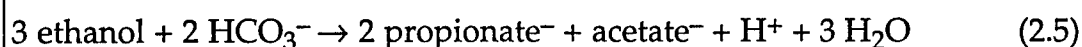
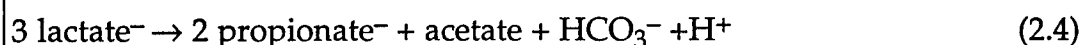
Syntrophobacter pfennigii [256] oxidizes propionate and lactate in combination with hydrogenotrophs and also grows on either of these substrates in the presence of sulfate. Additionally, some strains of propionate-oxidizing bacteria grew with fumarate as the sole substrate [174, 219], or as a terminal electron acceptor for propionate fermentation, essentially replacing the hydrogenotrophic partner [219]. *S. wolinii* was unable to grow in this way [219].

While many of the syntrophic propionate fermenters also couple the oxidation of propionate to the reduction of sulfate, one study showed that when grown in the presence of sulfate, at least one species, strain SYN7, could not out-compete a propionate-using sulfate reducing bacterium *Desulfobulbus* sp. for propionate [95].

2.E.6. Fermentation of Ethanol or Lactate to Propionate

During microcosm studies carried out in this laboratory [20, 209] significant amounts of propionate were detected in ethanol and lactic acid-fed enrichment cultures or microcosms.

Lactate and ethanol may be fermented to propionate under certain circumstances according to Equations 2.4 and 2.5.

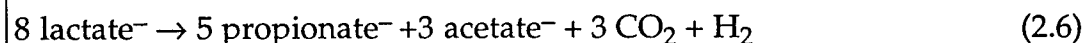


Fermentation of ethanol or lactate to propionate via these pathways is exergonic under the conditions studied and does not depend upon H_2 partial pressure. Lactate is fermented to propionate, acetate, and CO_2 by species that are often isolated from animal digestive systems—

Propionibacterium [132]; [253], *Clostridium* [132], *Selenomonas* [186], *Acetobacterium* [127], *Bacteriodes* [144], and *Megasphaera* [101].

Propionispira arboris was isolated by Schink from the wetwood of poplar trees [192]. Among other abilities, this organism ferments lactate to propionate, acetate, CO_2 , and traces of ethanol.

Lactate fermentation does depend upon H_2 partial pressure in the case of the metabolism of the *Veillonella* which oxidize lactate to propionate, acetate, CO_2 , and H_2 according to Equation 2.6 [55, 167, 207, 252].



Pelobacter propionicus is a 2,3-butanediol and acetoin fermenter that also ferments ethanol and lactate to acetate and propionate [187]. This organism was found to be unable to successfully compete with sulfate reducing bacteria for ethanol [228]

Schink *et al.* [191] studied several natural anoxic freshwater environments to determine the importance of ethanol as an intermediate and to compare pathways of ethanol degradation. Greater than 30 percent of the ethanol fermentation proceeded through propionate. Pure cultures of the propionate-forming ethanol fermenters isolated *were* inhibited by addition of H_2 in these environments. Even though most of the ethanol-oxidizing strains isolated did not form propionate, the propionate-forming ethanol fermenters could successfully compete for high levels of ethanol in those environments.

Clostridium neopropionicum was originally studied by Samain *et al.* in 1982 [183], but it was not fully described until 1992 [240]. This organism ferments ethanol via the acrylate pathway to propionate, acetate, propanol, and traces of butyrate. No H_2 is formed during this fermentation and H_2 added to the headspaces of these cultures did not affect the fermentation.

The sulfate-reducing bacterium *Desulfobulbus propionicus* oxidizes lactate, pyruvate, ethanol, propanol, and propionate to acetate while reducing sulfate to sulfide. In the absence of sulfate, this organism ferments lactate, pyruvate and ethanol to propionate and acetate [126, 218, 230, 263]. H_2 affected the fermentation in *D. propionicus* strain Lindhorst by causing a change in the stoichiometry of the reaction [126]. Under a H_2/CO_2 atmosphere the ethanol was quantitatively reduced to propionate.

Lactate or ethanol fermentation to propionate at a particular site would be expected to depend primarily upon the presence of organisms that carry out these reactions and sometimes, but not always, on the thermodynamic limitation of H_2 .

2.E.7. Other Pathways

Butyrate formation from the reductive decarboxylation of propionate was observed in anaerobic digester sludge, however, the organism(s) responsible were not isolated [239, 241].

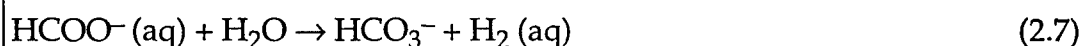
Clostridium acetobutylicum strain P262 utilizes lactate and acetate and produces butyrate, H₂ and CO₂ [58]. The metabolism of *Clostridium kluyveri* involves the conversion of acetate and ethanol to butyrate, caproate, and H₂ [234].

2.E.8. Energy Conservation in Syntrophic Associations

Organisms must conserve a finite amount of free energy—generally believed to be equivalent to the energy required to synthesize one-third of a mol of ATP [190]—in order to grow. This value is termed the “critical” Gibbs free energy or $\Delta G_{\text{critical}}$ and values have been reported for several syntrophic organisms. Seitz *et al.* [201] reported values of -5.2 to -5.5 kJ/mol H₂ produced (-10.4 to -11 kJ/mol ethanol used) for *Pelobacter acetylenicus*, *P. acetylenicus* GhAcy1, and *P. carbinolicum*. Similarly, Dwyer *et al.* [67] reported values of -8 kJ/mol H₂ formed (-16 kJ/mol butyrate used) for butyrate fermentation. Schink [190] presented calculations and suggested that $\Delta G_{\text{critical}}$ should be expected to be -20 kJ/mol substrate used; and experimental evidence was presented for butyrate fermentation for which a $\Delta G_{\text{critical}}$ of -23 kJ/mol butyrate was determined. When pure cultures of *S. wolfei* were amended with butyrate in the absence of hydrogenotrophs, butyrate was fermented until 6.3×10^{-4} atm of H₂ accumulated, then fermentation ceased. This H₂ partial pressure corresponded to a free energy of -26.3 kJ/mol butyrate [257].

2.F. Interspecies Electron Transfer— H_2 and Formate

"*D. ethenogenes*" and *D. restrictus* do not use formate, while *D. multivorans* does. The question of whether H_2 or formate is produced upon donor oxidation is thus a question of some importance; however, in a mixed microbial community, H_2 and formate are rapidly biologically exchanged with each other through the action of the formate- H_2 lyase enzyme system which is possessed by many different types of organisms. In aqueous systems, H_2 and formate undergo the microbially-mediated reversible reaction shown in Equation 2.7.



It is currently unclear whether during syntrophic degradation of fatty acids, H_2 transfer or formate transfer is the more important mechanism by which reducing equivalents are transferred from the obligate proton reducer to the hydrogenotroph. There is currently evidence to suggest that both H_2 transfer and formate transfer are important for interspecies syntrophic associations.

In studies with a mixed population containing a syntrophic ethanol-oxidizer (*Desulfovibrio vulgaris*), differences in electron carriers between dispersed cells and flocs were examined. Interspecies electron transfer was enhanced inside intact flocs made up of lattice-type cell arrangements where the electron carrier, H_2 , was not transferred to the bulk solution to any great extent. Furthermore, methanogenesis was independent of the bulk H_2 concentration [236]. The experiments suggested that some other carrier was responsible besides H_2 and this was confirmed to be formate in separate experiments with either ethanol or lactate oxidation [237]. In fact, the authors found that greater than 90 percent of the conversion of ethanol

to CH_4 could be accounted for by formate transfer while less than 10 percent was from H_2 transfer. In some cases a thermodynamic advantage would be afforded if formate rather than H_2 could be transferred. The authors proposed a model for a bicarbonate-formate electron shuttle mechanism.

S. wolfei was reported to produce only H_2 and not formate by McInerney *et al.* [155]. Formate added to cultures containing *S. wolfei* Göttingen strain and an H_2 - CO_2 -using methanogen, *Methanobacterium bryantii*, did not result in the formation of CH_4 —suggesting that *S. wolfei* has no formate dehydrogenase activity. However, this *S. wolfei* strain did grow better when cocultured with *Methanospirillum hungatei* which does use formate in addition to H_2 - CO_2 .

S. wolfei, strain LYB, did show formate dehydrogenase activity [27]. Furthermore, calculations were presented that suggested that because of the low solubility of H_2 and the much higher corresponding concentration of formate that would be in equilibrium with the H_2 , formate as an electron carrier would account for 98-fold more interspecies electron transfer in the system studied than would H_2 .

S. bryantii degraded butyrate in coculture with methanogens that use both H_2 and formate but not with methanogens that use only H_2 [65]. In the second case, butyrate fermentation was inhibited both by H_2 and formate. *S. bryantii* contains both a hydrogenase and formate dehydrogenase activity.

The syntrophic propionate degrader MPOB could couple propionate degradation with methanogens that use both formate and H_2 but could not couple with a methanogen that could only use H_2 unless a third organism was added which could convert formate to H_2 and CO_2 [66]. MPOB contained a hydrogenase and formate dehydrogenase and could

interconvert H_2 and formate; curiously, this enzyme system apparently only became active when H_2 was above 80 kPA and formate concentration was above 0.9 mM—higher than the levels required for energetically favorable syntrophic growth.

Formate diffusion rates were calculated to be about 100 times the rates of H_2 diffusion in cocultures of fatty acid oxidizers and methanogens [217]. Both H_2 and formate were produced by the butyrate-oxidizer *S. bryantii* when pentenoate was the electron acceptor and by the propionate-oxidizer MPOB when fumarate was used as the electron acceptor.

Bae and McCarty [10] reported that addition of formate (5 to 10 nM) to a butyric-acid-degrading consortium resulted in inhibition of butyric acid degradation. They suggested that the reason for the inhibition is that formate, not H_2 , is the electron transferring agent.

Labib *et al.* [130] also found inhibition of butyric acid degradation upon addition of formate to their butyrate-degrading attached-film reactor. They speculated that the inhibition was caused by H_2 which was formed upon degradation of the formate, however, they were not able to conclusively separate the effect of formate.

Thus, much of the information does suggest that formate exchange is very important—perhaps more important than H_2 transfer—in these types of cocultures. As long as other organisms are present that do have a formate- H_2 lyase system, it should make little difference whether the dechlorinator uses H_2 or formate since these two compounds will be rapidly interconverted by other members of the consortium.

2.G. Competition for H_2

Since H_2 is a key intermediate in the degradation of complex organic matter in sediments and anaerobic digestors, its production via fermentative activities and the subsequent competition for its use have been studied intensively. One of the most important competitions in natural environments is that between sulfate-reducing bacteria and methanogens. This competition has been analyzed by many researchers and it has been determined that when sulfate is not limiting, sulfate reducers have a higher affinity for H_2 and acetate than do methanogens [119, 180]. If substrate (i.e. sulfate, H_2 , or acetate) is not limiting, then both sets of organisms can live and grow. When H_2 or acetate is limiting, the sulfate-reducing bacteria can use these substrates down to levels that are lower than the threshold values of their methanogenic competitors and the methanogens are then unable to compete for substrate in the environment. The differences in threshold values for H_2 for different species is dependent upon the energetics of the bioreactions. The H_2 -consuming bioreaction that has the better energetics will dominate in mixed populations and the energetics of the H_2 -electron acceptor couple determines how low the threshold for H_2 for that reaction will be [50]. This has been termed "competitive exclusion" by Chapelle [40]. Interestingly, as was already described, when sulfate is depleted, some sulfate-reducing bacteria can switch to a syntrophic-type metabolism, oxidizing ethanol, lactate, or propionate to acetate and H_2 and then, methanogens become their syntrophic partners—pulling away the end products of the oxidation and keeping the reactions energetically favorable for the sulfate reducers.

It is important to determine where the dehalogenators sit in this competitive ladder. In a mixed culture containing "*D. ethenogenes*", a higher affinity for H_2 was exhibited by dechlorinators than for hydrogenotrophic methanogens, as quantified by measurement of K_S values [208, 210]. The $K_{S(H_2)dechlor}$ (K_S for H_2 use by dechlorinators) was 10-fold lower than that for the methanogens in the culture. Similarly, Ballapragada *et al.* have reported an even lower range of values for $K_{S(H_2)dechlor}$ in a mixed culture containing unidentified dechlorinator(s) [13]. Thus far, no direct study of H_2 threshold for "*D. ethenogenes*" has been performed, however data from this study does suggest a lower H_2 threshold for dechlorination than for methanogenesis (see Results Section 4.A.3.d). Also, no direct comparison of dechlorinators and sulfate-reducing bacteria has yet been performed. In studies with *D. tiedjei*, which is both a sulfate reducer and a dechlorinator, it was shown that reduction of sulfur oxyanions was favored over aryl dechlorination. Some researchers have speculated that this was because the reduction of sulfate pulled H_2 levels too low for dechlorination of 3-chlorobenzoate to be favorable [56].

2.H. Modeling Microbial Systems

The model developed during this study encompasses the kinetics of dechlorination, donor fermentation, methanogenic use of H_2 and acetate, and the growth of all involved microbial communities. A review of some of the work already reported in this area is given here. Additionally, peripherally pertinent to this work is the extensive field of modeling the transport, sorption, and degradation of pollutants in groundwater aquifers. It is hoped that the model developed as a part of this study may some day be useful as a "plug-in" to groundwater models. A review of some existing

pollutant fate-and-transport models is given with specific emphasis on the kinetic models for pollutant and primary electron donor degradation that are incorporated into them.

2.H.1. Pollutant Degradation Models

Many of the existing pollutant fate-and-transport models were developed for predicting petroleum hydrocarbon transport and degradation. Since these compounds are electron donors and are consumed by many types of ubiquitous microorganisms, their degradation is simpler to model and the outcomes may be more easily predicted. Some of these models are now being converted to application to chlorinated solvents. Since halogenated solvents serve as electron acceptors, and are used only by a limited number of microorganisms and with a limited number of electron donors, predicting their disappearance using the same models that have been developed for hydrocarbon use is inappropriate.

Also, accurately modeling pollutant detoxification is complicated by byproduct inhibition, competition, or toxicity; lack of knowledge about prevalence and substrate specificity of microorganisms on site; and changes in electron donor or acceptor conditions over time. These are among the reasons that the popularity of simplistic first-order models for pollutant degradation persists [21, 193]. Alexander has described in depth many different types of biodegradation models and has criticized the prevalent use of simplistic first-order or half-life kinetic models in environmental fate models as leading to incorrect conclusions about the fate and persistence of environmental contaminants [7]. The National Research Council has identified the lack of knowledge of transformation rates in the field and the uncertainty involved with extrapolating laboratory-measured

contaminant kinetics to the field as a major impediment to predicting the outcome of bioremediation [161].

For chlorinated solvent reduction, the use of such a simplistic approach is especially subject to error. First, the presence, availability, and kinetics of the crucially important electron donor are not even considered. Second, the reduction of the chlorinated compounds in the future are computed from half-lives based on historical data, then are extrapolated into the future. It is dangerous to assume that the same donor, redox, and microbial population conditions that were present in the past year or past 20 years will continue to exist into the future for 10, 20, or 30 years. Nonetheless, many examples of application of half-lives for predicting future chlorinated solvent biodegradation exist.

BIOSCREEN, a screening tool developed for simulating natural attenuation of hydrocarbons, is being converted to a screening tool, BIOCHLOR, for assessing chlorinated solvent natural attenuation [166]. It was reported that first-order decay would be used for modeling chlorinated solvents.

Brasaemle *et al.* [32] presented data supporting evidence for natural attenuation of *cis*-1,2-DCE and VC in a plume from a landfill. The U.S. EPA fate and transport model BIOSCREEN was utilized to predict the ultimate fate of the VC in the plume. A simple half-life (5 yr) was used to model biodegradation of the VC.

Leethem *et al.* [133] also presented data for a naturally attenuated VC plume at a manufacturing site. In anaerobic zones of the plume, ETH and ethane have been detected and additionally, at the aerobic perimeter of the plume, aerobic VC degradation is thought to be occurring. For this site,

MODFLOW was used to model groundwater flow and MT3D was used to model VC fate and transport with a half-life of 400 days for VC.

A risk analysis model was reported by Cline *et al.* for TCE degradation for which site-specific parameters could be incorporated. However, this model also uses simple, first-order decay incorporating half-lives for TCE and the other chlorinated break-down products to predict biodegradation [45].

BIOPLUME III was developed by Rifai [178] and is an extension of the popular and widely-used petroleum hydrocarbon model, BIOPLUME II, to anaerobic conditions. This model simulates aerobic and anaerobic processes; the transport and fate of the contaminant; and the transport and sequential use of the electron acceptors O_2 , NO_3^- , $Fe(III)$, SO_4^{2-} , and CO_2 . Organic contaminants are modeled as a "lumped" parameter, not as individual components. Depending upon the reaction, the model may utilize first-order or Monod kinetics; or, when biodegradation occurs rapidly in comparison to groundwater velocities, biodegradation is modeled instantaneously. The model does not (in the description available) include chlorinated solvents as one of the alternative electron acceptors.

Tonnaer *et al.* [243] incorporated more descriptive Monod kinetics that included both chlorinated species kinetics and kinetics for electron donor degradation in their model. They also recognized the need to account for the conversion of an applied donor, methanol, to other donors—acetate and H_2 —thought to be used for dechlorination. Competitive inhibition between PCE and TCE was also incorporated.

UTCHEM, a multiphase flow simulator with a biodegradation component also includes more complex biokinetic equations [25]. The

biodegradation portion of the model includes Monod kinetic equations incorporating both electron donor and electron acceptor kinetic expressions for multiple donors and/or acceptors. The model includes consideration of both attached microbial films and suspended growth within the bulk liquid. Inhibition terms can be included, as can expressions for (aerobic) co-metabolic degradation.

The draft version of the AFCEE Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater [264] provides an overview of the many analytical and numerical models that are currently available. Of eight analytical models described, seven incorporate first-order decay and the eighth has no listing for kinetic options. Of 23 numerical models listed, most are described as incorporating first-order decay as the kinetic model for contaminant degradation (some also have zero- or multiple-order options). Only two of those listed—RT3D, currently under development by researchers at Washington State University and Pacific Northwest National Laboratory [227], and BIOPLUME III—appear to incorporate more elaborate biodegradation schemes.

It is encouraging that several of the above models do incorporate more complex kinetic expressions for substrate degradation and microbial growth. None of them, however, incorporate the necessary kinetic expressions to model closely coupled H_2 formers and users—as is necessary to model associations of VFA- and alcohol-oxidizers with hydrogenotrophic dehalogenators. The following section discusses models for syntrophic associations. Some incorporation of these types of models is necessary for more complete description of chlorinated solvent reduction with H_2 as the electron donor.

2.H.2. Donor Fermentation and Syntrophic Microbial System Models

Powell has mathematically described the equalization of the specific growth rates for a tightly coupled syntrophic association of two organisms meeting the following conditions: species X utilizes substrate S and produces product P [175]. Because of thermodynamics, P inhibits the growth of X, but at the same time, P is the limiting substrate for the growth of organism Y. Powell found that the two organisms should exhibit stable coupled growth over a range of dilution rates in continuous culture systems [176]. Archer and Powell presented experimental evidence with syntrophic ethanol-degrading cocultures containing an ethanol fermenter and one of ten different methanogens, and showed that the maximum specific growth rates of cocultures were roughly proportional to the maximum specific growth rates of the corresponding individual methanogens [9].

Kreikenbohm and Bohl [118] presented their own mathematical extension of these syntrophic associations including thermodynamical considerations and they presented an equation of the form of Equation 2.8 for growth of the X organisms:

$$\mu_x = \frac{\mu_{\max} \left(S - \frac{P}{K_1} \right)}{K_2 + S + K_3 * P} \quad (2.8)$$

Where:

μ_x = the specific growth rate of organism X;

μ_{\max} = the maximum specific growth rate of organism X;

K_2 = a Michaelis-Menten-type constant;

K_3 = an inhibition constant related to the negative influence of P; and, further, they suggested that

K_1 = some constant between 0 and 1 that is related to the thermodynamics of the reaction;

$$K_1 := \exp\left(\frac{-\Delta G^{o'}}{RT}\right)$$

where $\Delta G^{o'}$ is the standard free energy change of the metabolic reactions involved.

The model of Labib *et al.* [128, 129, 130]—constructed for use in predicting the effect of transient pulse loads of acetate and H_2 on the degradation of butyric acid in a fluidized-bed reactor—included calculation of pH; gas production rates; partial pressures of CH_4 , CO_2 , and H_2 ; and the biomass concentrations for the various microbial groups that would be expected to be growing in the biofilm. The model for butyrate degradation kinetics was based on reversible Michaelis-Menten enzyme kinetics [181, 199] taking the form of Equation 2.9:

$$v_{\text{net}} = \frac{V_{\max, f} \left([S] - \frac{[P]}{K_{\text{eq}}} \right)}{K_s \left(1 + [P]/K_p \right) + [S]} \quad (2.9)$$

Where $\frac{[P]}{K_{eq}} = [S]_{eq}$

$[S]$ = substrate concentration;

$[P]$ = product concentration;

$[S]_{eq}$ = the equilibrium substrate concentration given K_{eq} and the concentration of $[P]$;

K_{eq} = equilibrium constant;

K_S = half velocity coefficient;

K_P = inhibition constant for P;

$V_{max, f}$ = the maximum forward rate of substrate degradation;
and

v_{net} = the rate of substrate degradation.

This is identical in form to the model of Kreikenbohm and Bohl.

The model was further modified by Labib as per Equations 2.10 and 2.11:

$$[S]_{eq} = [S] * \exp(\Delta G / RT) \quad (2.10)$$

$$v_{net} = \frac{V_{max, f} [S] (1 - \exp \Delta G / RT)}{K_s (1 + [P] / K_p) + [S]} \quad (2.11)$$

The final form of the model used by Labib *et al.* to predict the degradation of butyrate and responses to shock loads of H_2 or acetate is Equation 2.12:

$$r_{sB} = \frac{k_{mB} X_B S_B (1 - \exp^{\Delta G_{rB} / RT})}{K_{sB} (K_A S_A + K_H S_H) + S_B} \quad (2.12)$$

Where:

- r_{sB} = rate of butyrate use;
- k_{mB} = maximum specific rate of butyrate utilization;
- X_B = concentration of butyrate-degrading biomass;
- S_B = concentration of butyrate;
- K_{sB} = half-velocity coefficient for butyrate;
- K_A = acetate inhibition coefficient;
- S_A = acetate concentration;
- K_H = H_2 inhibition coefficient;
- S_H = H_2 concentration; and
- ΔG_{rB} = the free energy change for the butyrate reaction under physiological conditions.

Hoh and Cord-Ruwisch [102] presented an "equilibrium-based model" for modeling anaerobic processes that often operate near the point of thermodynamic equilibrium—i.e. processes depending upon syntrophic associations. Their model is also based upon reversible reaction kinetics and takes the form shown in Equation 2.13.

$$v = \frac{v_{\max(F)} \times S \left(1 - \frac{\Gamma}{K}\right)}{k_m(s) + S \left(1 + \frac{\Gamma}{K}\right)} \quad (2.13)$$

Where:

- v = the rate of reaction;
- $v_{\max}(F)$ = the reaction rate at substrate saturation;
- S = substrate concentration;
- $k_m(S)$ = the Michaelis-Menten constant for S ;
- K = equilibrium constant (ratio of [products] over [substrates] at dynamic equilibrium); and
- Γ = the mass action ratio (actual ratio of [products] over [substrates]).

When the concentrations of the substrates are much higher than those of the products (far from equilibrium), Γ/K becomes small and the model reduces to classic Michaelis-Menten kinetics. As the reaction approaches equilibrium, Γ/K approaches unity and the rate of reaction approaches zero. This model is also similar in form to those of Labib *et al.* and Kreikenbohm and Bohl.

Thus far, none of the groundwater models (that this author has examined) have incorporated this type of thermodynamically-controlled donor fermentation model. For H_2 modeling, this will be critical. These types of models will be useful for more fully describing the donor fate in dechlorinating systems.

CHAPTER THREE

MATERIALS AND METHODS

3.A. *Source Cultures*

Source cultures were operated as reservoirs for fresh culture to be used in serum bottle experiments and for other purposes described in this chapter.

3.A.1. General Source Culture Operation

A diagram of a source culture reactor is shown in Figure 3.1. Each source culture reactor consisted of a *circa* 9 L Pyrex® bottle containing a stirbar that was closed by a Teflon®-lined steel top with a three-way stainless-steel valve to accommodate addition of basal medium and removal of waste culture and evolved biogas [59]. A 0.5-inch diameter hole in the side accommodated a septum which was held in place by a stainless-steel hose clamp that had a small hole at the point where the clamp crossed the septum. Gas and liquid samples could be removed and liquid substrates could be added via syringe through the septum. The septum could be removed to allow introduction of anoxic purge gas via cannula. The anoxic purge gas was a 70% N₂ (high purity N₂, 99.99%, Matheson Gas Products) and 30% CO₂ (anaerobic CO₂, 99.99%, Matheson Gas Products) gas mixture that was bubbled vigorously through a titanium-citrate complex to remove any traces of O₂ prior to use in cultures. For purging cultures to remove volatile end products, the hose clamp was loosened and slipped off to allow the septum to be popped out while purging cannula were quickly inserted into the hole.

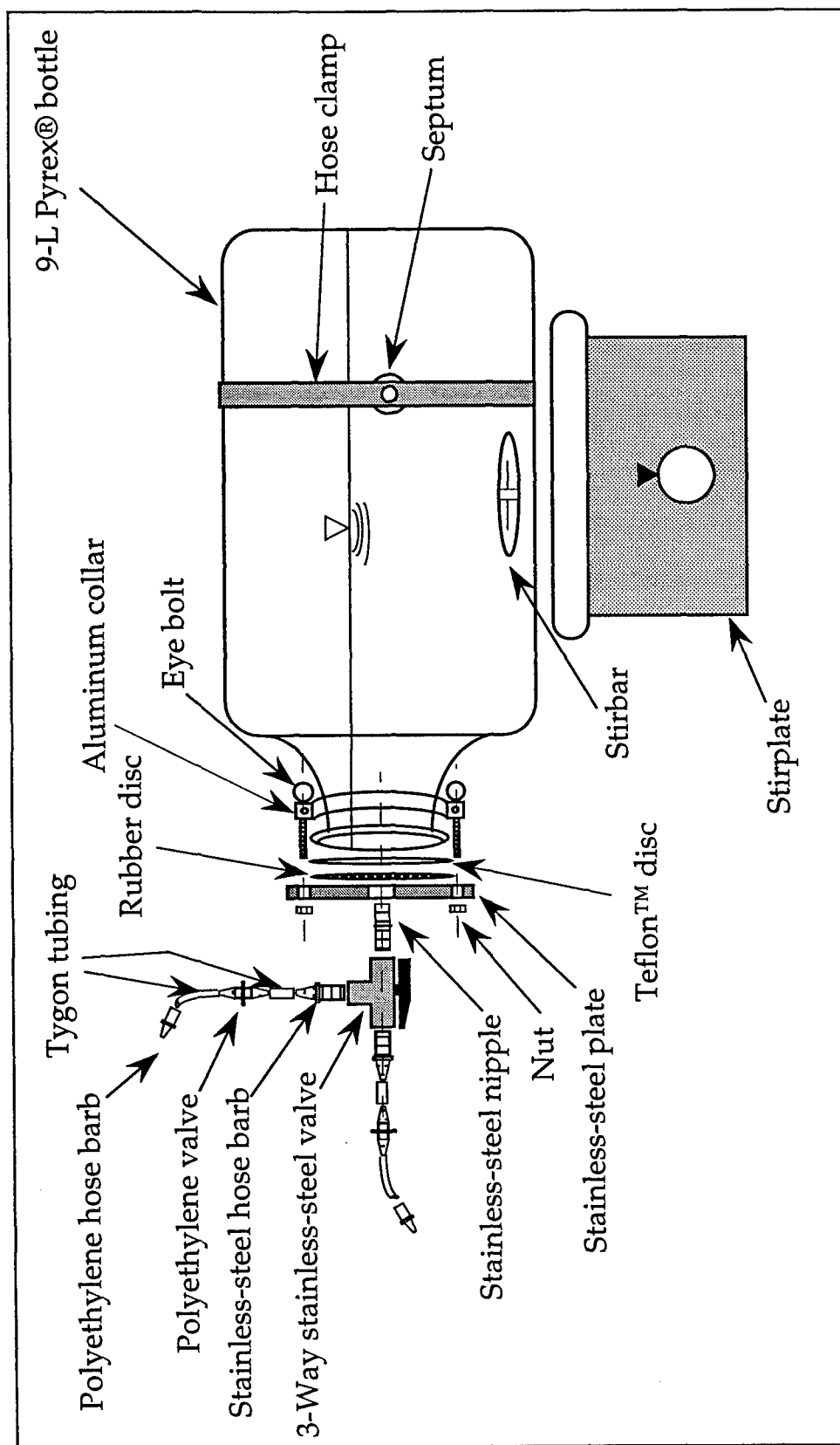


Figure 3.1. Schematic of source culture reactor.

Each source culture reactor was held on its side in a rack with a stirplate beneath and was stirred constantly. Reactors were incubated at 35°C in a constant-temperature chamber.

The source culture reactors were operated with the same liquid-to gas-space volume ratio (1.67 L liquid to 1 L gas space) as serum bottles to enable the use of the same calibration standards for each. A list of source culture reactor volumes is shown in Table 3.1.

The source cultures were fed PCE, electron donor, and nutrient supplement every second day. Every fourth day, after liquid and gas samples were removed, source cultures were purged to remove accumulated volatile compounds. A hydraulic retention time (HRT) of 40 days was maintained by a fill and draw exchange of 10 percent of the liquid every fourth day. Basal medium was added to the reactor from a pressurized 19-L basal medium container. During transfers, purge gas was introduced continuously to the basal medium container through two diffusing stones to maintain pressure. The liquid withdrawal line was connected to the "in" port of the 3-way valve on the reactor. The 3-way valve was positioned to allow basal medium to flow into and out of the valve to flush out air. With the basal medium flowing, the "out" port of the 3-way valve was closed, the valve in the basal medium withdrawal line was closed and then the 3-way valve was positioned to allow basal medium to flow into the reactor. The valve in the basal medium withdrawal line was opened and basal medium was allowed to flow into the reactor under pressure until the required amount had been added. The source culture reactor was shaken vigorously, then positioned on its side. The 3-way valve was positioned to allow the well-mixed culture to flow out, under

pressure built up by addition of fresh basal medium, into a graduated cylinder. When the correct amount of waste had been expelled, the 3-way valve was closed. PCE, electron donor, and nutrients were then added via syringe.

Table 3.1. Volumes of source culture reactors.

	Bottle Volume (L)	Liquid Volume (L)	Gas Volume (L)	Waste Volume (L)
Low-PCE/Butyric Acid-Enriched Source Culture I	9.1	5.7	3.4	0.57
Low-PCE/Butyric Acid-Enriched Source Culture II	9.3	5.8	3.5	0.58
High-PCE/Methanol- Enriched Source Culture	9.6	6	3.6	0.6

3.A.2. High-PCE/Methanol Source Culture

The high-PCE/methanol culture [59] has been operated for 6 years. This culture served as inoculum for most of the experiments and for the start up of low-PCE/butyric acid source cultures. The high-PCE/methanol source culture was maintained throughout this study by J.M. Gossett. The culture was maintained with the protocol shown in Table 3.2 at 35°C and was occasionally monitored for ethenes, CH₄ and pH to ascertain culture health. The culture was brought to a PCE concentration of approximately 550 µM or 91 mg/L (concentration excluding partitioning to the headspace) every second day. This PCE concentration is inhibitory to methanogens and consequently there is very little methanogenic activity, either

acetotrophic or methanogenic. Since most experiments described here used PCE doses of 110 μM (one fifth the level employed with the source culture), a 20 percent dilution of the source culture was used as starting material for some of the experiments. The lack of acetotrophic activity made this culture especially suitable for experimentation. Fermentation of the added electron donors proceeded primarily to acetic acid and H_2 , with no conversion of acetic acid to CH_4 . Therefore, all H_2 equivalents which were channeled to CH_4 formation could be easily accounted for. Without the inhibitory PCE concentrations, however, acetotrophic activity was expected to begin eventually. Unfortunately, it did occur in some experiments and after that time, a strict accounting of CH_4 formed from H_2 was not possible.

Table 3.2. Operational protocol for the high-PCE/methanol source culture (35°C).

	Second Day	Fourth Day
Anoxic Purge	no	yes
Waste	no	yes
PCE (mmol)	3.5	3.5
Methanol (mmol)	9.5	9.5
Yeast Extract (mg)	125	125

* Yeast extract solution contained 50 g/L yeast extract and 2.5 mL of this solution was added.

3.A.3. Low-PCE/Butyric Acid Source Cultures

Low-PCE/butyric acid source cultures were started using the high-PCE/methanol source culture as inoculum. The rationale for the start-up of the butyric acid cultures was to provide a long-term test for butyric acid

as an effective H_2 donor that would support significant dechlorination in a culture operated at non-inhibitory PCE concentrations that also supported healthy methanogenic populations. These cultures also served as inoculum for experiments described here, for experimentation by other members of our group [210], and as a source of cell extracts used as a nutritional supplement for the support of the pure culture isolates [152].

To start up the first enrichment, a 9.1-L reactor was filled with distilled water, and anoxic purge gas was started via cannula in the hole which normally accommodated the septum. The distilled water was allowed to exit the bottle through the three-way valve as the bottle was slowly filled with the anoxic purge gas. After the water was drained, the bottle was purged for an additional 1 hour to ensure anoxic conditions. Next, 5 L of basal medium and 600 mL of the high-PCE/methanol culture was delivered to the new bottle through the hole as the bottle was purged. Thus, the culture was started with a 10.5 percent inoculum. The bottle was then closed, shaken thoroughly, and fed PCE, butyric acid, and pre-fermented yeast extract to start the new protocol. The second source culture was started as above, but using 6.8 percent inoculum from the original low-PCE/ butyric acid source culture.

The operating protocol for the cultures, which were incubated at 35°C, is shown in Table 3.3. At each feeding, PCE was added to obtain a nominal concentration (neglecting partitioning to the headspace) of 110 μ M. Butyric acid was added at a 2:1 ratio to PCE on an equivalents basis. The amount added at each feeding was 440 μ M (38.72 mg/L). Pre-fermented yeast extract solution containing 50 g yeast extract/L was added at each feeding to obtain 20 mg FYE/L in the culture. Vitamin solution

addition was begun on Day 125 and after Day 289, the concentration of vitamin B₁₂ in the vitamin solution and in the cultures was increased by ten-fold.

Table 3. 3. Operational protocol for low-PCE/butyric acid source cultures (35°C).

	Second Day	Fourth Day
Headspace Sample	yes	yes
pH Measurement	no	yes
VFA Measurement	no	yes
Gas Production Measured	no	yes
Anoxic Purge	no	yes
Basal Medium Added (mL)	no	569
Culture Wasted (mL)	no	569
PCE (μmol)	626	626
Butyric Acid (μmol)	2504	2504
Pre-Fermented Yeast Extract (mg) ^a	115	115
Vitamin Solution (mL) ^b	no	2.9

^a Pre-fermented yeast extract solution contained 50 g/L yeast extract and 2.4 mL of this solution was added.

^b After day 125.

3.B. Serum Bottle Studies

In this section, general information about serum bottle set-up and handling is presented. Serum bottle cultures were studied both over long-term, semi-continuous operation, and during short-term time-intensive studies. Each serum bottle test had somewhat different procedures and

more detailed information concerning specific tests is presented where necessary in the Results sections. All serum bottle tests were performed at 35°C.

3.B.1. Set-up of Serum Bottles From Source Cultures

Experiments were performed in 160-mL serum bottles containing 100 mL of culture and 60 mL of gas. Previously autoclaved TeflonTM-backed, gray-butyl rubber septa (Wheaton Industries) and aluminum crimp caps with tear-off seals were used to close the bottles. Serum bottles were prepared either with 100 percent source culture directly from a source culture reactor, or from a 20 percent inoculum that was prepared in the glovebox.

For preparation directly from source cultures, tared bottles were filled with distilled water and inverted into a large container of distilled water. A cannula delivering anoxic gas was introduced into the bottle and the distilled water was replaced with anoxic gas. The bottle was removed from the container and placed upright while still purging. A source culture reactor which had been purged and charged with excess basal medium was connected via the 3-way valve to the suction side of a Unispense II pump (Wheaton Industries). The discharge side of the pump terminated in a double cannula. One side of the double cannula dispensed the discharged culture from the pump and one side discharged anoxic purge gas. The pump was calibrated with distilled water prior to use to ensure delivery of *circa* 100 mL of liquid during each timed dispensing cycle. The 3-way valve was situated to connect the reactor contents with the suction side of the pump which was then first operated for one cycle

with the discharge collected in a waste container to flush the pump tubing. The double cannula was then inserted into the prepared serum bottle, the pump was activated and 100 mL of the culture was delivered to the serum bottle. The double cannula was then withdrawn, leaving the original anoxic purge cannula in place. A pre-tared septum was placed onto the bottle and pressed snugly down into the opening of the bottle while the purging cannula was simultaneously withdrawn. A pre-tared aluminum crimp cap was placed over the septum and crimped. After the culture was delivered to the bottle it was weighed and the weight and corresponding volume of culture which had been delivered was determined. If the volume deviated more than 1 mL from the desired 100-mL volume, the volume was adjusted by removing or adding culture via a gas-tight, locking syringe (Dynatech Precision Sampling Corp.).

Serum bottles with a 20 percent dilution of the source culture were prepared by transferring required volumes of source culture and basal medium in purged, sealed bottles to an anaerobic glovebox and mixing these together in bulk to prepare a 20 percent dilution. The mixture was dispensed via a 100-mL volumetric pipette to 160-mL serum bottles. The serum bottles were capped with previously autoclaved, gray-butyl, Teflon®-lined septa (Wheaton Industries) and crimped with aluminum caps. Upon removal from the glovebox, each bottle was purged anoxically for 2 min to remove H_2 —a potential electron donor which was present in the glovebox atmosphere—then were re-capped.

3.B.2. General Protocol for Serum Bottle Operation

Substrates were added in liquid form to serum bottles via syringe. Butyric acid, ethanol, lactic acid, and propionic acid were added in neat form using microsyringes (Hamilton Company). PCE was added in neat form by microliter syringe (Hamilton Company) except during time-intensive studies, when PCE was pre-solubilized in basal medium prior to addition to bottles. H_2 , when added as an electron donor, was added via a gas-tight, locking syringe (Dynatech Precision Sampling Corp.). The temperature and pressure were noted and the volume of pure H_2 required to give the needed molar amount was computed using the ideal gas law. Yeast extract—added as a nutrient source in an anoxic aqueous, pre-fermented form—and an anoxic aqueous mixture of vitamins were added using a gas-tight, locking syringe (Dynatech Precision Sampling Corp.).

Hydrogen donors (except where specifically noted) were added at a 1:1 or 2:1 ratio to PCE on an equivalent basis based on the fermentation of the donor to H_2 as shown in Table 3.4.

Table 3.4. Equivalent values for hydrogen donors (on the basis of fermentation to H_2) and the amendment amounts for 1:1 and 2:1 donor to PCE ratios.

Substrate	eq/mol	Amount Added 1:1 ratio (μmol)	Amount Added 2:1 ratio (μmol)
PCE	8	11	11
Butyric Acid	4	22	44
Ethanol	4	22	44
Lactic Acid	4	22	44
Propionic Acid	6	14.7	29.4
H_2	2	44	88

When acetotrophic activity was present in cultures, it was no longer possible to quantify the CH_4 produced via H_2 —in that case, CH_4 was produced from H_2 and acetate. Under those circumstances, it was useful to present results with donor available or released via fermentation on a CO_2 equivalents basis. The CO_2 equivalent conversion factors were 20, 12, 12, and 14 $\mu\text{eq}/\mu\text{mol}$ donor for butyric acid, ethanol, lactic acid, and propionic acid, respectively.

3.B.3. Protocol for Long-Term Operation of Serum Bottles

Every second day during long-term operation, a 0.1-mL headspace sample was removed from each bottle for dechlorination products, H_2 , and CH_4 analysis; then, PCE, electron donor (if any), and FYE were added. As will be shown later, because of its slow rate of degradation, propionic acid was sometimes withheld to avoid its accumulation in the cultures. Every fourth day, after headspace samples were removed, 10 mL of culture was removed via an anoxically purged, gas-tight, locking syringe (Dynatech Precision Sampling Corp.), 10 mL of fresh basal medium was added in the same manner, the septum and crimp cap were removed, the bottle was purged for 5 minutes with a cannula and/or glass diffusing rod, and then re-capped using a fresh, tared septum. The removed liquid was used for measurement of pH, VFAs, ethanol, and lactic acid. After the exchange and purge, the bottles were fed neat PCE, neat electron donor (if any), FYE, and vitamin solution. During long-term operation, bottles were incubated in a 35°C walk-in chamber, in a slanted, inverted position on a orbital platform shaker (Innova 2000, New Brunswick Scientific Co., Inc.) at 165 rpm.

3.B.4. Protocol for Time-Intensive Studies in Serum Bottles

After headspace samples were analyzed, 10 mL of culture was removed, the bottles were purged for 5 min with a cannula and/or glass diffusing rod and then re-capped with a fresh tared septum.

Approximately 11 μ mol of pre-dissolved PCE was delivered by adding 9 mL of PCE-saturated basal medium and 1 mL of regular basal medium. Excess gas pressure of 7 mL was allowed to remain in each bottle to facilitate the removal of the gas and liquid samples without resulting in vacuum—and danger of introduction of air—in the bottle. After vitamin solution was added, the electron donor and any additional supplements were injected (Time = 0). Except where noted, FYE was not added during the time-intensive studies for the comparison of electron donors to allow more accurate determination of the fate of reduction equivalents provided by donor alone. Bottles were incubated in a slanted, inverted position in a rotary-shaker water bath (Gyrotary Water Bath Shaker Model G76D, New Brunswick Scientific Co, Inc.) at 165 rpm and 35°C.

During short-term, time-intensive tests, numerous gas samples (0.1- or 0.5-mL) for chloroethenes, ETH, CH₄ and H₂ analysis were withdrawn via a gas-tight, locking syringe (Dynatech Precision Sampling Corp.); and six 0.5-mL samples or eight 0.25-mL liquid samples for donor and VFA analyses were withdrawn via a 1-mL luerlock syringe (Hamilton Company). Study durations were from approximately 10 to 48 hr.

3.C. Analytical Methods

3.C.1. Reagents and Solutions

Butyric acid, (Aldrich Chemical Co., 99%), ethanol (campus supplier, 95% by volume), methanol (HPLC grade, 99.9%, Fisher Scientific Co.) lactic acid (Fisher Scientific Co., 87.6%), propionic acid (Eastman Kodak Co., 99%), and PCE (Eastman Kodak Co., 99.9%) were routinely used as direct culture amendments and for preparation of analytical standards. Glacial acetic acid (Mallinckrodt, Inc., 99.5 to 100.5%), isobutyric acid (Fisher Scientific Co., 99%), isovaleric acid (Aldrich Chemical Co., 99%), and hexanoic acid (Aldrich Chemical Co., 99.5%) were used for preparation of analytical standards and, during one experiment for preparation of a surrogate fermented yeast extract. TCE (Fisher Scientific Co., 99%), DCE isomers—*cis*-1,2, *trans*-1,2, and 1,1—obtained in neat form in ampules (Supelco, Inc.), VC (Matheson Gas Products, 99%), ETH (Matheson Gas Products), CH₄ (Scott Specialty Gases), H₂ (Airco, 1% in N₂ and Matheson Gas Products, ultra high purity), were used for preparation of analytical standards.

H₂SO₄ (Fisher Scientific CO., 95.9%) and H₃PO₄ (Mallinckrodt, Inc., 95.9%) were used to prepare 6 N and 8 N solutions, respectively, for preservation of liquid samples. Acidification of samples was at a rate of 10 µL per prefiltered 0.5-mL sample.

3.C.1.a. Basal salts medium. Cultures were grown in a basal salts medium which has been used to develop and work with this culture. The solution was adapted by Freedman [79] from one described by Zeikus [277] for methanogens. The composition of the medium is shown in Table 3.5. The solution was prepared in 15-L batches and was stored at 35°C under a

pressurized anoxic atmosphere as previously described to prevent the infiltration of air.

3.C.1.b. PCE-saturated basal medium. During time-intensive studies, PCE was added to serum bottles in a pre-dissolved form to prevent the dissolution of the PCE from affecting the rate of PCE degradation. PCE-saturated basal medium was prepared in serum bottles by anoxically delivering 100 mL of basal medium to a 160-mL serum bottle and adding 50 μ L of neat PCE via syringe to the bottle. The bottle was agitated on a wrist-action shaker (Burrell Inc., Wrist Action Shaker, Model 75) for 3 days at 35°C then allowed to rest at a slant—so that the droplets of undissolved PCE would settle to the side of the bottle, but not on the septum—for at least one day prior to use. The basal medium contained approximately 200 mg PCE/L. A 9- or 9.5-mL volume of this basal medium combined with 1- or 0.5-mL of regular basal medium was used during the basal medium exchange at the initiation of time-intensive studies to deliver approximately 11 μ mol pre-dissolved PCE to each serum bottle.

3.C.1.c. Yeast extract solution. Yeast extract served as a trace nutrient source for the high-PCE/methanol culture. To 100 mL of distilled water, 5 g of yeast extract powder (Difco Laboratories) was added. The solution was purged for 30 min with anoxic gas, and was then capped with a gray-butyl septum and aluminum crimp cap. The solution was stored refrigerated. Prior to removing yeast extract solution from the bottle, the same volume of anoxic gas was delivered to prevent a vacuum from forming in the bottle.

Table 3.5. Basal salts medium.

Compound	Quantity (per L distilled water)
NH ₄ Cl	0.2 g
K ₂ HPO ₄ •3H ₂ O	0.1 g
KH ₂ PO ₄	0.055 g
MgCl ₂ •6H ₂ O	0.2 g
Resazurin	0.001 g
Trace Metal Solution [†]	10 mL
FeCl ₂ •4H ₂ O	0.1 g
Na ₂ S•9H ₂ O	0.5 g
NaHCO ₃	6.0 g

The first seven items were added, and an N₂ purge was maintained until the solution changed from blue to pink, the purge was changed to a 70%N₂/30% CO₂ purge and then the two remaining items were added quickly. The solution was black and changed to pink if oxygen was present.

[†] Trace Metal Solution contained: 0.1 g/L MnCl₂•4H₂O; 0.17 g/L CoCl₂•6H₂O; 0.10 g/L ZnCl₂; 0.251 g/L CaCl₂•2H₂O; 0.019 g/L H₃BO₃; 0.05 g/L NiCl₂•6 H₂O; 0.02 g/L Na₂MoO₄•2H₂O. Adjusted to pH 7 with 8 N NaOH.

3.C.1.d. Pre-fermented yeast extract (FYE) solution. For low-PCE/butyric acid source cultures and all serum bottle experiments, the yeast extract was allowed to ferment prior to use to reduce the amount of contributed electron donor. This allowed for a more accurate accounting of reducing equivalents added from the primary electron donor. FYE was

prepared as for yeast extract solution except that the yeast extract was mixed into 90 mL of distilled water. After purging and crimping the cap and septum onto the bottle, 10 mL of the high-PCE/methanol culture was added via syringe and the solution was mixed by shaking. This mixture was allowed to ferment quiescently for 10 days prior to use. Excess gas was vented from the bottle daily through a needle inserted through the septum. During the fermentation period and throughout use, the solution was stored inverted and quiescently at 35°C. FYE was added to cultures at a rate of 20 μ L (for 1:1 donor to PCE ratios) or 40 μ L (for 2:1 donor to PCE ratios) per 100 mL culture at each feeding. Some samples of the FYE were examined for volatile fatty acid (VFA) content (see Section 5.K).

The concentration of reducing equivalents available in FYE was determined from short-term time-intensive tests which are described in detail in Appendix II. Cultures fed FYE and PCE were monitored to determine the total amount of equivalents (from FYE and endogenous decay) channeled to dechlorination and methanogenesis during 2 days of operation. Other culture bottles, fed PCE only to determine the amount of equivalents from endogenous decay alone, were also maintained. FYE addition was estimated to result in the formation of about 31 μ eq of reduction products per 40 μ L per 2 days at 35°C. In the FYE batches analyzed, roughly 60 percent of the reducing equivalents in FYE were accounted for by the contributions from measured concentrations of the VFAs: propionic, butyric, isobutyric, isovaleric, and hexanoic acids.

3.C.1.e. Surrogate FYE (SFYE). SFYE—used in some experiments to replace the electron-donating capacity of FYE—was prepared by adding neat reagent-grade, individual VFAs to 100 mL of anoxically purged distilled

water to form an aqueous solution of the VFAs at their measured, FYE concentrations. However, since a portion of the reducing equivalents determined to be available in FYE were unaccounted-for by VFA analysis, this difference was made up by employing additional butyric acid. The final VFA content of SFYE is shown in Table 3.6. SFYE, where employed, was also added at a rate of 40 μL per 100 mL culture for a 2:1 donor to PCE ratio and provided approximately 30 $\mu\text{eq}/40 \mu\text{L}$.

Table 3.6. Surrogate FYE composition.

Volatile Fatty Acid	Concentration in SFYE (mM)
Acetic acid	94
Propionic acid	18
Butyric acid	125
Isobutyric acid	6.6
Isovaleric acid	10.6
Hexanoic acid	7.5

3.C.1.f. Vitamin solution. An anoxic, aqueous vitamin solution described in Table 3.7 was added to cultures excepted where noted. The solution was prepared with crystalline or powdered forms of the vitamins (all 99.9%, Sigma Chemical Co.), and purged with anoxic gas as in the preparation of FYE described earlier. The solution was refrigerated inverted for storage.

3.C.1.g. Titanium chloride scrubbing solution. Anoxic gas consisting of a mixture of 70% N_2 /30% CO_2 used to purge cultures and prepare stock solutions was continuously bubbled through a reducing solution prior to

use to remove trace amounts of oxygen. The solution was prepared by adding 10 mL of 20% titanous chloride solution (Fisher Scientific Co.), 12.5 g sodium bicarbonate (Fisher Scientific Co.), and 4.412 g of citric acid, trisodium salt dihydrate (99%, Aldrich Chemical Co., Inc.) to 1 L of distilled water. The titanium (III) citrate complex forms a violet/blue solution which loses its color upon oxidation [275].

Table 3.7. Vitamin solution^a for amendment of cultures.

Constituent	Quantity (mg/L)
d-biotin	20
folic acid	20
pyridoxine hydrochloride	100
thiamin hydrochloride	50
riboflavin	50
nicotinic acid	50
DL-calcium pantothenate	50
vitamin B ₁₂ (cyanocobalamin)	10
p-aminobenzoic acid	50
lipoic acid	50

^a Previously unamended cultures were amended with 0.05 mL vitamin solution per 10 mL of culture and thereafter 0.05 mL vitamin solution per 10 mL fresh basal medium was added.

3.C.2. Chlorinated Ethenes, CH₄, and H₂ Analyses

Analysis of PCE, TCE, DCEs, VC, ETH, CH₄ and H₂ was performed with two Perkin-Elmer Corporation model 8500 gas chromatographs which were equipped with flame ionization detectors (FID) and thermal conductivity detectors (TCD); and a stand-alone Trace Analytical Corp.

reduction gas detector (RGD). A diagram of the chromatography system is shown in Figure 3.2. A single 0.1- or 0.5-mL headspace sample removed from the reactor or serum bottle headspace via a gas-tight, locking syringe (Dynatech Precision Sampling Corp.) was injected into the system.

Two columns were used to separate components and two air-actuated four-port switching valves (Valco Inc.) were used to direct the carrier gas streams and the components to be detected to one of the three different detector types. The first column in series was a 1/8-inch diameter, 8-ft stainless-steel column packed with 1% SP-1000 on 60/80 Carbowpack-B (Supelco, Inc.). The second column was a 1/8-inch diameter, 10-ft stainless-steel column packed with 100/120 Carbowieve-G (Supelco, Inc.). Both columns were contained in the oven of GC #1 and were subjected to the same temperature program. N₂ gas (ultra high purity, 99.998%, Matheson Gas Co.), at 30 to 35 mL/min was the carrier flow. Prior to passing into the GC system, the carrier was first passed through a catalytic combustion filter (Trace Analytical Corp.) to remove the RGD contaminants CO and H₂; and through a molecular sieve (Supelco, Inc.) to remove water and hydrocarbons.

The FIDs were maintained with H₂ and air. The TCD was maintained with N₂ carrier and reference gas flows at 30 to 35 mL/min. The outputs from these detectors were integrated by their respective GC integration systems and the results from each were printed on Perkin-Elmer GP-100 printers.

The RGD consists of a bed of mercuric oxide (HgO) maintained at approximately 280°C. As H₂ passes over it, mercury vapor is produced in proportion to the amount of H₂ present according to Equation 3.1:

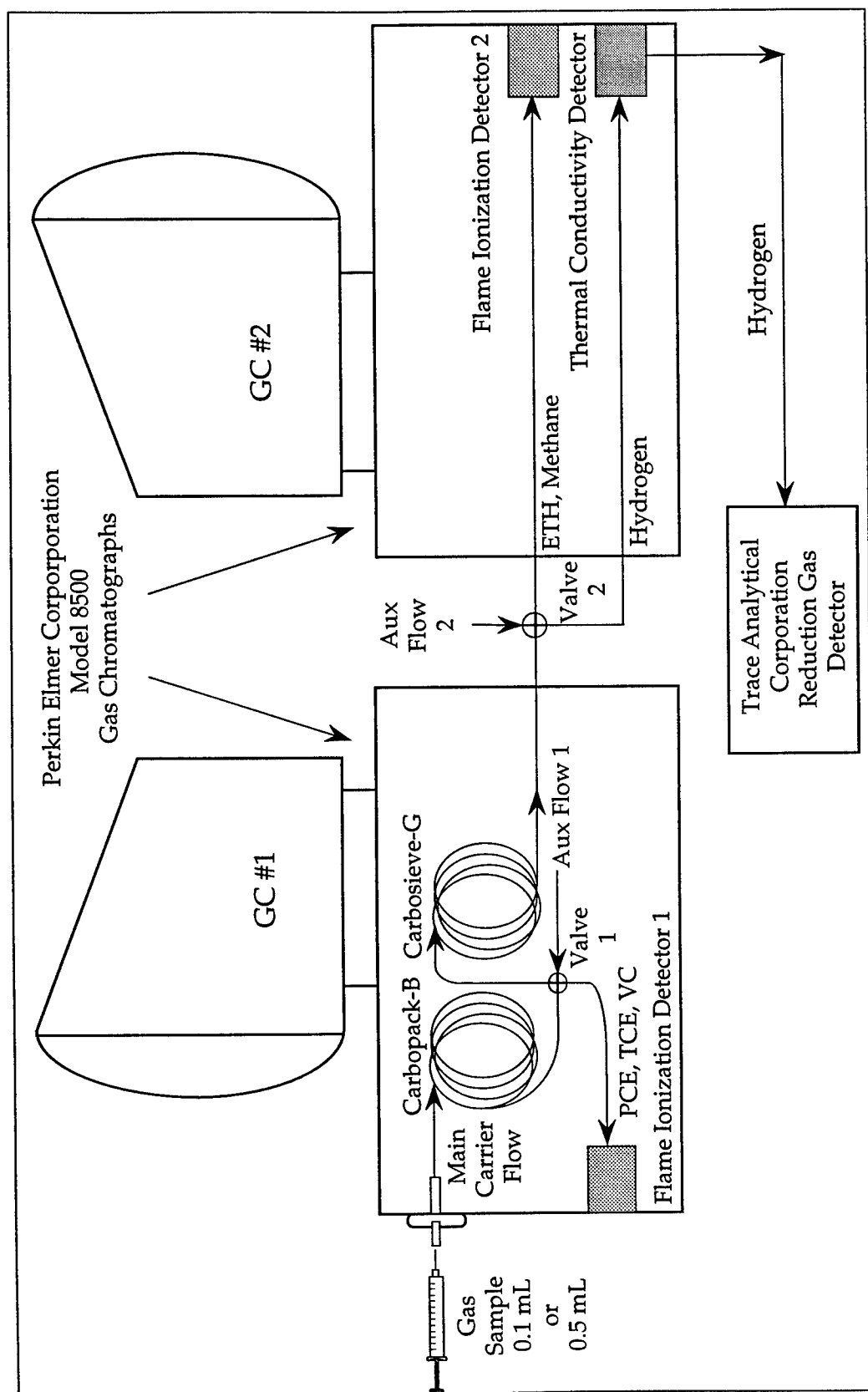
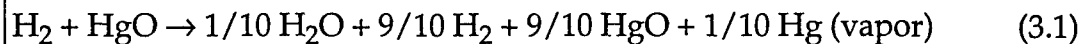


Figure 3.2. Gas chromatography system for headspace analysis of volatile compounds.



The gas stream then passes to a 200- μL optical cell with a split-beam 254-nm ultraviolet photometer. A signal detector monitors changes in light absorption in the optical cell due to changes in mercury vapor concentration. The output from this detector was integrated by a Perkin-Elmer LCI-100 integrator.

When a sample was injected, the GC system was activated and relays programmed to actuate the switching valves at specific times controlled to which detector the separated compounds were directed. The oven temperature was maintained at 90°C for the first 2.8 min and was then ramped to 200°C at 30°C per min. The temperature was held at 200°C for an additional 9.1 min. The injector temperature was 200°C and the detector temperature was 250°C. The main carrier gas flow was directed through the two columns to the TCD in GC #2 and the RGD for the first 1.38 min, while H_2 passed rapidly through the columns and entered the TCD and the RGD, in that order.

Early in the experimentation, measurements of higher H_2 levels (greater than about 2 μmol per bottle) were via a separate 0.5-mL injection on GC #2 to a 3.2 mm \times 3.05 m stainless-steel column packed with 100/120 Carboseive S-II (Supelco, Inc.) and connected to the TCD. The column was held isothermally at 150°C. Later in the study, the TCD was placed in the carrier flow line prior to the RGD, and during periods when H_2 was above the detection range of the RGD, all measurements were obtained with a single 0.5-mL injection, while when H_2 levels were low—i.e. within the detection sensitivity of the RGD—a single 0.1-mL injection was used.

After 1.38 minutes, Valve 2 switched positions and the main carrier gas flow was then connected to FID 2 and auxiliary flow 2 was connected to the RGD. CH₄ and ETH passed relatively quickly through the Carbopack column and entered the Carbosieve where they were separated and detected by FID 2. After 1.4 minutes Valve 1 changed positions and the main carrier gas flow passed through the Carbopack column which separated the chlorinated ethenes PCE, TCE, and VC. The DCE isomers came out together on this column. PCE, TCE, DCEs, and VC were eluted from the Carbopack column to FID 1. Auxiliary flow 1 flowed through the Carbosieve column and continued to elute CH₄ and VC to FID 2. Over the time period of this study, flow rates and programming times changed somewhat, however, typical retention times of the compounds are shown in Table 3.8.

Table 3.8. Retention times for compounds from single-injection gas chromatography analysis.

Compound	Retention Time (min)
PCE	14.5
TCE	8.9
DCE (all isomers)	6.0
VC	2.3
ETH	8.4
CH ₄	3.1
H ₂	1.1

When necessary *cis*-1,2-DCE and *trans*-1,2-DCE were separated by a separate 0.1-mL injection on GC #1 to a 1/8-inch diameter, 8-ft stainless-steel column packed with 20% SP-2100 on 80/100 Supelcoport (Supleco, Inc.) which was connected to an FID. The column was maintained isothermally at 60°C with a 35 mL/min N₂ carrier flow. Retention times for the DCE isomers on this column were 2.16 min for *trans* and 2.77 min for *cis*.

Calibration factors for PCE, TCE, DCEs, VC, ETH, and CH₄, were determined every one or two months depending upon the experiments in progress. A methanol/PCE/TCE/DCE stock was prepared by adding approximately 100 µL of neat PCE, TCE, and when needed DCE isomers, via a gas-tight, locking syringe (Dynatech Precision Sampling Corp.) to a known mass of methanol (approximately 10 mL) in a 14-mL serum vial sealed with a Teflon[®]-backed septum. The masses of PCE, TCE, and DCEs present in the stock were determined gravimetrically by weighing the vial after each addition. 100 µL of the methanol/PCE/TCE/DCE stock was delivered to each of four duplicate 160-mL serum bottles which contained 100 mL of distilled water and were sealed with crimp caps and Teflon[®]-backed septa. The mass of stock delivered was determined from the difference in the weight of the full syringe and the weight of the empty syringe after delivery to the standard bottle. 500 µL each of gaseous VC, ETH, and CH₄ was added via gastight syringe to the four standard bottles. The temperature and barometric pressure were noted at the time of the transfer and the mass of each gas added was determined from the ideal gas law.

The standard bottles were allowed to equilibrate at 35°C in an orbital shaking water bath (Gyrotary Water Bath Shaker Model G76D, New Brunswick Scientific Co, Inc.) at 165 rpm for at least 3 hr and then were analyzed (either with a 0.1- or 0.5-mL injection volume) on the GC system. The calibration factor for each component (μmol component per peak area of output) was calculated as a mean of the factors obtained from the four standard bottles. The calibration factors for 0.1-mL injections had a coefficient of variation ($100 \times \text{standard deviation}/\text{mean}$) of 0.1 to 5 percent while those for the 0.5-mL injections were 0.3 to 2.9 percent.

H_2 standards were prepared and run every one to two months (during long-term testing), and/or within one week of the running of each time-intensive study, since it was important during these tests to have more accurate measurement of H_2 levels. Since the sensitivity of the RGD changed somewhat over time, the upper limit of H_2 detection (before the detector became saturated) was from 1 to 2 μmol H_2 per bottle. The lower limit was about 5 nmol per bottle with a 0.1-mL injection (approximately 1 ppb). The TCD could detect a minimum of approximately 0.25 μmol per bottle for a 0.5-mL injection and about 2 μmol per bottle for a 0.1-mL injection. The TCD response was linear and a single linear fit sufficed. The RGD response was nonlinear and thus standard curves were plotted as either two different linear portions or the entire curve was fitted with a second-order curve. Standards of 0 to 8 μmol per 100 mL of distilled water were prepared by adding known volumes of H_2 (1% in N_2 or pure) to 160-mL serum bottles containing 100 mL of distilled water and sealed with crimp caps and Teflon®-backed septa. To avoid interference from atmospheric H_2 in the lower level bottles to be analyzed on the RGD, the

distilled water was purged for 5 min with a 35 mL/min high-purity, catalytically filtered N₂ prior to use. The temperature and barometric pressure were noted at the time of the gas addition and the mass of H₂ delivered to each bottle was calculated from the ideal gas law. Bottles were incubated inverted and at a slant and agitated in an orbital shaker bath at 35°C and 165 rpm for 1 hr prior to analysis.

3.C.3. Volatile Acids Analysis

A Perkin-Elmer Corporation Autosystem gas chromatograph with a 0.53-mm x 15-m Nukol[®] capillary column (Supelco, Inc.) and an FID was used for analysis of the VFAs acetic, propionic, isobutyric, butyric, valeric, isovaleric, and hexanoic acids [206]. The N₂ carrier gas flow rate was at 90 PSI and 10 mL/min, the injector temperature was 200°C and the detector temperature was 250°C. The flame was maintained with H₂ (30 PSI, 45 mL/min) and air (30 PSI, 450 mL/min).

For VFA analysis including only acetic, propionic, isobutyric, and butyric acids, a 0.5-μL sample was injected onto the column which was held at 90°C for 9 min. The retention times for this run were: acetic acid, 2.9 min; propionic acid, 4.5 min; isobutyric acid, 5 min; and butyric acid, 7 min.

For VFA analysis that also included valeric, isovaleric, and hexanoic acids, a 0.5-μL sample was injected onto the column which was held at 90°C for 8 min, then ramped at 25°C/min to 110°C and held for an additional 3 min. The retention times for this run were: acetic acid, 2.9 min; propionic acid, 4.5 min; isobutyric acid, 5 min; butyric acid, 7 min; isovaleric acid, 8 min; valeric acid, 9 min and hexanoic acid, 11 min.

Detector output was integrated by a PE Nelson model 1022 integration system. Output was printed on an Okidata Microline 320 printer.

A glass injector liner was used in the injector and the Nukol® column was connected to a deactivated 5-m guard column at its ends between the column and injector and column and detector. It was important to change the septum and replace the liner with a clean liner every 50 to 60 VFA injections to rid the system of accumulated buildup of contaminants. It was hypothesized that portions of the accumulated contaminants deposited on the liner and in the precolumn were subsequently "steamed" off with each new injection. Frequent liner changeout reduced this problem. A loop of the guard column was removed periodically for the same reason. Initially, a ramped temperature program with an ultimate final temperature of 150°C was investigated for VFA analysis, but it was observed that increasing the column temperature to higher values at the end of a run resulted in elution of heavier, accumulated contaminants and an unstable baseline that required lengthy stabilization time. The time required for restabilization of the baseline was greater than the time saved from operation at elevated temperature. Isothermal operation with frequent cleaning procedures was superior.

Samples of 0.25 or 0.5 mL were removed from reactors or serum bottles via a 1-mL luerlock syringe (Hamilton Company) and were immediately filtered through a 0.2- or 0.45- μ m PTFE filter (Gelman Sciences) into a 2-mL vial. The samples were acidified by the addition of 8 N H_3PO_4 (10 μ L per 0.5 mL of sample) to obtain a pH of between 1 and 2, and refrigerated until analysis. Identification of the volatile acids was

through comparison of retention times with those of known standards. Volatile acids stock solutions were prepared by adding known gravimetric amounts of neat acids to 1 L of distilled water. Standards were prepared by adding volumes of the stock solutions to a 100-mL volumetric flask. The flask was filled to just below the 100-mL mark with distilled water. The pH of the solution was adjusted to between 1 and 2 by the addition of 8 N H_3PO_4 and the volumetric flask was then filled to the 100-mL mark with distilled water.

3.C.4. Ethanol Analysis

The Perkin-Elmer Corporation Autosystem gas chromatograph with a 0.53-mm x 15-m Nukol[®] capillary column (Supelco, Inc.) and an FID, described above, was used for analysis of ethanol. A 3- μL sample was injected onto the column which was held isothermally at 70°C for 1 min. The same baseline deterioration from accumulated contaminants as was described with VFA analysis was observed with ethanol—and in fact was even more severe. A clean glass liner was inserted every 15 to 20 injections.

An ethanol stock solution was prepared by adding a known gravimetric amount of 95% ethanol to a 500-mL flask half-filled with distilled water. The flask was filled to the 500-mL mark and mixed well. Standards were prepared by adding known volumes of the stock solution to a 100-mL volumetric flask. The flask was filled to just below the 100-mL mark with distilled water. The pH of the solution was adjusted to between 1 and 2 by the addition of 8 N H_3PO_4 and the volumetric flask was then

filled to the 100-mL mark with distilled water. Samples were prepared as for volatile acids analysis.

3.C.5. Lactic Acid and Volatile Fatty Acids Analysis by HPLC

Lactic acid (and the VFA content of time-intensive-studies samples from lactic-acid-fed cultures) was determined with a Hewlett Packard 1090 high performance liquid chromatograph (HPLC) with either a 300-mm x 7.8-mm HPX-87H ion-exclusion column operated at 65°C, or a 100-mm by 7.8-mm Fast-Acid column operated at ambient temperature (Bio-Rad Laboratories), and a diode-array detector at 210 nm. The mobile phase was 0.013 N H₂SO₄, at 0.65 mL/min for the HPX-87H column and 0.7 mL/min for the Fast-Acid column. Samples of 0.5 mL were removed from serum bottles via a 1-mL luerlock syringe. They were immediately filtered through a 0.2- or 0.45-μm PTFE filter (Gelman Sciences) into an HPLC vial, preserved with 10 μL of 6 N H₂SO₄, and capped with a septum and crimp cap and refrigerated. The HPLC injection volume was either 60 or 100 μL. Standards were prepared by adding neat acids gravimetrically to HPLC grade water (Fisher Scientific Co.) and acidifying in the same manner as the prepared samples. During this study many problems were experienced in attempting to resolve lactic acid with the HPLC. It was finally determined that the standard reagent (lactic acid, Fisher Scientific Co., 87.6%) contained an unknown, recalcitrant contaminant that was eluted just prior to the lactic acid peak. This peak became more prominent with respect to lactic acid as the lactic acid was degraded and its peak area became small. These two peaks then began to overlap each other to a fused peak. For many samples, these could not be resolved. For this reason, some of the lactic

acid analyses were determined by subtracting the constant peak area of the unknown peak from the total peak area.

3.C.6. Solids Analysis

Solids analyses were performed according to Standard Methods 209D. Sample volumes of 100 mL were filtered through GF/F glass fiber filters (Whatman International Ltd.).

3.C.7. Particulate Organic Nitrogen Analysis

Traditional VSS analysis was used as an estimate of biomass early in the study. However, the VSS content of the basal salts medium alone was determined to be approximately 30 mg/L. This interference exerted a significant influence on the VSS test. For most of the study, therefore, as a better method, biomass was estimated from the particulate organic nitrogen (PON) content of samples. A microbial cell composition of $C_5H_7O_2N$ was assumed [153]. A 100-mL volume of enrichment culture sample or a basal medium blank was filtered through a SUPOR-200, 0.2- μ m filter (Gelman Sciences). After the sample had just passed through the filter, 50 mL of phosphate buffer was filtered through to remove free ammonia from the solids. The filters with the captured and rinsed solids were placed in glass vials and frozen until analysis. Total Kjeldahl nitrogen (TKN) analysis was performed on the prepared filters according to Standard Methods (421). Occasionally, a glycine standard containing 2 mg organic N was carried through the TKN analysis to check the procedure. These standards were measured to be within 1 to 2 percent of the expected organic nitrogen content. The following calculations were performed to convert from nitrogen content to biomass (volatile suspended solids, VSS):

mg organic-N=

$$\begin{aligned} & [\text{mL titrant for culture} - \text{mL titrant for basal medium}] \times \\ & [0.28 \text{ mg organic-N/mL titrant}] \end{aligned}$$

mg biomass ($\text{C}_5\text{H}_7\text{O}_2\text{N}$)/L =

$$\begin{aligned} & [\text{mg organic-N/ L sample}] \times \\ & [\text{mg biomass } (\text{C}_5\text{H}_7\text{O}_2\text{N})/0.125 \text{ mg organic-N}] \end{aligned}$$

CHAPTER FOUR

EXPERIMENTAL RESULTS

4.A. Comparison of the Electron Donors Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid

Eighteen serum bottles were prepared using a 20 percent dilution of the high-PCE/methanol source culture. The dilution was prepared in the glovebox as described in Section 3.B.1. After preparation, six bottles were immediately sacrificed for particulate organic nitrogen (PON) analysis, and the remaining 12 bottles were anoxically purged to rid them of the glovebox atmosphere, which contained H_2 . The bottles were then fed (see Section 3.B.3) according to the 1:1 operational protocol shown in Table 4.1. Vitamin solution (0.5 mL) was added to bring the vitamin content of the previously unamended culture to the correct concentration. Every two days, in addition to the donor and PCE, each bottle was amended with FYE (20 μ L during the 1:1 ratio operational protocol and 40 μ L during the 2:1 operational protocol) every second day and vitamin solution every fourth day. All bottles were incubated at 35°C.

Initially, bottles were operated with a 1:1 ratio of electron donor to PCE on an H_2 equivalent basis. Seven of the bottles were fed only H_2 in order to develop healthy methanogenic populations (later blended with a dechlorinating mixed culture as described below). Two of these bottles were discarded after a few days when all bottles appeared to contain healthy cultures. Four bottles were operated with one of the H_2 donors and PCE to develop healthy fermenter and dechlorinator populations. One control

bottle which received only FYE and PCE (but no other electron donor) was also maintained.

Table 4.1. Protocol for long-term operation of cultures for comparison of electron donors at 1:1 and 2:1 ratios of donor to PCE

Culture Bottle Set	Electron Donor	PCE ^a (μmol)	Electron Donor ^a (μmol)	Electron Donor ^a (μeq) ^b
1:1 Ratio				
Methanogenic Cultures	H ₂	0	44	88
Dechlorinating Cultures and Dechlorinating /Methanogenic Mixtures	Butyric Acid	11	22	88
	Ethanol	11	22	88
	Lactic Acid	11	22	88
	Propionic Acid	11	14.7	88
	FYE Only	11	--	≈15 ^c
2:1 Ratio				
Dechlorinating /Methanogenic Mixtures	Butyric Acid	11	44	176
	Ethanol	11	44	176
	Lactic Acid	11	44	176
	Propionic Acid	11	29.4	176
	FYE Only	11	--	≈30 ^c

^a Indicated quantities were repetitively added every two days.

^b Assumptions for computation of equivalents (μeq/μmol): PCE, 8; H₂, 2; Butyric Acid, 4; Ethanol, 4; Lactic Acid, 4; and Propionic Acid, 6.

^c The approximate amount of reducing equivalents available from FYE was determined by observation of the amount of CH₄ and dechlorination products formed.

For the first 52 days (56 days for the butyric-acid-fed bottle) the 1:1 ratio protocol of Table 4.1 was followed. On Days 36, 40, or 52, one or more of the dechlorinating cultures was subjected to time-intensive studies (TISs) (Section 3.B.4). After Day 52 (Day 56 for the butyric-acid-fed bottle), each of the dechlorinating cultures and the FYE-PCE control bottle were (in the glovebox) combined with one of the H₂-fed methanogenic cultures,

mixed, redistributed to duplicate bottles, purged and re-capped, then continued on the 1:1 donor to PCE ratio protocol. By mixing a dechlorinating culture fed PCE with an H_2 -fed, methanogenic culture, it was ensured that any perceived advantage (i.e. low levels of methanogenesis) was a result of advantageous patterns of H_2 production, and not because of an unhealthy methanogenic population. The duplicate bottles formed from mixing the methanogenic and dechlorinating cultures were continued with the 1:1 ratio protocol until Day 80 when one of the duplicates was switched to a 2:1 ratio of electron donor to PCE as per Table 4.1. The other duplicates were maintained with the 1:1 ratio protocol. The run continued until Day 128 or Day 134 when TISs were performed on the 2:1 bottles. Afterwards, all bottles were sacrificed and PON analysis was performed on each one.

4.A.1. Results From Hydrogen-Amended Methanogenic Cultures

The H_2 -amended, methanogenic cultures that were maintained for the first 52-56 days behaved similarly and produced approximately 11 to 15 μmol of CH_4 every two days. Complete stoichiometric conversion of the added H_2 (44 μmol) should have produced 11 μmol CH_4 —the excess was produced as a result of additional contributions from FYE. A exemplary graph of an H_2 -fed methanogenic bottle is shown in Figure 4.1. The saw-tooth configuration of the graph results from the depiction of the cumulative fate of the 44 μmol H_2 added every second day. Since bottles were purged only every fourth day, every other data point depicts the CH_4 produced from the total 88 μmol of H_2 which had been added up to that time.

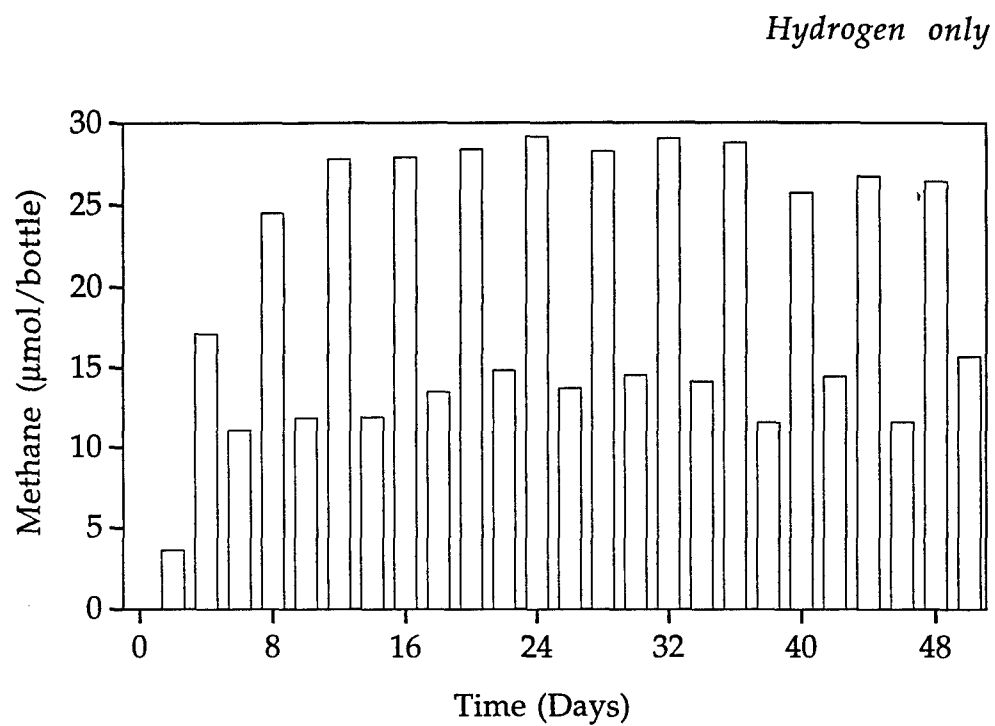


Figure 4.1. Long-term operation with hydrogen and no PCE at a 1:1 ratio. *Blended with a dechlorinating culture on Day 52.*

4.A.2. Results From Long-Term Comparison of Hydrogen Donors

Results for the entire period of operation for each particular donor and for the FYE control are presented in a series of four graphs: (a) dechlorination; (b) CH_4 ; (c) reduction products; and (d) VFAs. Graph (c), reduction products, is computed for a two-day incremental basis. For example, the results for Day 2 are plotted, then results for Day 2 are subtracted from those of Day 4 to get the incremental product formation for Days 2 to 4, and so on.

4.A.2.a. Butyric-acid-amended cultures. Long-term results for a butyric-acid-fed bottle are shown in Figure 4.2. Figure 4.2a shows dechlorination-product formation. The saw-tooth configuration of the graph results from the depiction of the cumulative fate of the 11 μmol PCE added every second day. Since bottles were purged only every fourth day, every other data point depicts the dechlorination products of the total 22 μmol of PCE that had been added up to that time.

Butyric acid served as an excellent donor over the long-term with all the PCE being readily dechlorinated to VC and ETH. The increase of the donor from a 1:1 to a 2:1 basis on Day 80 resulted in an approximate doubling of the amount of ETH formed. The CH_4 production in butyric-acid-amended cultures during the 1:1 donor to PCE ratio operational period was approximately 4 μmol per feeding and this increased to about 6 μmol after the culture was mixed with its methanogenic counterpart on Day 56 (Figure 4.2b). During 1:1 ratio operation (through Day 80), about 70 percent of the equivalents were channeled to dechlorination product formation,

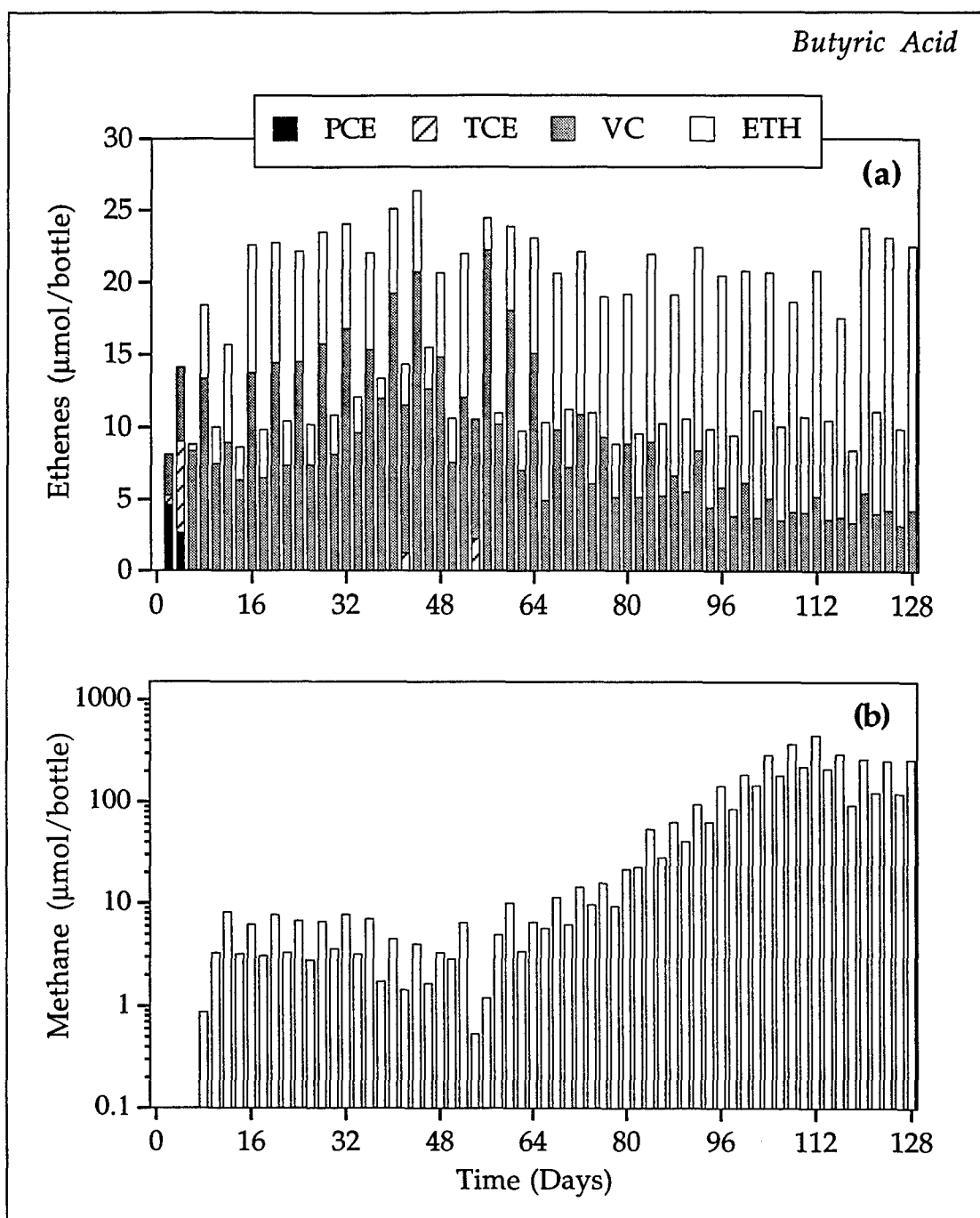
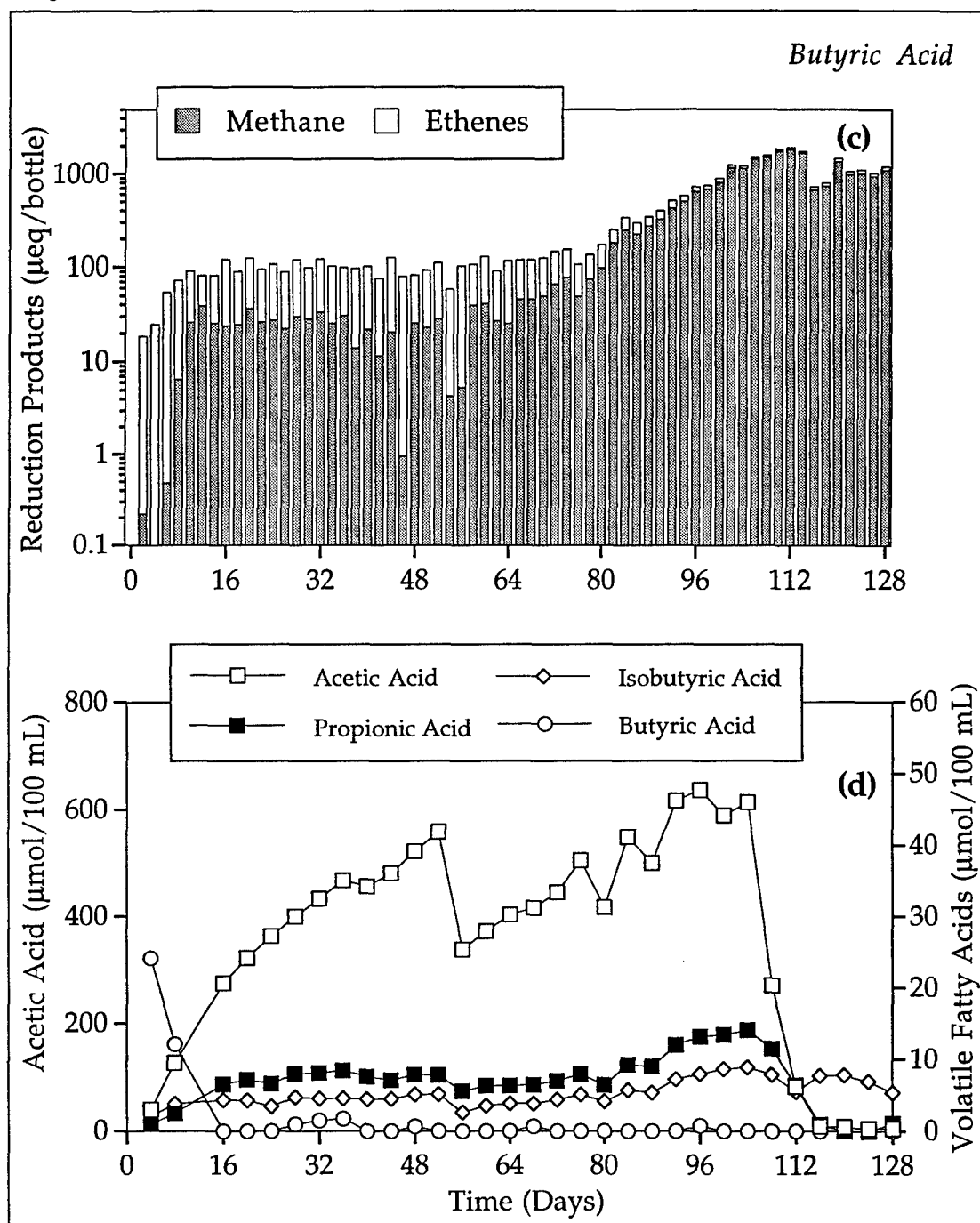


Figure 4.2. Long-term operation with butyric acid as an electron donor: (a) dechlorination; (b) methane; (c) reduction products; and (d) VFAs. 1:1 donor to PCE ratio Day 0 to Day 80; blended with methanogenic culture on Day 56; 2:1 ratio after Day 80; Time-Intensive Studies performed on Day 36, 40, 52, and 128.

Figure 4.2 (Continued)



while 30 percent were channeled to methanogenesis (Figure 4.2c). The decrease in the amount of acetate on day 56 was caused by the dilution effect of mixing the methanogenic and dechlorinating cultures (Figure 4.2d).

After Day 80, the 2:1 donor to PCE ratio protocol was initiated and dechlorination improved—more ETH and less VC was formed. CH_4 production more than doubled, then increased to much higher levels (Figure 4.2.b) as the accumulated acetate began to degrade (Figure 4.2.d) through the onset of acetotrophic activity. Propionic acid (contributed by FYE) and isobutyric acid (contributed by FYE and perhaps by isomerization of some of the added butyric acid) were present from the beginning of the test and these increased after Day 80 when the donor amendment (and FYE amendment) were doubled. The amount of isobutyric acid present was higher than that expected if the only source was FYE. Propionic acid was close to that expected from FYE contribution (see Section 4.A.2.f). After acetotrophic activity began on Day 108, propionic acid was also depleted, probably because the thermodynamics of its fermentation were improved by the removal of the acetate. Isobutyric acid persisted.

4.A.2.b. Ethanol-amended cultures. The results from an ethanol-fed culture are shown in Figure 4.3. Added PCE was dechlorinated to VC and ETH except during Day 36 to Day 64 when PCE and TCE remained (Figure 4.3a). This period of poor dechlorination was at first thought to be an indication of selective advantage to the methanogens—caused by high H_2 levels. However, the cultures eventually recovered completely and it is possible that this temporary failure was the result of the upsetting nature of TISs which were performed on Days 36 and 40. On Day 52, the culture was

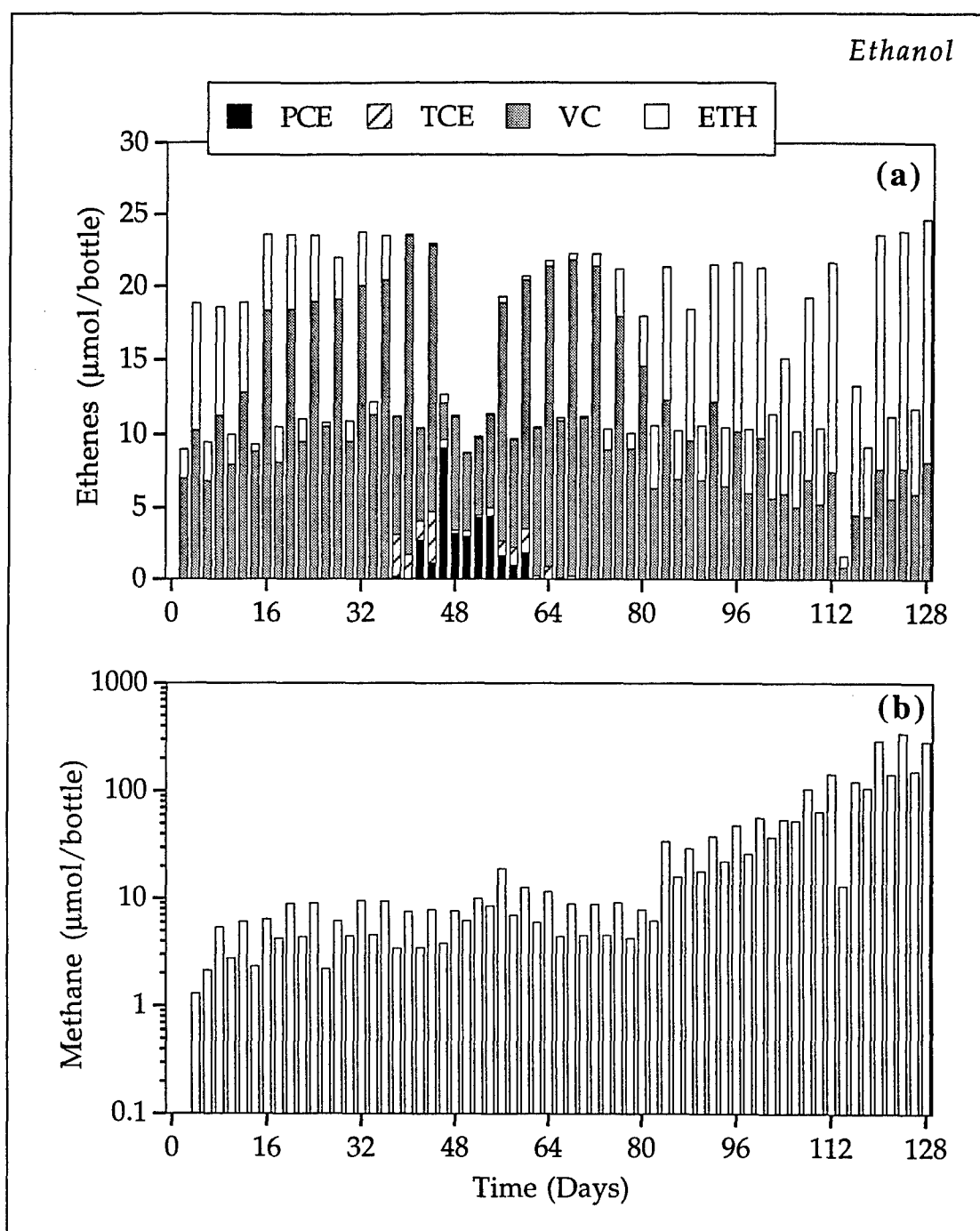
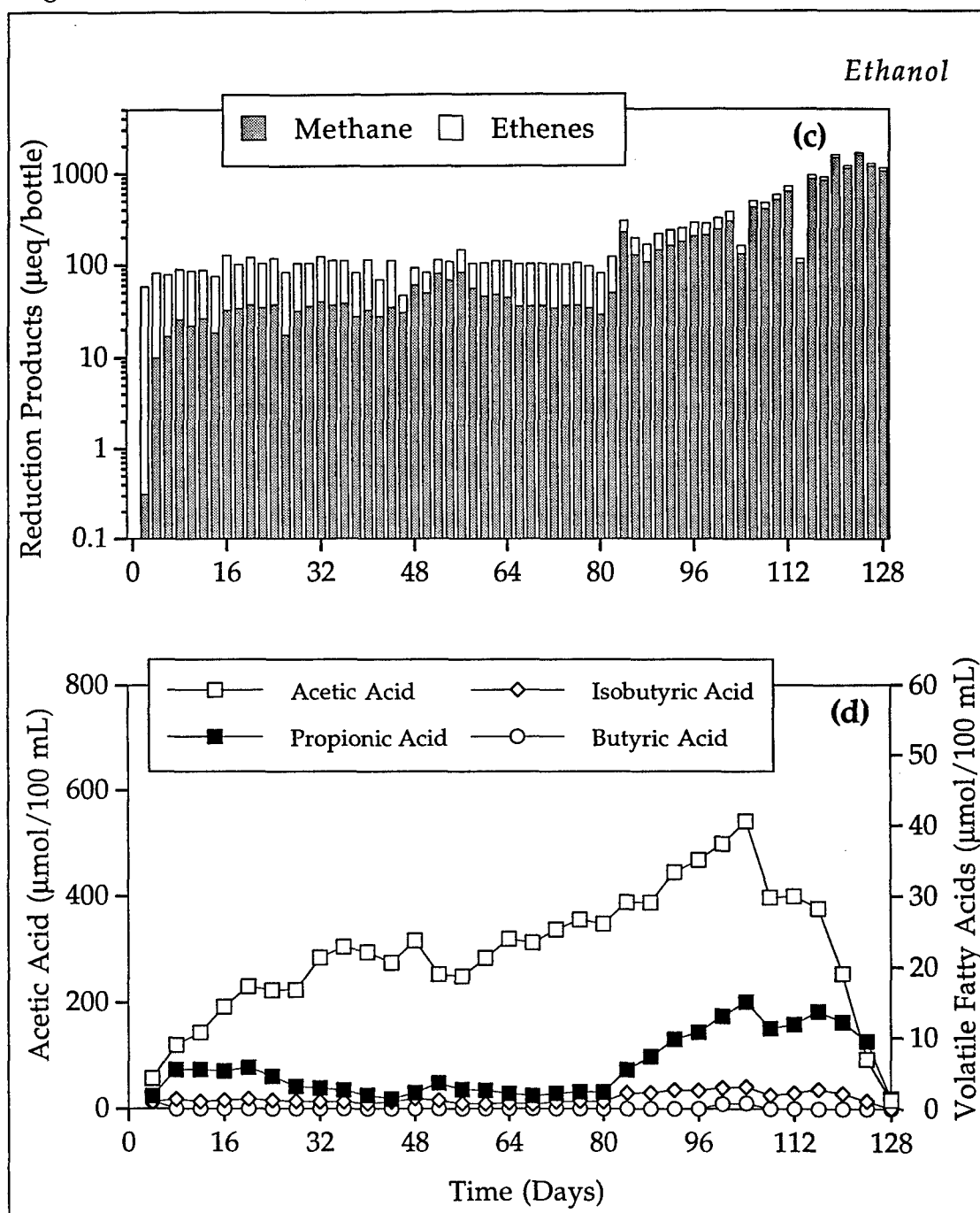


Figure 4.3. Long-term operation with ethanol as an electron donor: (a) dechlorination; (b) methane; (c) reduction products; and (d) VFAs.

1:1 donor to PCE ratio Day 0 to Day 80; blended with methanogenic culture on Day 52; 2:1 ratio after Day 80; Time-Intensive Studies performed on Day 36, 40 and 128.

Figure 4.3 (Continued)



blended with its H_2 -fed methanogenic counterpart. After blending, there was no significant increase in methanogenesis (Figure 4.3b) over that which had already been observed ($6\ \mu\text{mol}/\text{feeding}$). After Day 84, however, a significant increase in methanogenesis *was* observed and was caused by the onset of acetotrophic activity. During 1:1 ratio operation (through Day 80), about 60 percent of the equivalents were channeled to dechlorination product formation, while 40 percent were channeled to methanogenesis (Figure 4.3c). Acetic acid, which had accumulated from the degradation of ethanol, was rapidly degraded after Day 100 as acetotrophic activity became significant (Figure 4.3d). Propionic acid increased when the ethanol loading (and concurrently the FYE loading) was increased to a 2:1 donor to PCE ratio (Figure 4.3d). From the analysis of the FYE content, however, the expected propionic acid concentration from FYE (if none was fermented) would only reach about $13\ \mu\text{mol}/100\ \text{mL}$ —approximately that observed in the ethanol-fed bottle (see Section 4.A.2.f). The accumulation of propionic acid in ethanol-fed microcosms has been observed in our laboratory [209]. It is difficult to say in this case if propionate was produced upon ethanol fermentation.

4.A.2.c. Lactic-acid-amended cultures. The results from a lactic-acid-fed culture are shown in Figure 4.4. Lactic acid supported somewhat better dechlorination than ethanol (Figure 4.4a). CH_4 production developed much more slowly than in butyric-acid- or ethanol-fed cultures, but eventually reached about $4\ \mu\text{mol}$ per feeding during the 1:1 ratio period (Figure 4.4b). During the 1:1 ratio operational period, the amount of lactic acid channeled to dechlorination ranged from 100 percent at the beginning of the test, to 70 percent when methanogenesis stabilized, around Day 50

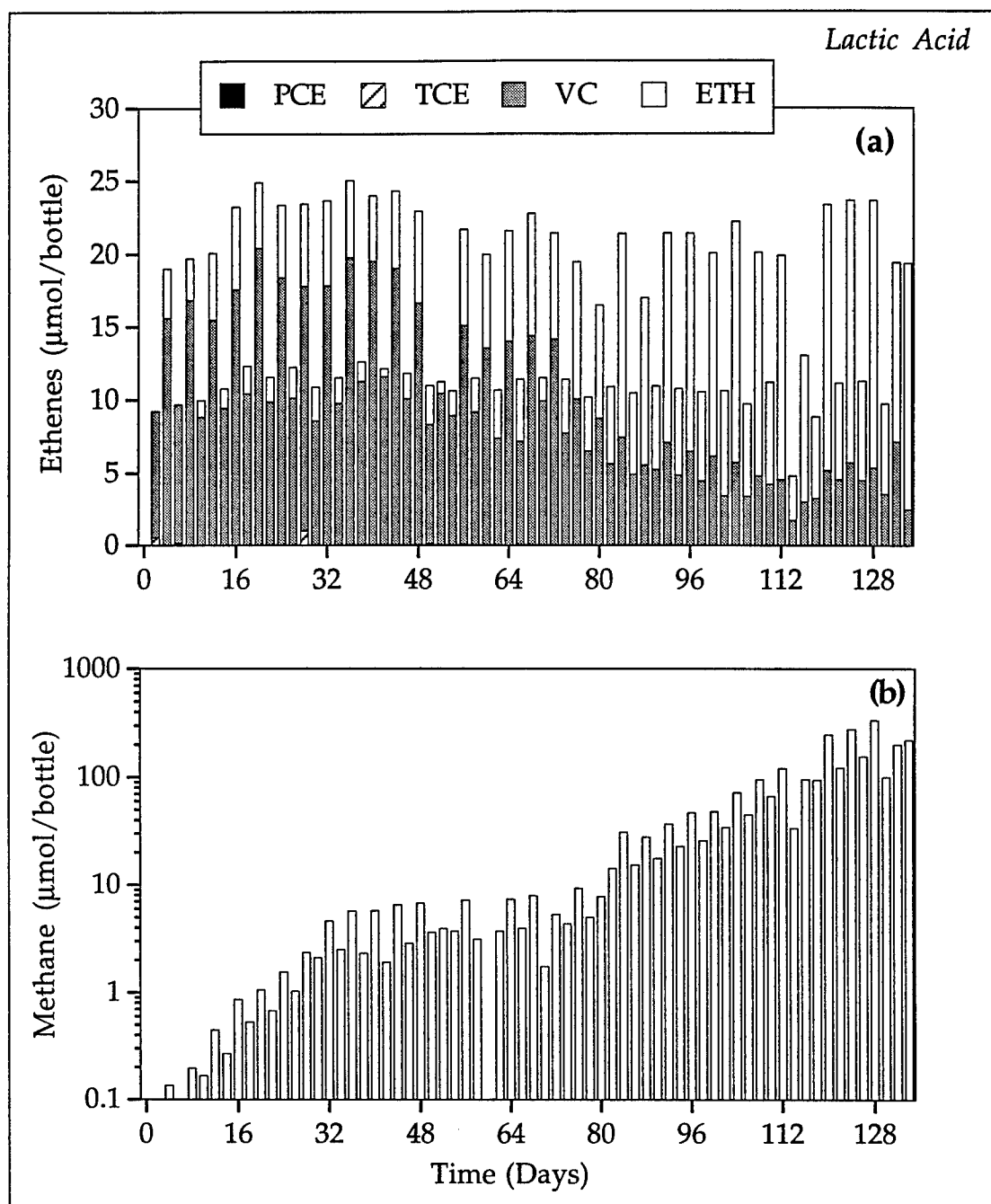
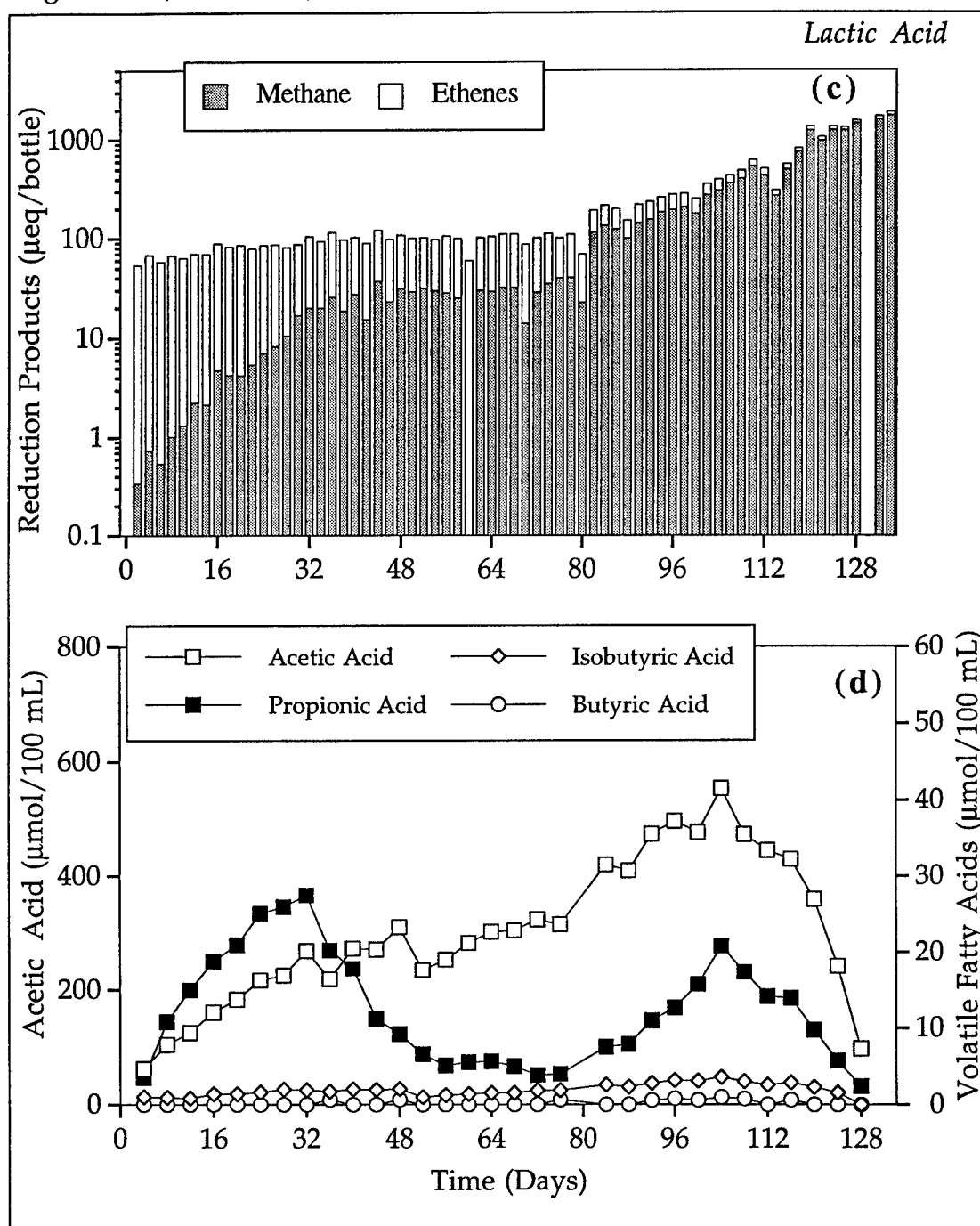


Figure 4.4. Long-term operation with lactic acid as an electron donor: (a) dechlorination; (b) methane; (c) reduction products; and (d) VFAs.
 1:1 donor to PCE ratio Day 0 to Day 80; blended with methanogenic culture on Day 52; 2:1 ratio after Day 80; Time-Intensive Studies performed on Day 36, 40, and 134.

Figure 4.4 (Continued)



(Figure 4.4c). Mixing with the methanogenic counterpart did not apparently result in any significant change in the amount of CH_4 formed. Dechlorination was excellent after the increase from a 1:1 to a 2:1 donor to PCE ratio. The VFA profile of this donor yielded interesting results (Figure 4.4d). Propionic acid accumulated in the bottle to levels significantly greater than could have been contributed by FYE (see Section 4.A.2.f) both initially ($30\ \mu\text{mol}/100\ \text{mL}$), and then again after the donor addition was increased to a 2:1 ratio beyond Day 80 ($20\ \mu\text{mol}/100\ \text{mL}$). It seems likely that a significant portion of the lactic acid was being fermented to propionate. Thus, at least at 1:1 donor to PCE ratio operation, the lactic acid-amended culture was very similar to a propionic-acid-amended culture. Fermentation of lactic acid to propionate was also observed in microcosm studies [209].

4.A.2.d. Propionic-acid-amended cultures. The results from a propionic acid-fed culture are shown in Figure 4.5. Dechlorination was comparable to other donors (Figure 4.5a). Culture development on propionic acid had a significant exclusionary effect on the development of a methanogenic population (Figure 4.5b). Prior to blending with its methanogenic counterpart, only trace amounts of CH_4 were produced. After blending, CH_4 production was still only about one-fourth that of cultures amended with ethanol. After Day 80, when propionic acid addition was increased to a 2:1 donor to PCE ratio, CH_4 production began to increase and dechlorination improved. Towards the end of the test, this CH_4 increase was probably associated with the onset of acetotrophic activity. Practically all of the added propionic acid was channeled to dechlorination prior to mixing with the methanogenic counterpart. After mixing,

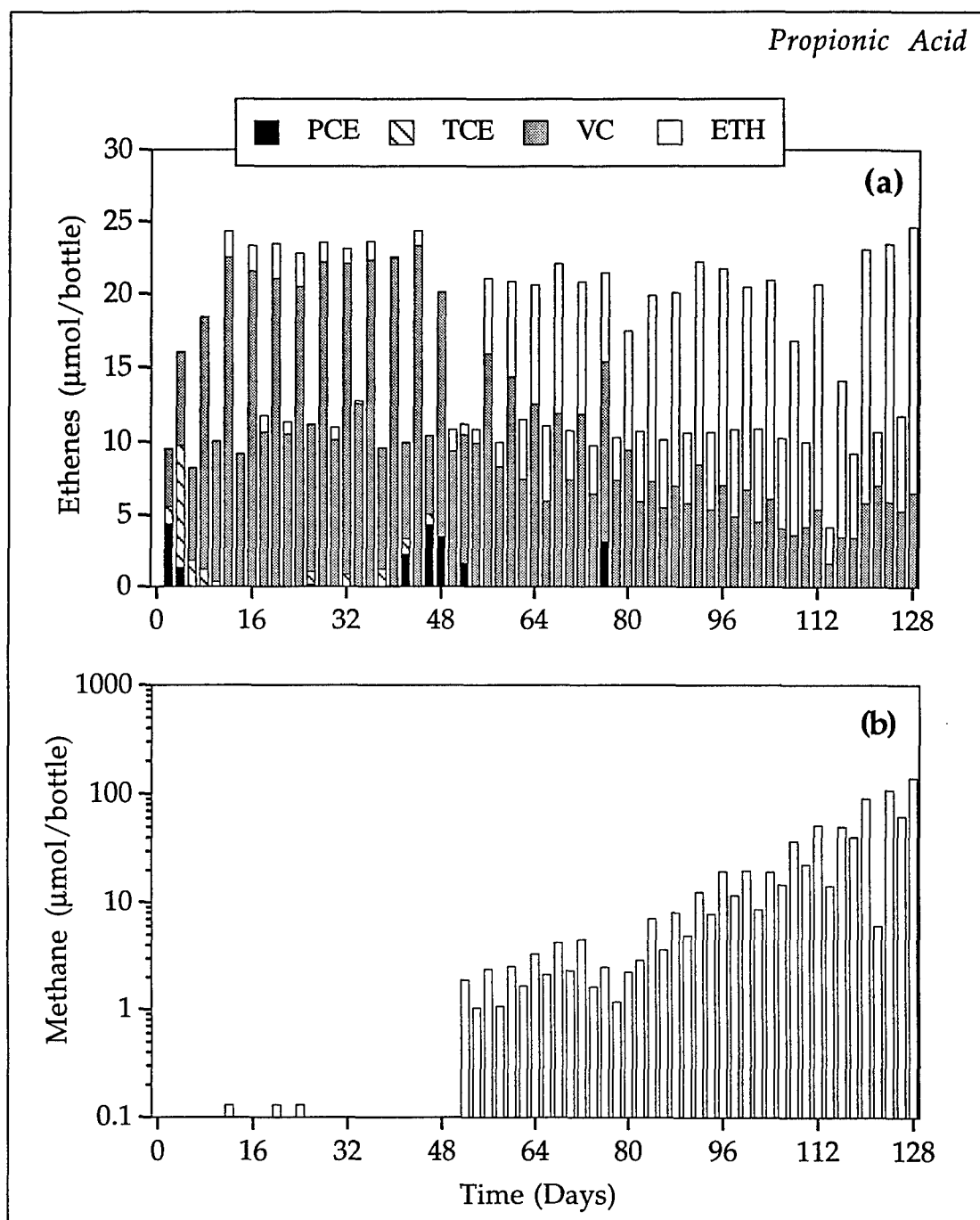
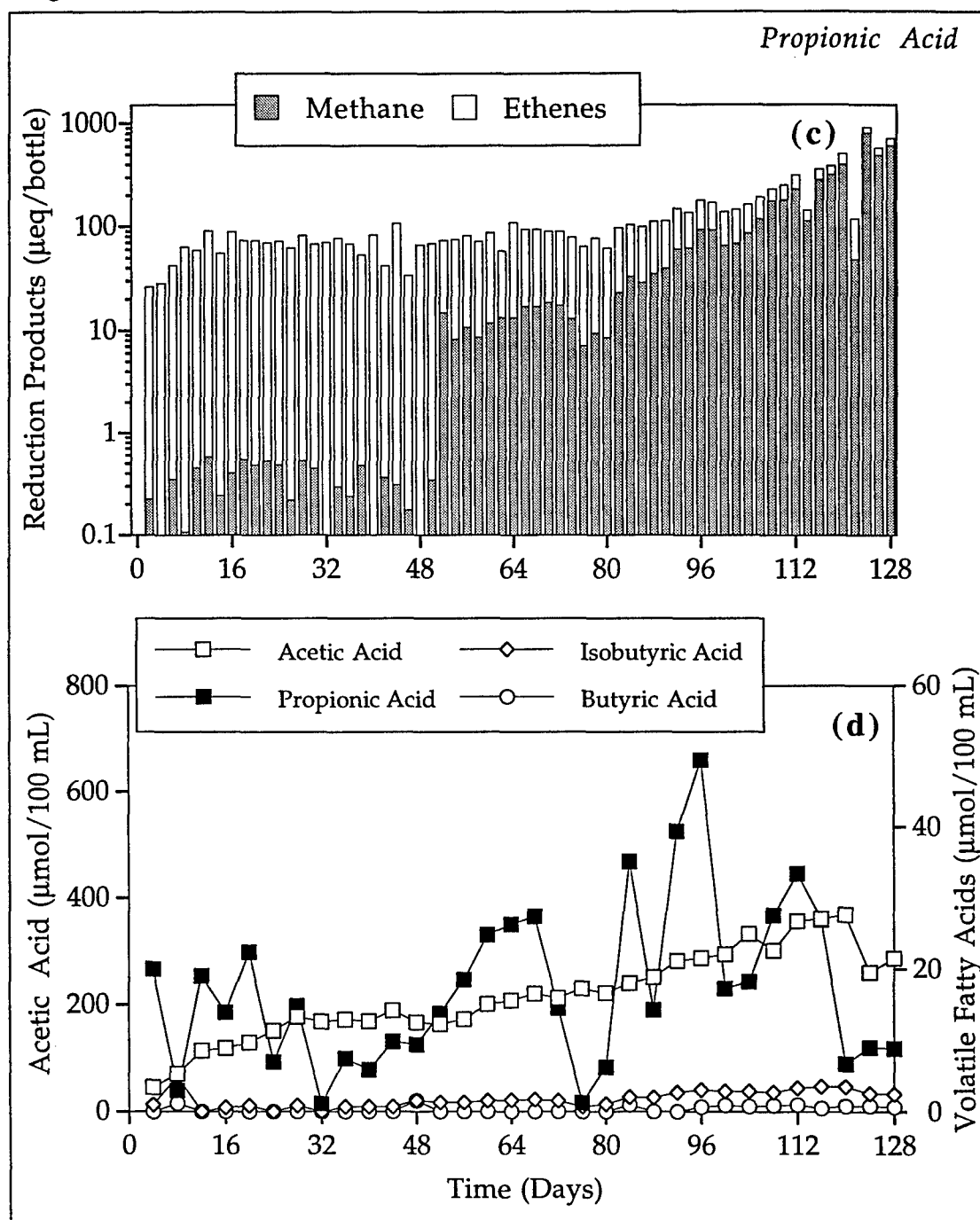


Figure 4.5. Long-term operation with propionic acid as an electron donor: (a) dechlorination; (b) methane; (c) reduction products; and (d) VFAs. 1:1 donor to PCE ratio Day 0 to Day 80; blended with methanogenic culture Day 52; 2:1 ratio after Day 80; Time-Intensive Studies performed on Day 36, 40, and 128.

Figure 4.5 (Continued)



approximately 10 to 25 percent of the reduction products formed was CH_4 (Figure 4.5c). Propionic acid was degraded slowly and was not completely consumed within the two-day period between additions, but tended to accumulate (Figure 4.5d). The slow degradation rate did not allow precise doubling of the electron donor to PCE ratio from 1:1 to 2:1, since propionic acid was sometimes withheld to avoid a significant increase above the desired level of amendment. Prior to TISs, for example, propionic acid was withheld so that it would be depleted. The depletion of propionic acid resulted in residual PCE on several occasions.

4.A.2.e. FYE controls. The control bottles, which were amended with FYE but no other electron donor (Figure 4.6), exhibited incomplete dechlorination with significant amounts of remaining PCE and TCE (Figure 4.6a) and trace amounts of CH_4 formation (Figure 4.6b). The total reduction equivalent formation from FYE and endogenous decay was approximately 20 μeq when amended at a 1:1 ratio (20 μL) and about 40 μeq during 2:1 ratio (40 μL) (Figure 4.6c).

4.A.2.f. Summary of long-term results. Results of long-term operation were not significantly different among H_2 donors, in terms of the amount and extent of dechlorination that was observed. All donors facilitated dechlorination to VC and ETH in comparable amounts. Mixing the dechlorinating cultures with the methanogenic cultures on Day 52 or Day 56 resulted in more methanogenesis in some of the donor-amended bottles. Furthermore, increasing the donor to PCE ratio from 1:1 to 2:1 resulted in the production of more ETH in all cultures. Duplicate bottles run at a 1:1 donor to PCE ratio (formed after mixing the dechlorinating cultures with the methanogenic cultures) behaved similarly.

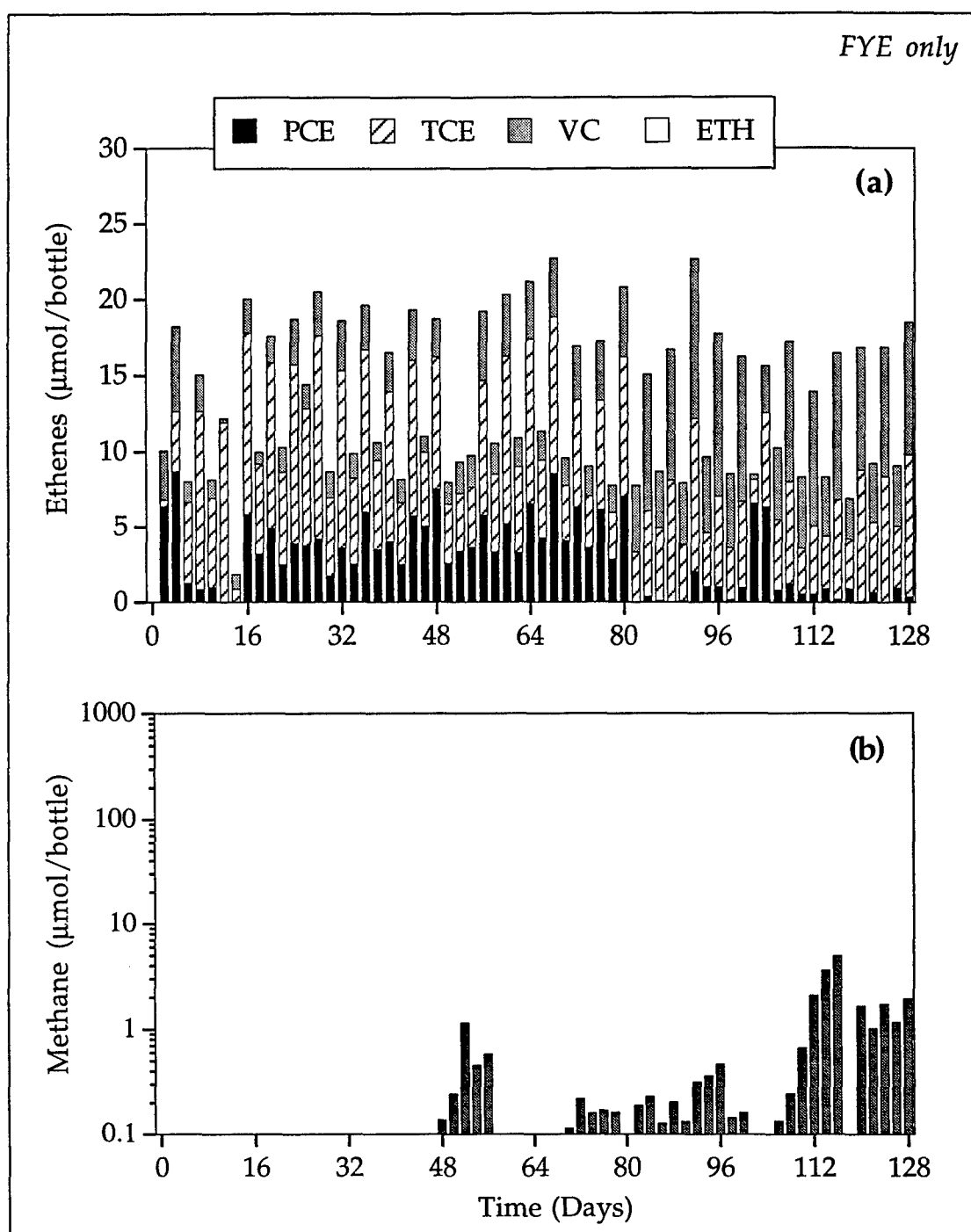
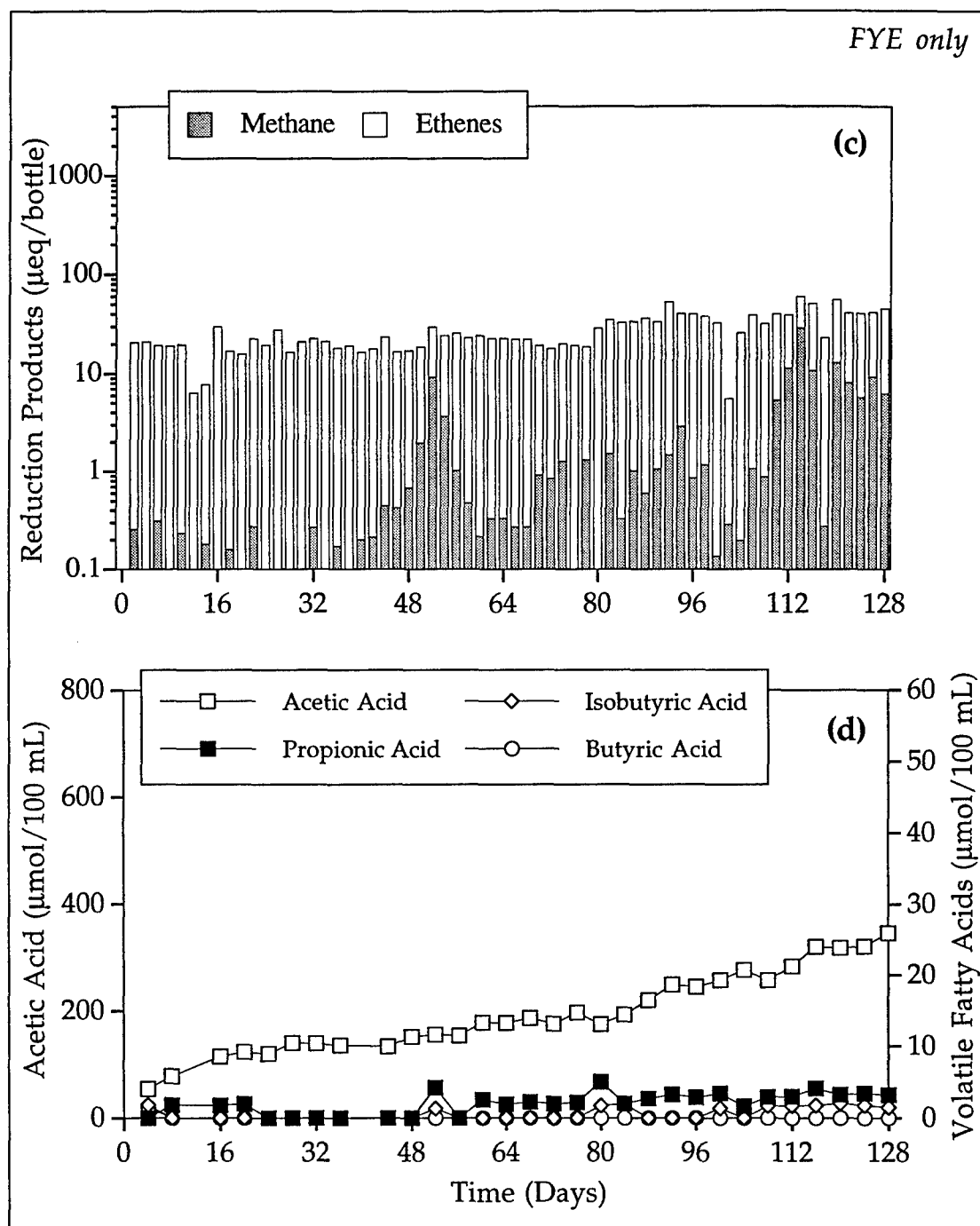


Figure 4.6. Results of long-term operation with fermented yeast extract and no electron donor: (a) dechlorination; (b) methane; (c) reduction products; and (d) VFAs. 1:1 donor to PCE ratio Day 0 to 80; blended with methanogenic culture Day 52; 2:1 ratio after Day 80.

Figure 4.6 (Continued)



All bottles contained traces of propionic and isobutyric acids. Using analyses of FYE content (Section 5.K.2 and Appendix II) and assuming that none of the FYE-contributed propionic or isobutyric acid was degraded, FYE-amendment would result in a steady-state concentration of approximately 6.5 μmol propionic acid/100 mL and 3.5 μmol isobutyric acid/100 mL at a 1:1 ratio; and 13 μmol propionic acid/100 mL and 7 μmol isobutyric acid/100 mL at a 2:1 ratio. These amounts can be compared to the levels observed in each bottle and serve as a benchmark to determine if other sources of these compounds (such as fermentation of the primary donor) were present. Of course, it is possible that production and consumption of either of these compounds might fortuitously produce the same levels expected from steady-state FYE addition. It appeared from this comparison that isobutyric acid may have been produced in the butyric-acid-amended cultures and that propionic acid was produced in the lactic-acid-amended cultures and perhaps in the ethanol-amended cultures.

4.A.3. Time-Intensive Studies Comparing Electron Donors.

Results for TISs at a 1:1 and a 2:1 basis for each H_2 donor are presented in a series of six graphs for each TIS: (a) dechlorination; (b) CH_4 ; (c) H_2 ; (d) donor and VFAs; (e) reduction products; and (f) free-energy analysis. For each donor, two TISs were performed at a 1:1 donor to PCE ratio and one TIS was performed at a 2:1 donor to PCE ratio. One of the 1:1 TIS data sets and the 2:1 TIS data set for each donor is presented. The data set for the second 1:1 TIS is included in Chapter 6, Model Results, for

comparison to model output. Except where noted, the results of the two 1:1 TIS tests were similar.

The Gibbs free energy of reaction (ΔG_{rxn}) was computed for each time step of TISs to determine the free-energy condition of the enrichments. For each analytical interval, concentrations of electron donor and acetate were available, and the H_2 concentration could be calculated since the molar H_2 content of the bottle was also measured at or within a few minutes of the liquid measurements. The aqueous-phase H_2 concentration was calculated according to Equation 4.1, assuming gas-liquid equilibrium.

$$C_{\text{wH}_2} = \frac{M_{\text{tH}_2}}{V_{\text{g}} \times H_{\text{cH}_2} + V_{\text{w}}} \quad (4.1)$$

Where:

- C_{wH_2} = aqueous H_2 concentration ($\mu\text{mol/L}$);
- M_{tH_2} = total amount of H_2 in the bottle (μmol);
- H_{cH_2} = Henry's constant for H_2 ;
- V_{g} = gaseous volume (L); and
- V_{w} = liquid volume (L).

The ΔG_{rxn} was calculated using the Nernst equation, Equation 5.6 in Section 5.D. ΔG° values used for these calculations are shown in Appendix VI.

The pH was regularly measured at the end of each 48 hr. An average pH of 7.3 (based on observations of many pH measurements) was chosen for these calculations. It is not known what the actual pH was at each time step, but it is unlikely that it varied greatly in the heavily buffered basal

medium. This is a limitation since pH does participate in Equation 5.6 for some of the reactions.

While the comparison of electron donors over the long-term showed little difference in terms of the final dechlorination results, TISs did show marked differences among the donors.

4.A.3.a. Butyric-acid-amended cultures. Figure 4.7 shows results from one of the two TISs (HBUtIS 3) of butyric acid at a 1:1 donor to PCE ratio. HBUtIS 3 (Figure 4.7) was performed on Day 52, and a previous study HBUtIS 2 was performed on Day 40.

PCE was dechlorinated to TCE, and VC (Figure 4.7a). While about 4 μmol TCE (2 μmol during HBUtIS 2) was formed, it was not normally detected at the end of a 48-hour period during long-term studies (see Figure 4.2a). Since this test was run for only 24 hr, the gradual disappearance of the TCE was not observed. Also, since FYE was withheld, fewer reducing equivalents were available than during normal feeding. During HBUtIS 3 a steady H_2 level of $10^{-4.8}$ atm (30 nmol/bottle) or less was maintained (Figure 4.7b). The H_2 level during HBUtIS 2 reached $10^{-4.7}$ atm (60 nmol/bottle). Note that a small amount of CH_4 was produced during the first 14 hr as the H_2 level reached 10^{-5} atm (25 nmol/bottle or 0.008 μM) then leveled off as H_2 fell to below about $10^{-5.1}$ atm (20 nmol/bottle) (Figure 4.7c).

Butyric acid was degraded readily (Figure 4.7d) under an acetate concentration of about 5 mM at a free energy of approximately -20 kJ/mol butyric acid (Figure 4.7f). Reduction product formation matched the amount of butyric acid fermented (Figure 4.7e) and dechlorination products

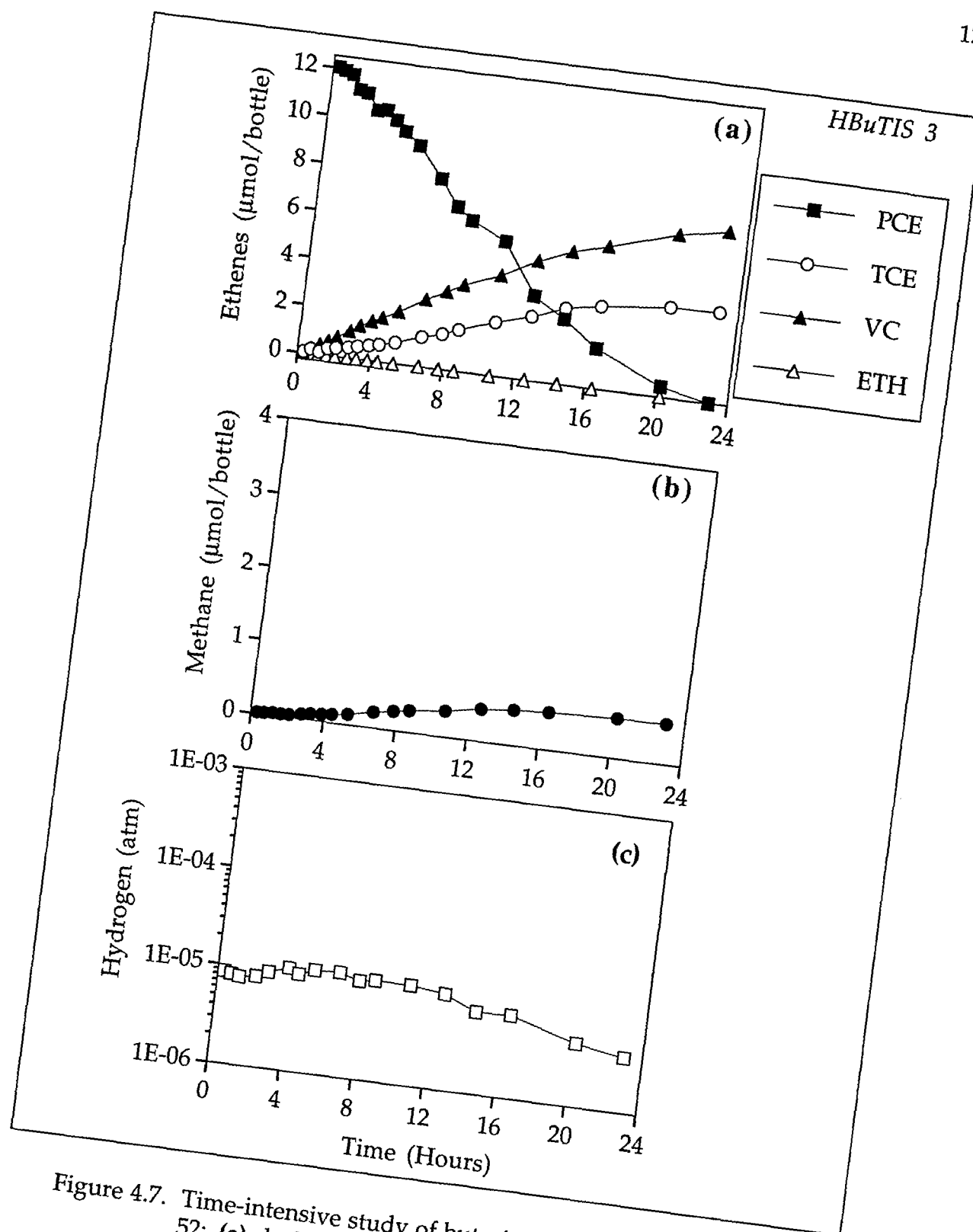
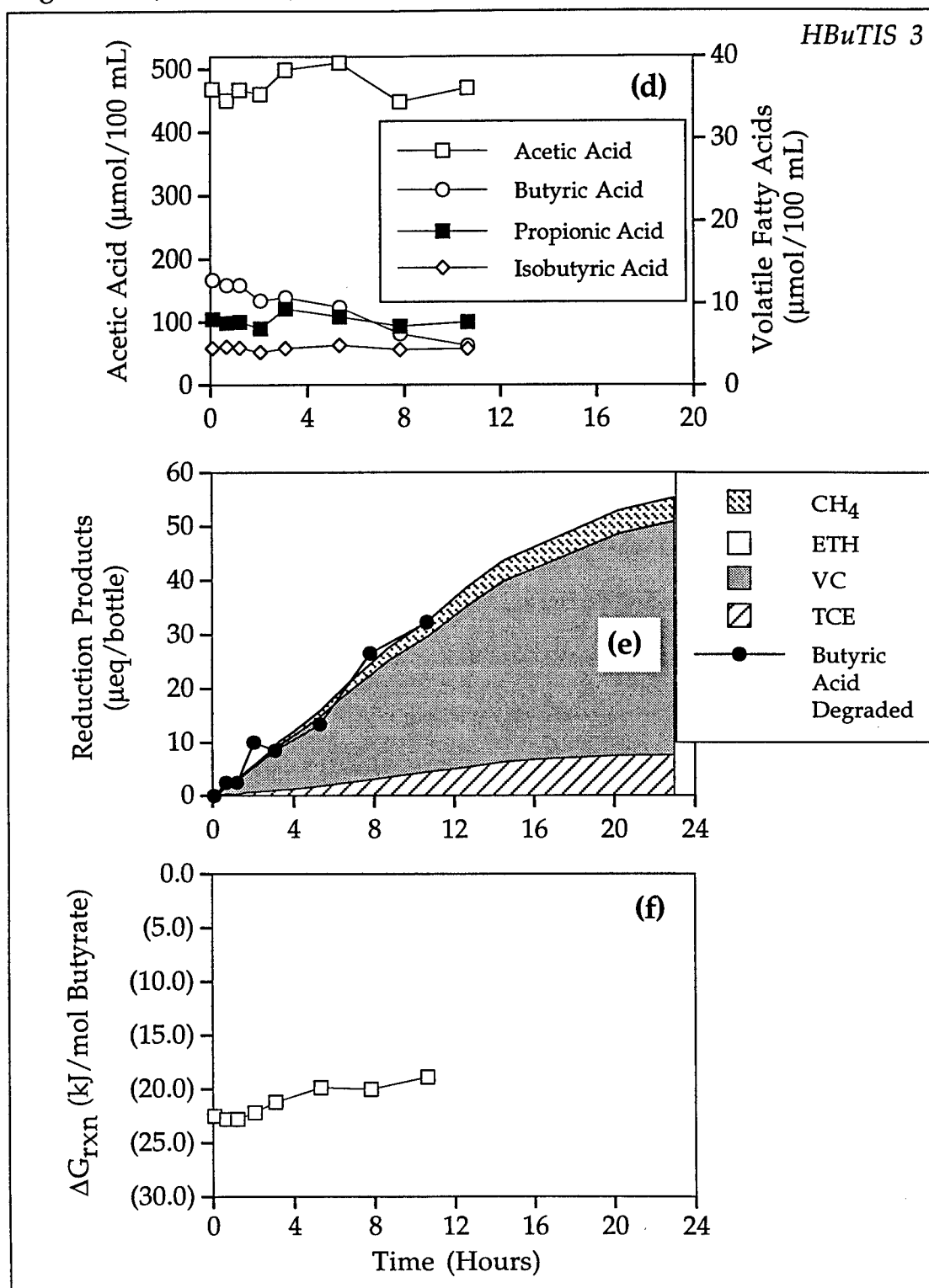


Figure 4.7. Time-intensive study of butyric acid at a 1:1 ratio, Day 52: (a) dechlorination; (b) methane; (c) hydrogen; (d) VFAs (e) reduction products; and (f) free-energy analysis.

Figure 4.7 (Continued)



accounted for about 90 percent of the total amount of reduction products formed.

During the 2:1 butyric-acid to PCE TIS (HBU-TIS 4) performed on Day 128, TCE was not a prominent intermediate—all of the PCE was dechlorinated to VC and ETH (Figure 4.8a). Since acetotrophic activity was onset at this time, a significant amount of CH_4 was formed primarily from the methanogenic conversion of the produced acetate (Figure 4.8b). H_2 accumulated to $10^{-4.2}$ atm (150 nmol/bottle) (Figure 4.8c). Reduction product formation is shown in Figure 4.8d. In this figure, the butyric acid equivalents are defined on the basis of conversion to CO_2 (20 $\mu\text{eq}/\mu\text{mol}$) and acetate is included as a product in the equivalents balance. Dechlorination accounted for 44 percent of the H_2 equivalents that were released by the fermentation of butyric acid. Butyric acid was fermented to acetate which was slowly converted to CH_4 during the test (Figure 4.8d). The free-energy status was not stable during this test and approached values as high as -13.6 kJ/mol butyric acid (Figure 4.8f).

4.A.3.b. Ethanol-amended cultures. Results of EtOHTIS 1 with ethanol at a 1:1 donor to PCE ratio are shown in Figure 4.9. Dechlorination proceeded rapidly for the first 3.5 hr (Figure 4.9a), then slowed drastically and a significant PCE residual remained. CH_4 was also produced rapidly during the initial 3.5 hr and then production ceased (Figure 4.9b). H_2 production occurred in a burst of $10^{-2.9}$ atm (3000 nmol/bottle) within 2 hr (Figure 4.9c) as the ethanol was rapidly degraded (Figure 4.9d). Background acetic acid was approximately 2.5 mM and propionic acid was approximately 30 μM during this test (as determined from measurements made at bottle set-up the day of the test), but specific measurements of

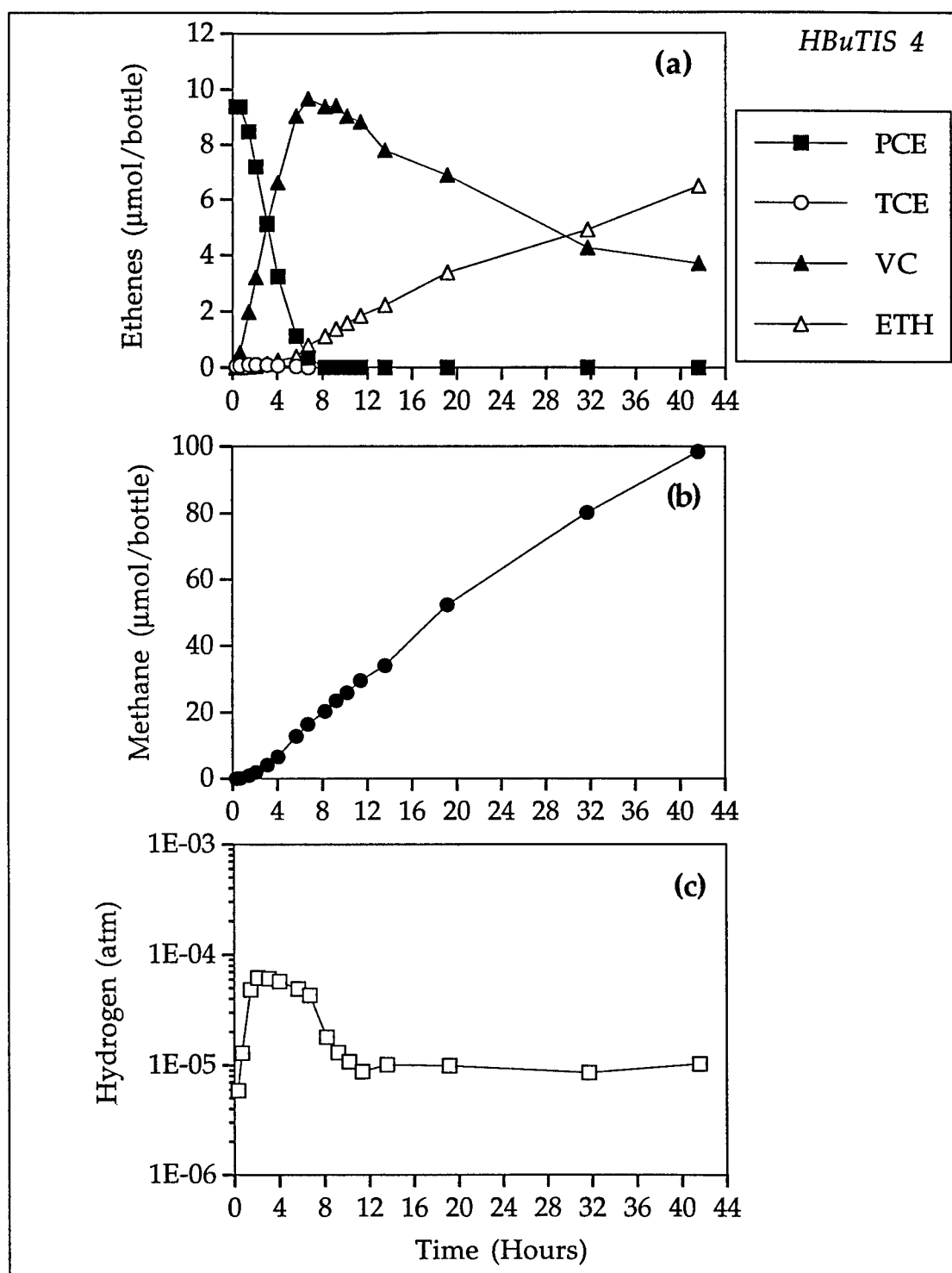
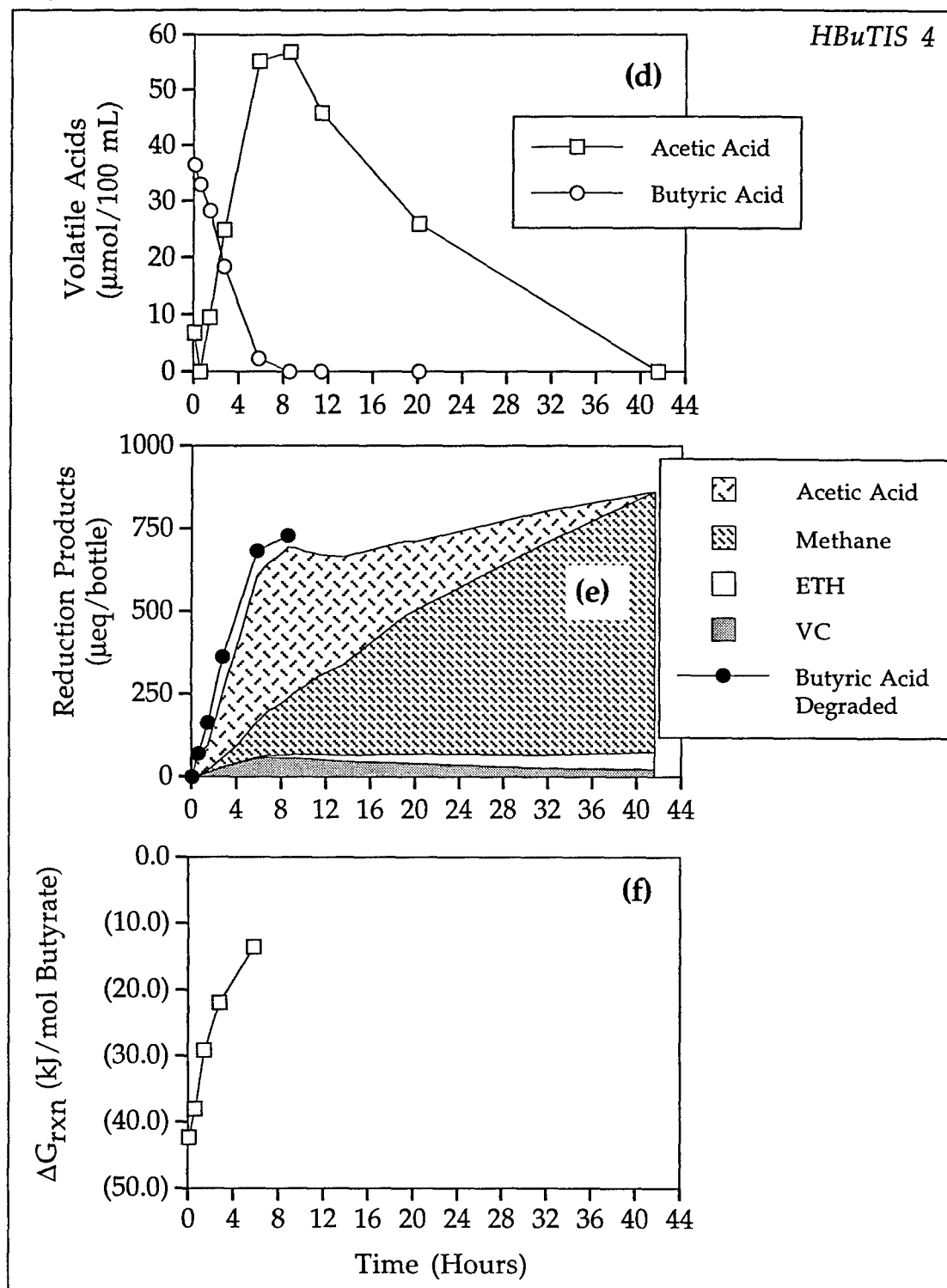


Figure 4.8. Time-intensive study of butyric acid at a 2:1 ratio, Day 128: (a) dechlorination; (b) methane; (c) hydrogen; (d) VFAs (e) reduction products; and (f) free-energy analysis.

Figure 4.8 (Continued)



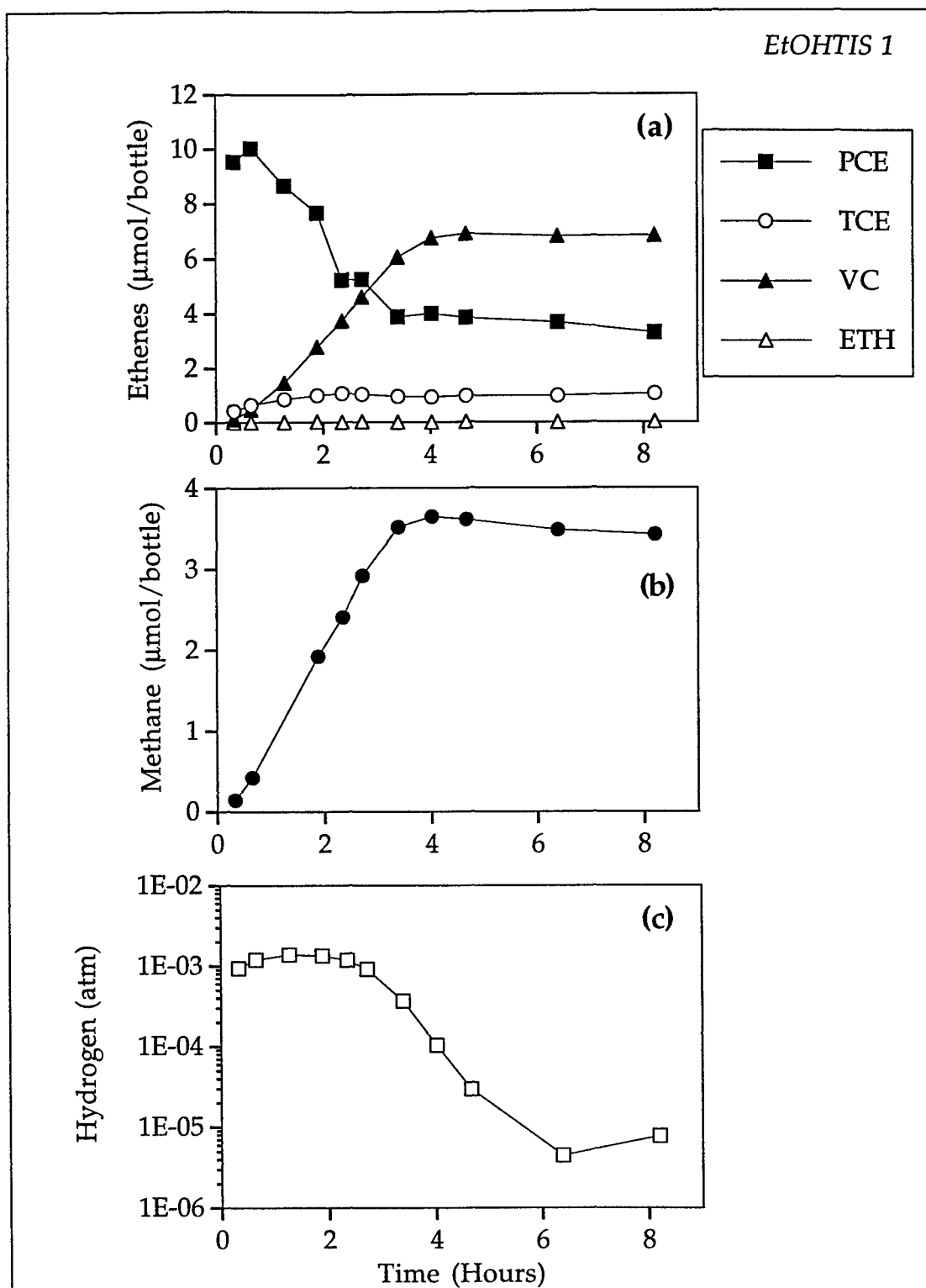
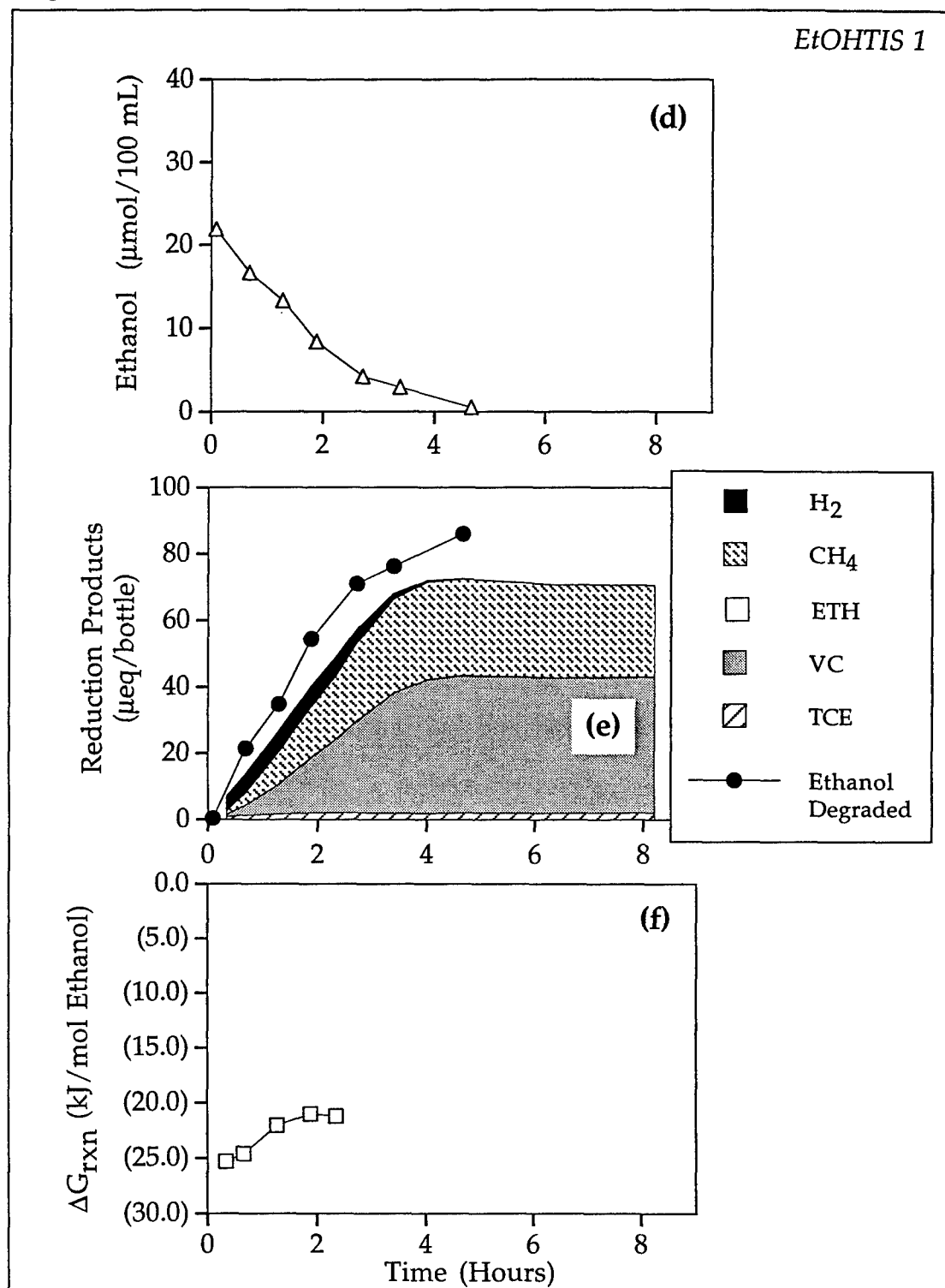


Figure 4.9. Time-intensive study of ethanol at a 1:1 ratio, Day 36: (a) dechlorination; (b) methane; (c) hydrogen; (d) ethanol (e) reduction products; and (f) free-energy analysis.

Figure 4.9 (Continued)



VFAs were not made during this test. Reduction product formation roughly equaled the amount of ethanol degraded (Figure 4.9e) and the dechlorination products accounted for 61 percent of the reduction products formed. The free energy of reaction was less than -20 kJ/mol ethanol (Figure 4.9f). EtOHTIS 2 yielded very similar results.

Results of EtOHTIS 4 performed on Day 128 at a 2:1 donor to PCE ratio are shown in Figure 4.10. Dechlorination was rapid initially, while after about 4 hr when ethanol and the produced H_2 were depleted, a sharp break in the rate was observed, and thereafter, proceeded very slowly (Figure 4.10a). Methanogenesis was also rapid initially, but there was a break in the rate after 4 hr after which a significant portion of CH_4 was produced from the acetate formed during the fermentation of the ethanol. H_2 reached levels of $10^{-2.6}$ atm (5500 nmol/bottle) before being rapidly depleted through use by dechlorination and methanogenesis (Figure 4.10c). Ethanol was entirely depleted after 3 hr and the produced acetate reached 350 μ M (Figure 4.10d). Reduction products formed were less than the total amount of ethanol degraded (on a CO_2 equivalents basis, 12 μ eq/ μ mol ethanol) (Figure 4.10e). Dechlorination accounted for 37 percent of the use of the H_2 produced by ethanol fermentation. The free-energy status of the test is shown in Figure 4.10f. The free energy for ethanol fermentation was less than -24 kJ/mol ethanol throughout the test.

4.A.3.c. Lactic-acid-amended cultures. Results from the lactic acid TISs were not as precise as were results from the other donors because the lactic acid and VFA analyses for these bottles were performed using the HPLC, which was less reliable than the GC method of VFA analysis.

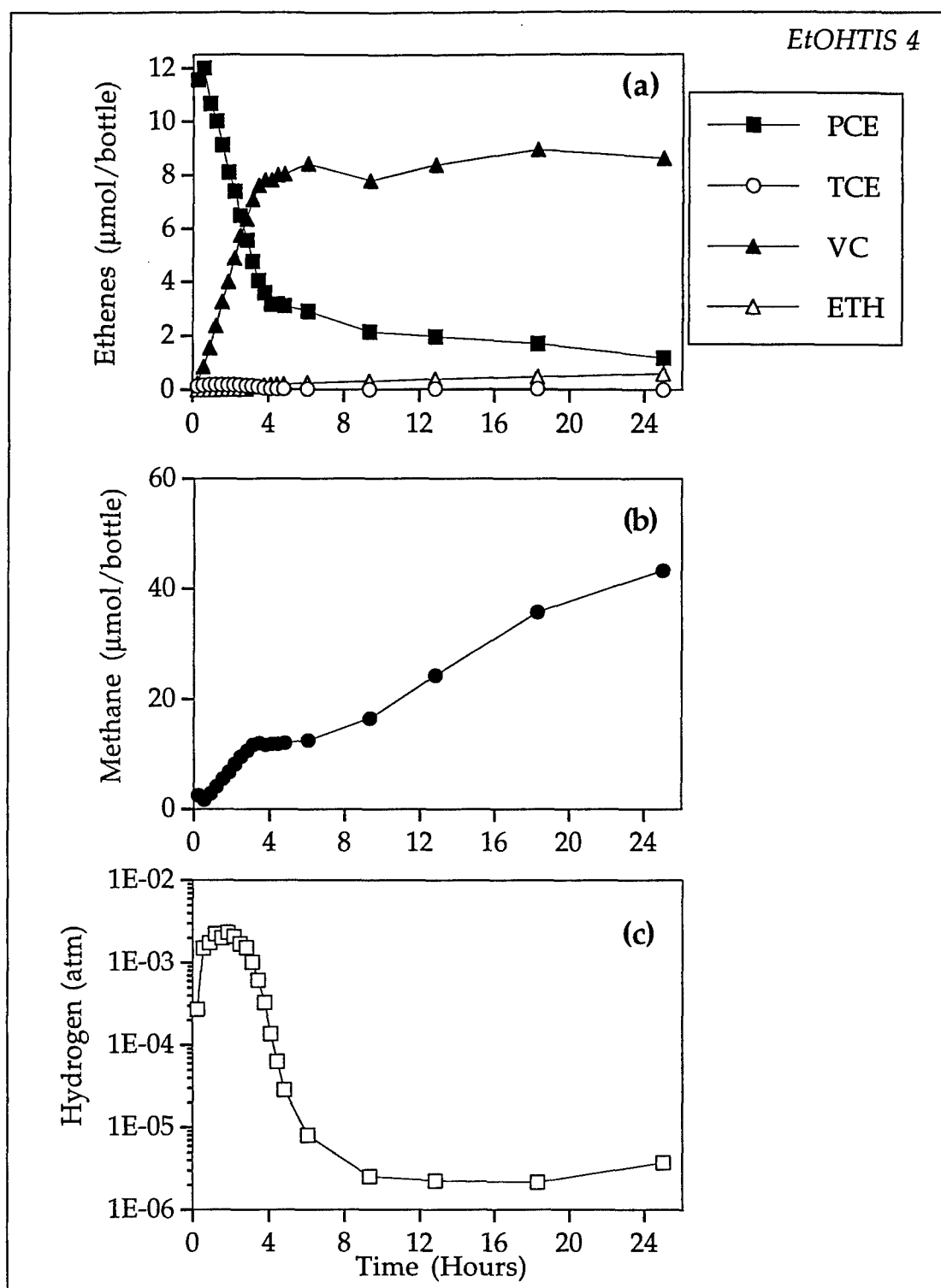
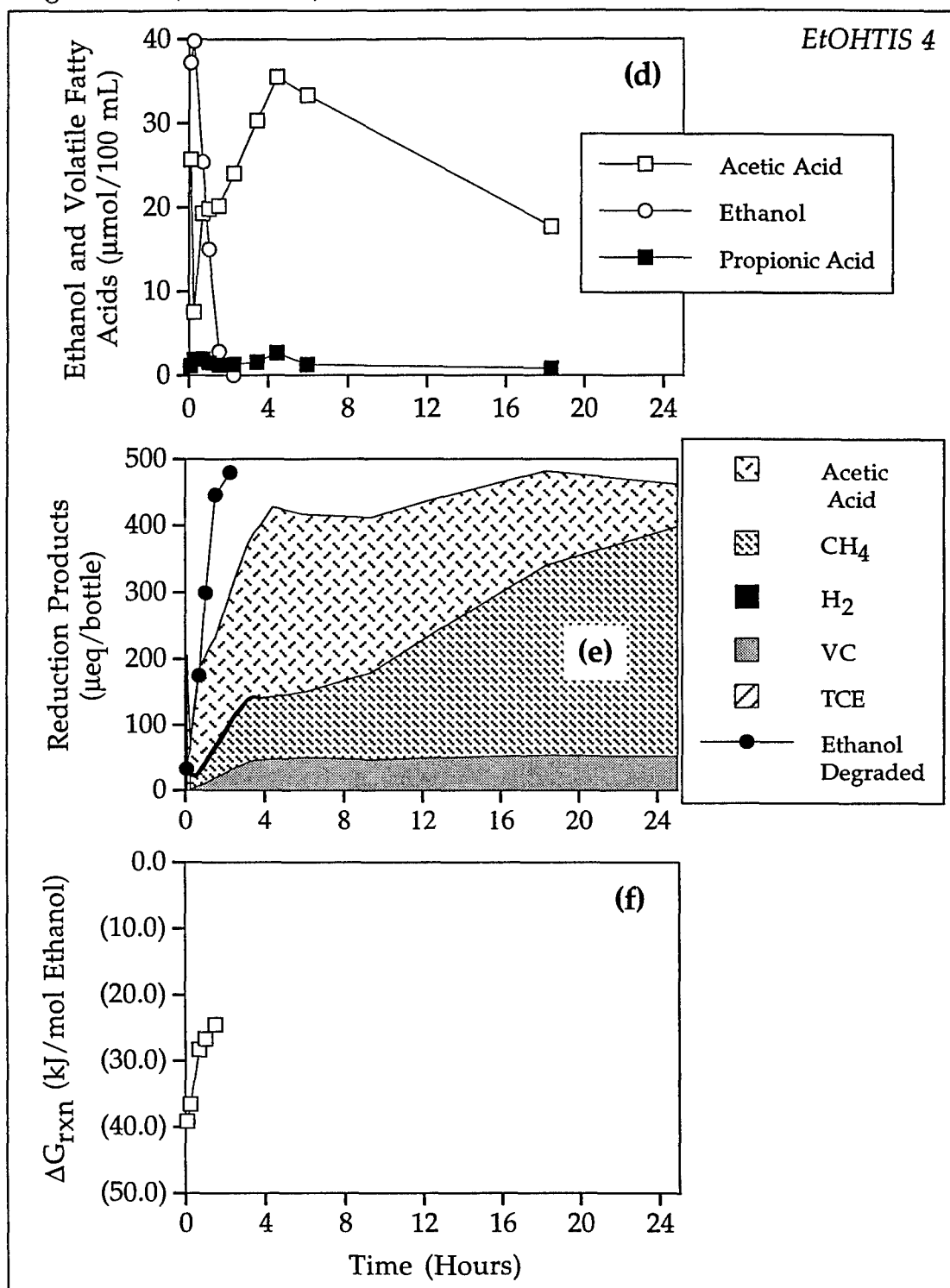


Figure 4.10. Time-intensive study of ethanol at a 2:1 ratio, Day 128: (a) dechlorination; (b) methane; (c) hydrogen; (d) ethanol and VFAs; (e) reduction products; and (f) free-energy analysis.

Figure 4.10 (Continued)



The results from lactic acid TISs differed depending upon whether the donor was fed at a 1:1 donor to PCE ratio or a 2:1 donor to PCE ratio.

Results of LacTIS 1 performed at a 1:1 ratio on Day 36 are shown in Figure 4.11. PCE dechlorination (Figure 4.11a) occurred rapidly while lactic acid was degraded and H_2 was being produced (Figure 4.11c), and it continued at a slightly reduced rate after lactic acid was depleted at about 6 hr. At a 1:1 ratio, degradation of lactic acid produced a peak of H_2 of only 10^{-4} atm (250 nmol/bottle) (Figure 4.11c). The continued dechlorination was probably stimulated by the presence of a significant pool of propionic acid (Figure 4.11d) which was apparently produced during lactic acid fermentation and then slowly degraded after lactic acid was depleted. CH_4 was initially produced at a rapid rate, then leveled off because the H_2 level dropped to 10^{-5} atm (18 nmol/bottle) at 16 hr. After PCE was depleted, H_2 increased somewhat to $10^{-4.3}$ atm (120 nmol/bottle) at 40 hr and methanogenesis had resumed at that time (Figure 4.11b). Reduction product formation, including the production of propionate, closely matched the degradation of lactic acid (Figure 4.11e). Of the H_2 produced from this fermentation, 81 percent was channeled to dechlorination. The free-energy status of this run assuming fermentation of lactic acid to acetate and H_2 is shown in Figure 4.11f. The energetics were highly favorable and would have been even more so for fermentation of the lactic acid to propionate. LacTIS 2 yielded similar results, but was run for only 18 hr.

The results of LacTIS 4 at a 2:1 donor to PCE ratio are shown in Figure 4.12. The results at a 2:1 ratio are similar to the results for ethanol. Initially rapid dechlorination of PCE to VC (Figure 4.12a) and

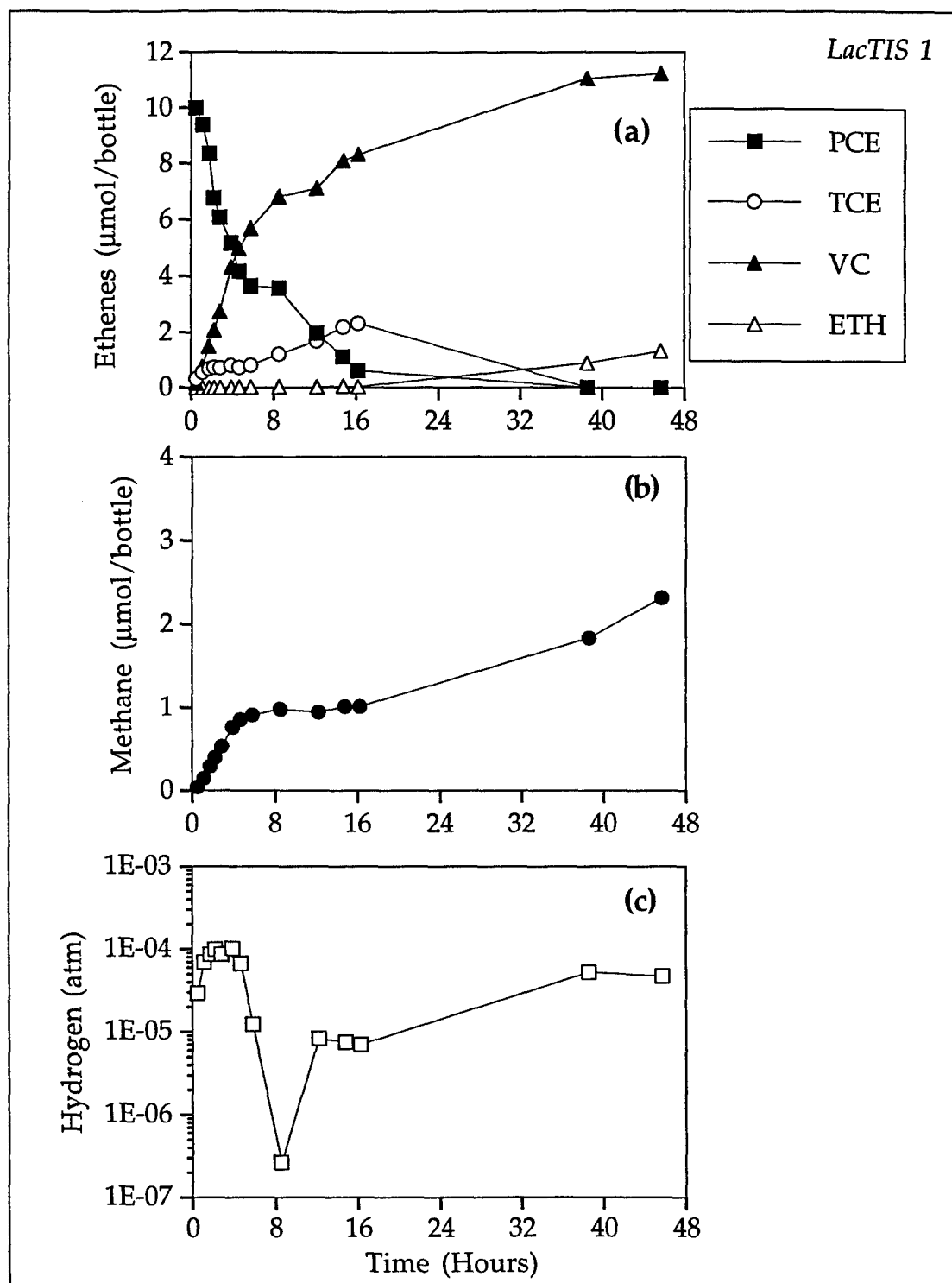
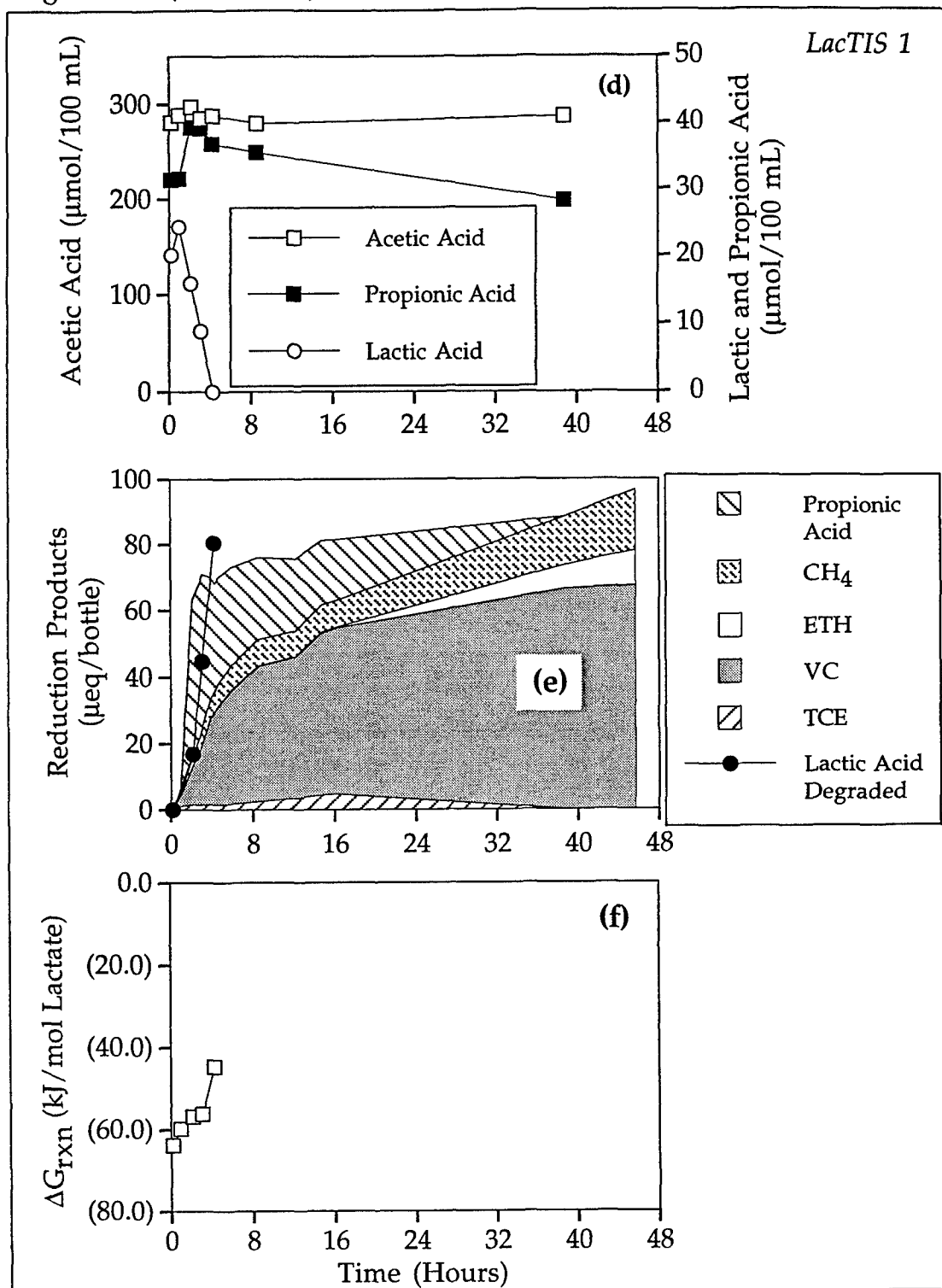


Figure 4.11. Time-intensive study of lactic acid at a 1:1 ratio, Day 36: (a) dechlorination; (b) methane; (c) hydrogen; (d) lactic acid and VFAs; (e) reduction products; and (f) free-energy analysis.

Figure 4.11 (Continued)



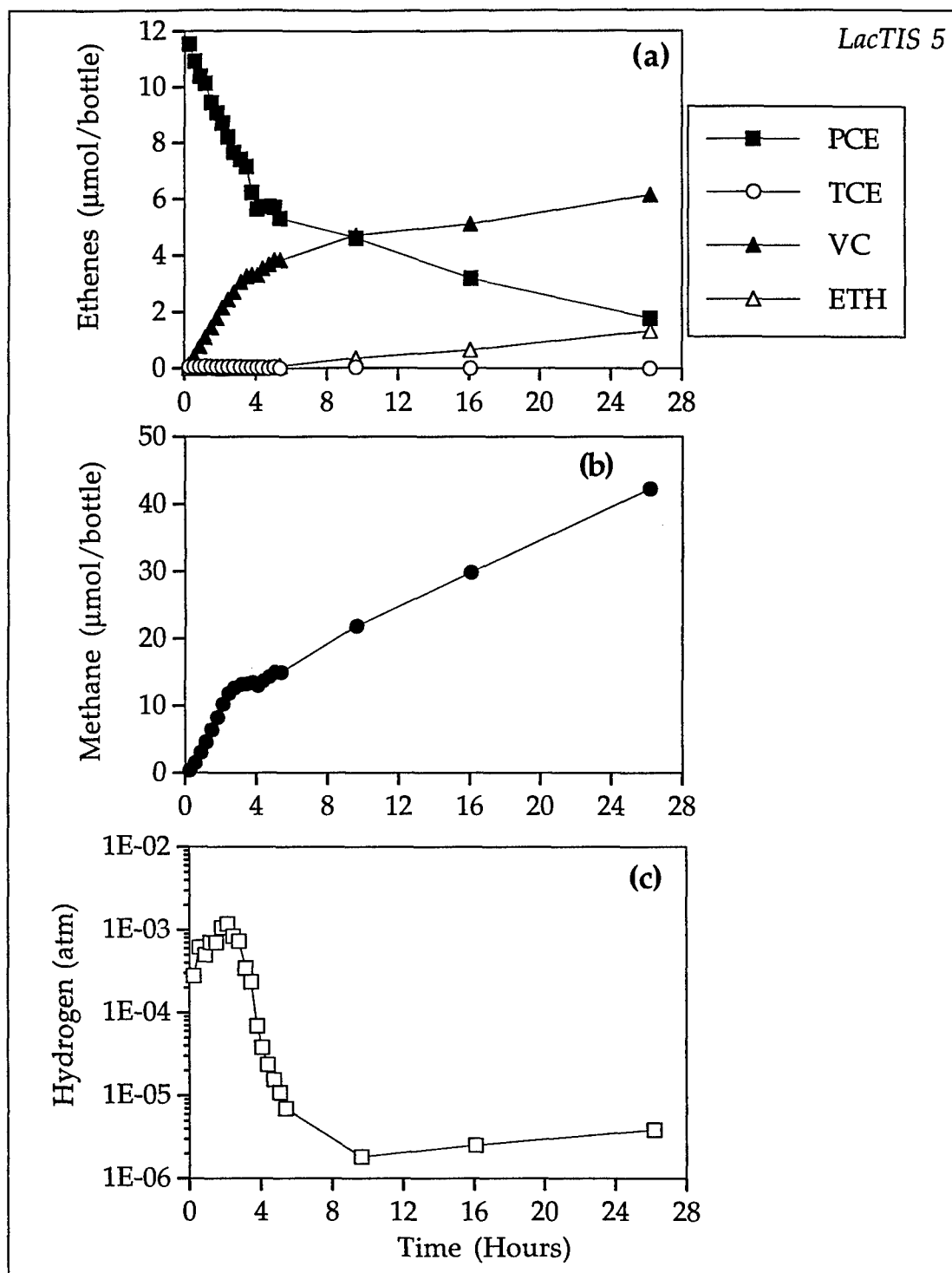
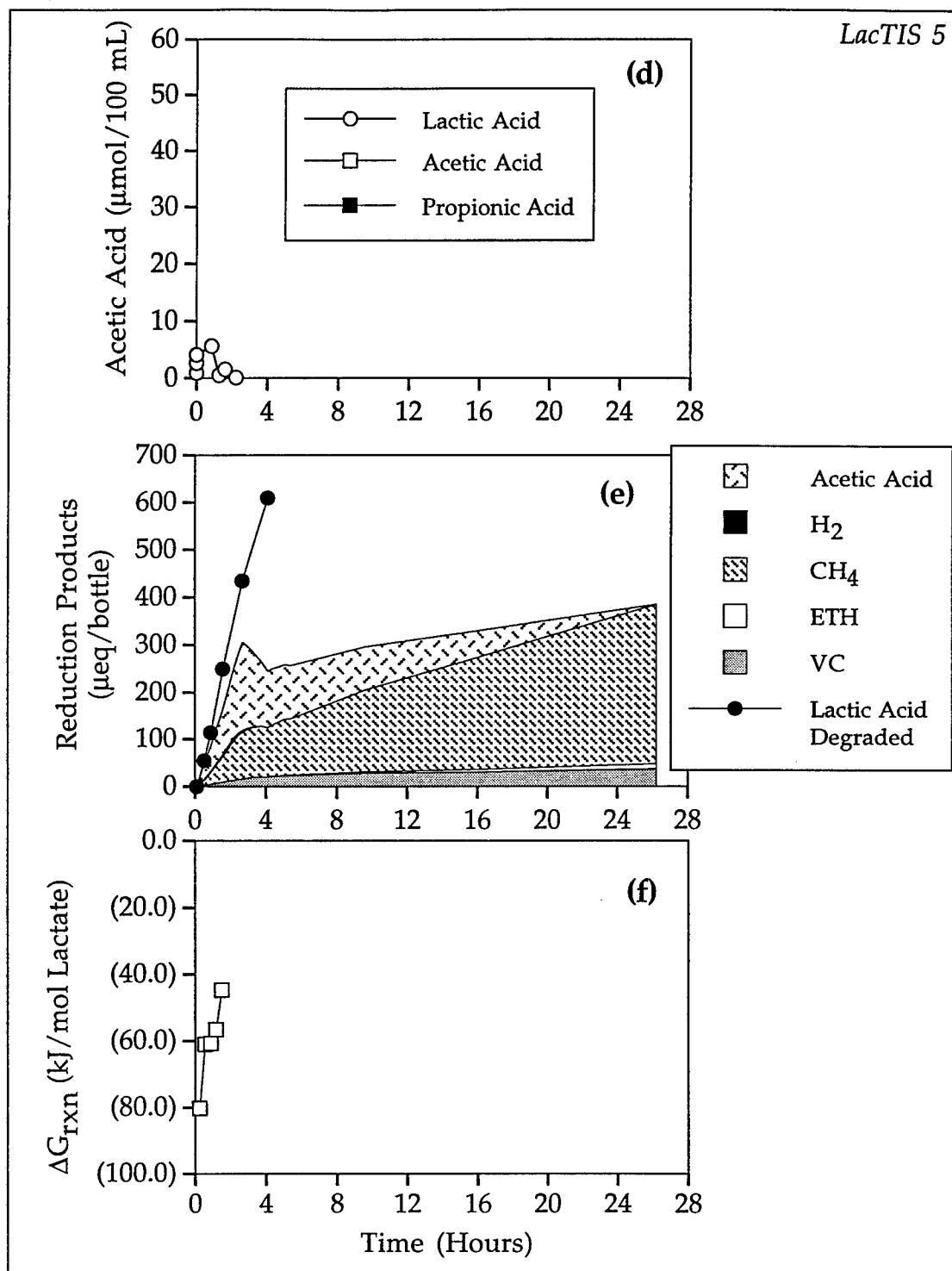


Figure 4.12. Time-intensive study of lactic acid at a 2:1 ratio, Day 134: (a) dechlorination; (b) methane; (c) hydrogen; (d) lactic acid and VFAs; (e) reduction products; and (f) free-energy analysis.

Figure 4.12 (Continued)



methanogenesis (Figure 4.12b) were fueled by the high H_2 peak of $10^{-2.9}$ atm (3000 nmol/bottle) (Figure 4.12c). The lactic acid was depleted after 4 hr and from the HPLC analysis only a small amount of acetate and only a trace amount of propionate were detected (Figure 4.12e). However, after 4 hr, dechlorination did continue slowly under an H_2 partial pressure of $10^{-5.6}$ atm (7 nmol/bottle). The reduction products formed did not match the amount of lactic acid degraded (on a CO_2 equivalents basis, 12 $\mu eq/\mu mol$ lactic acid) (Figure 4.12d). Clearly, there are some inconsistencies with the data set. The HPLC analysis is suspect in this case since the GC measurements were always consistent during this study. The free energy status of this TIS is shown in Figure 4.12f. As expected with lactic acid, the free energy of the reaction is highly negative, assuming fermentation to H_2 and acetate, and would have been even more negative for fermentation to propionate.

4.A.3.d. Propionic-acid-amended cultures. Results of PropTIS 1, performed on Day 30, of propionic acid at a 1:1 donor to PCE ratio are shown in Figure 4.13. PCE was slowly, but steadily, dechlorinated to VC over the 44-hr period of testing (Figure 4.13a). TCE that initially accumulated was also dechlorinated. Only a trace amount of CH_4 was detected (Figure 4.13b), and this was undoubtedly the result of the very low H_2 levels $10^{-5.1}$ atm (20 nmol/bottle) that were maintained (Figure 4.13c). Propionic acid was degraded very slowly under an acetate concentration of 1.75 mM (Figure 4.13d). A graph of reduction products formed (Figure 4.13e) is also indicative of the nearly complete channeling of reduction equivalents to dechlorination (CH_4 was too low to be evident on the graph). The free-energy status of the culture is shown in Figure 4.13f. The

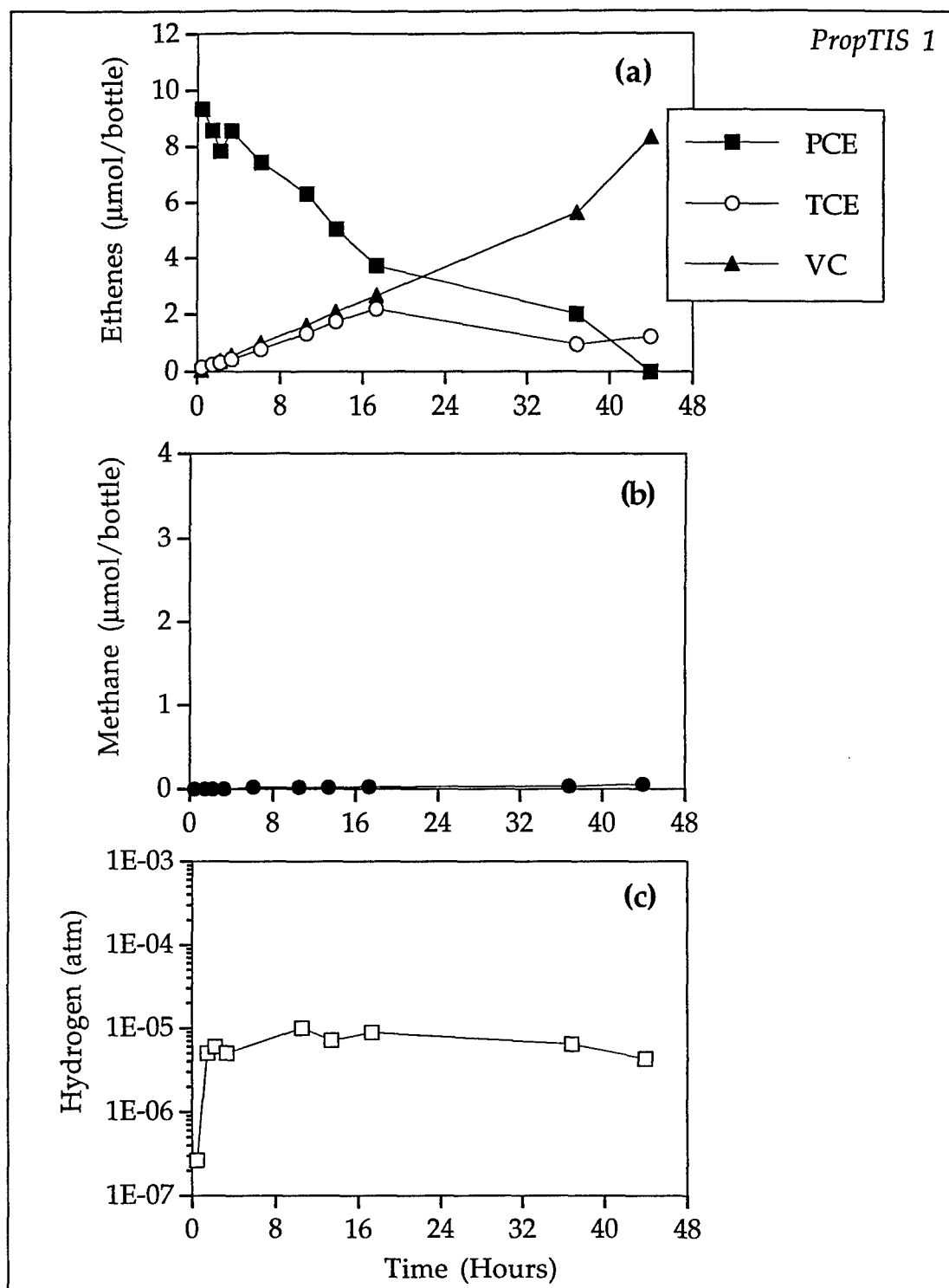
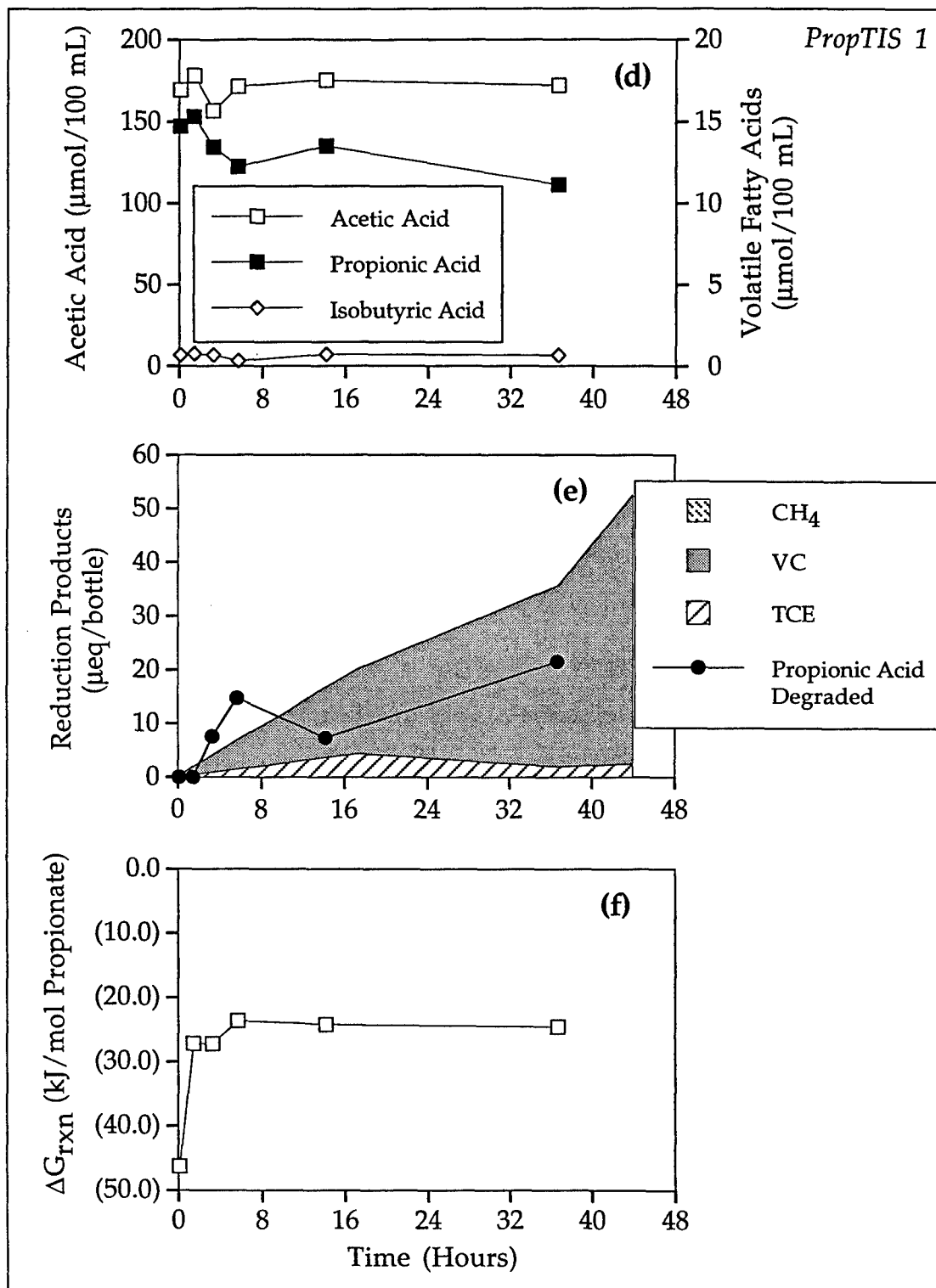


Figure 4.13. Time-intensive study of propionic acid at a 1:1 ratio, Day 36: (a) dechlorination; (b) methane; (c) hydrogen; (d) VFAs; (e) reduction products; and (f) free-energy analysis.

Figure 4.13 (Continued)



free energy of the reaction was fairly constant for most of the experiment at approximately -25 kJ/mol propionate. Interestingly, it can be seen from this data set that dechlorination occurred at an H_2 partial pressure of $10^{-5.4}$ atm (10 nmol H_2 /bottle). The aqueous H_2 concentration at that partial pressure (assuming equilibrium between the gas and liquid phase—a good assumption considering the very steady H_2 levels) was approximately 3 nM. In results from other bottles during other experiments when no donor was fed (data not shown), dechlorination could be seen to proceed at H_2 levels as low as 1.5 nM. These values are below most of the reported thresholds for H_2 use by methanogens (see Table A1.5).

Results of PropTIS 4 performed on Day 128 are shown in Figure 4.14. Dechlorination of PCE was complete to VC and ETH (Figure 4.14a). Methanogenesis was continuous, but because of the onset of acetotrophic methanogenesis, no CH_4 production pattern from H_2 alone could be obtained (Figure 4.14b). H_2 levels remained near $10^{-5.2}$ atm (17 nmol/bottle) until after PCE was depleted, then it increased to $10^{-4.5}$ atm (70 nmol/bottle) (Figure 4.14c). Propionic acid was degraded, and since acetotrophic activity had begun in the bottle, so was acetate (Figure 4.14d). Reduction-product formation was plotted differently in this case. The final acetate mass was subtracted from each point along the progress curve and this difference was plotted as an area. The amount of propionic acid remaining at each point was also plotted as an area. The amount of total equivalents in the system (based on CO_2 equivalents basis, 14 $\mu\text{eq}/\mu\text{mol}$ propionic acid) was fairly constant over 44 hr, indicating a good balance on reactants and products (Figure 4.14d). The free-energy analysis is shown in Figure 4.14f. Initially the free energy was fairly constant at -25 to -20 kJ/mol

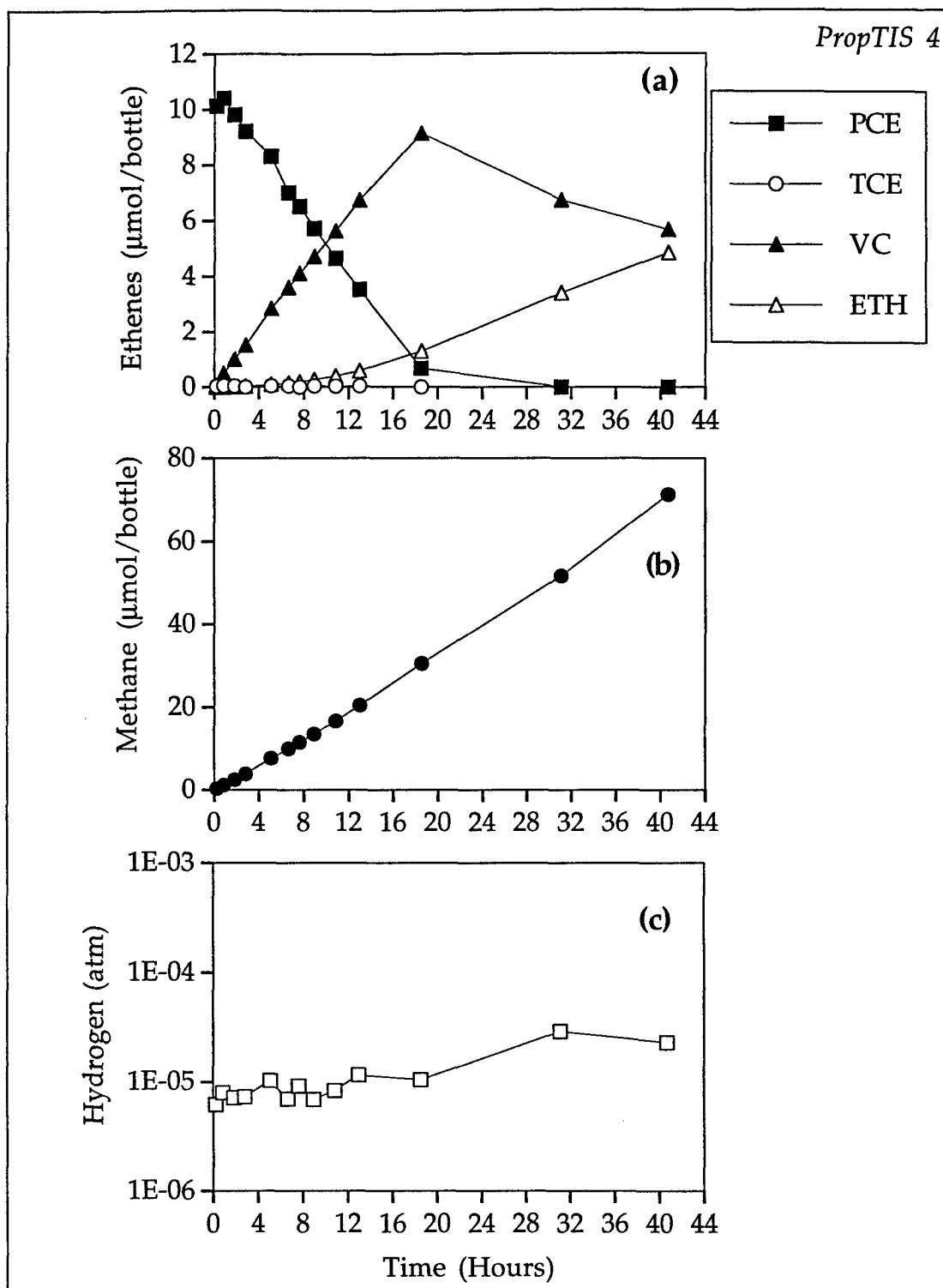
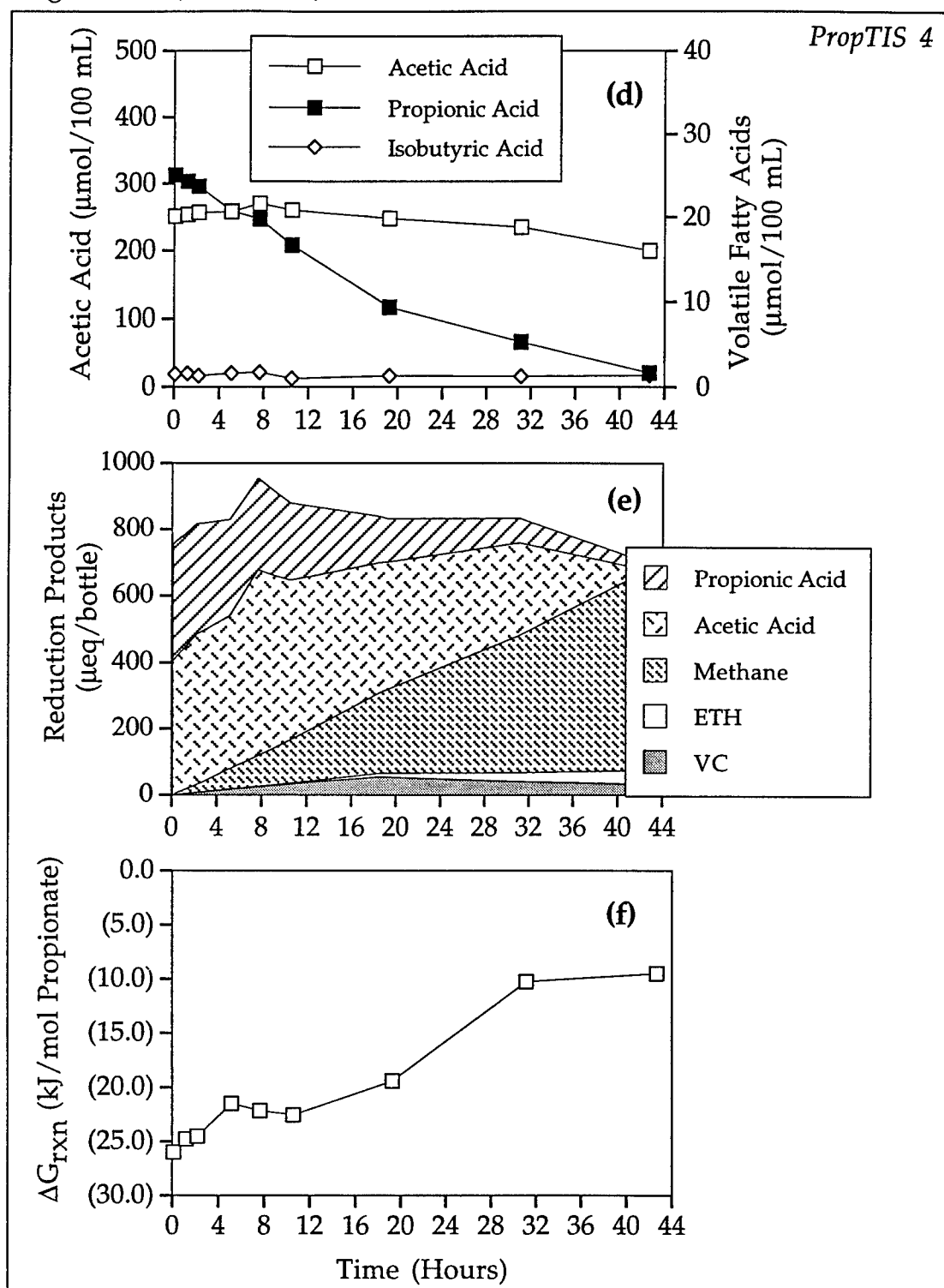


Figure 4.14. Time-intensive study of propionic acid at a 2:1 ratio, Day 128: (a) dechlorination; (b) methane; (c) hydrogen; (d) VFAs; (e) reduction products; and (f) free-energy analysis.

Figure 4.14 (Continued)



propionic acid. Later, when the H_2 level increased to $10^{-4.5}$ atm, the free energy increased to near -10 kJ/mol propionic acid. As will be shown in the Model Results Section 6.B.8, the increase in the free energy of reaction between 20 and 30 hr made this data set difficult to explain and to model.

4.A.3.e. Summary of fate of reduction equivalents for each donor during time-intensive studies. The fate of the added donor on an H_2 equivalent basis is shown in Table 4.2. Note that during 2:1 studies, the CH_4 produced from H_2 could not be quantified.

Table 4.2. Reduction equivalents released and reduction products formed during time-intensive studies.

			Dechlorination Products	
Study	Donor Degraded (μeq released)^a	CH_4 From H_2 (μeq)	(μeq)	As % of H_2 From Donor
Butyric Acid				
1:1	51.4	4.3	51	99
2:1	146	na	74.1	50.7
Ethanol				
1:1	87.6	27.4	43.2	49
2:1	160	na	61.2	38
Lactic Acid				
1:1	80.5	18.6	70.1	87
2:1	203.3	na	47.4	23
Propionic Acid				
1:1	50	0.5	52.6	100
2:1	140	na	72.8	52

^a Based on H_2 equivalents.

4.A.3.f. Comparison of H₂ levels for different hydrogen donors.

Figure 4.15 shows a comparison of the H₂ levels produced by each of the donors at 1:1 and 2:1 donor to PCE ratios. Peak H₂ levels from propionic acid, butyric acid, ethanol, and lactic acid at a 1:1 ratio were approximately 10^{-5.1}, 10⁻⁵, 10^{-2.9}, 10⁻⁴. At a 2:1 ratio the peak H₂ levels from propionic acid, butyric acid, ethanol, and lactic acid were approximately 10^{-5.1}, 10^{-4.2}, 10^{-2.7}, and 10^{-2.9} atm, respectively.

4.A.3.g. Biomass concentrations. Biomass concentrations were estimated from PON analyses conducted on each culture at the end of the long-term study (Table 4.3). These results were used as an aid in modeling. It is obvious from these measurements that biomass changed and reached different concentrations during different treatments; undoubtedly the fractions of the different populations also changed, but these were not determined.

Table 4.3. Beginning and ending biomass for the hydrogen donor comparison study.

Enrichment Cultures		Biomass (mg VSS/L)	
Donor	1:1 Enrichment	2:1 Enrichment	
Butyric Acid	36.8	69.9	
Ethanol	32.3	35.5	
Lactic Acid	35.1	39.6	
Propionic Acid	22.8	65.3	
FYE Control	15.6	25.7	
Starting Culture (20 percent dilution of high-PCE/methanol culture)		Biomass (mg VSS/L)	
		43.7	

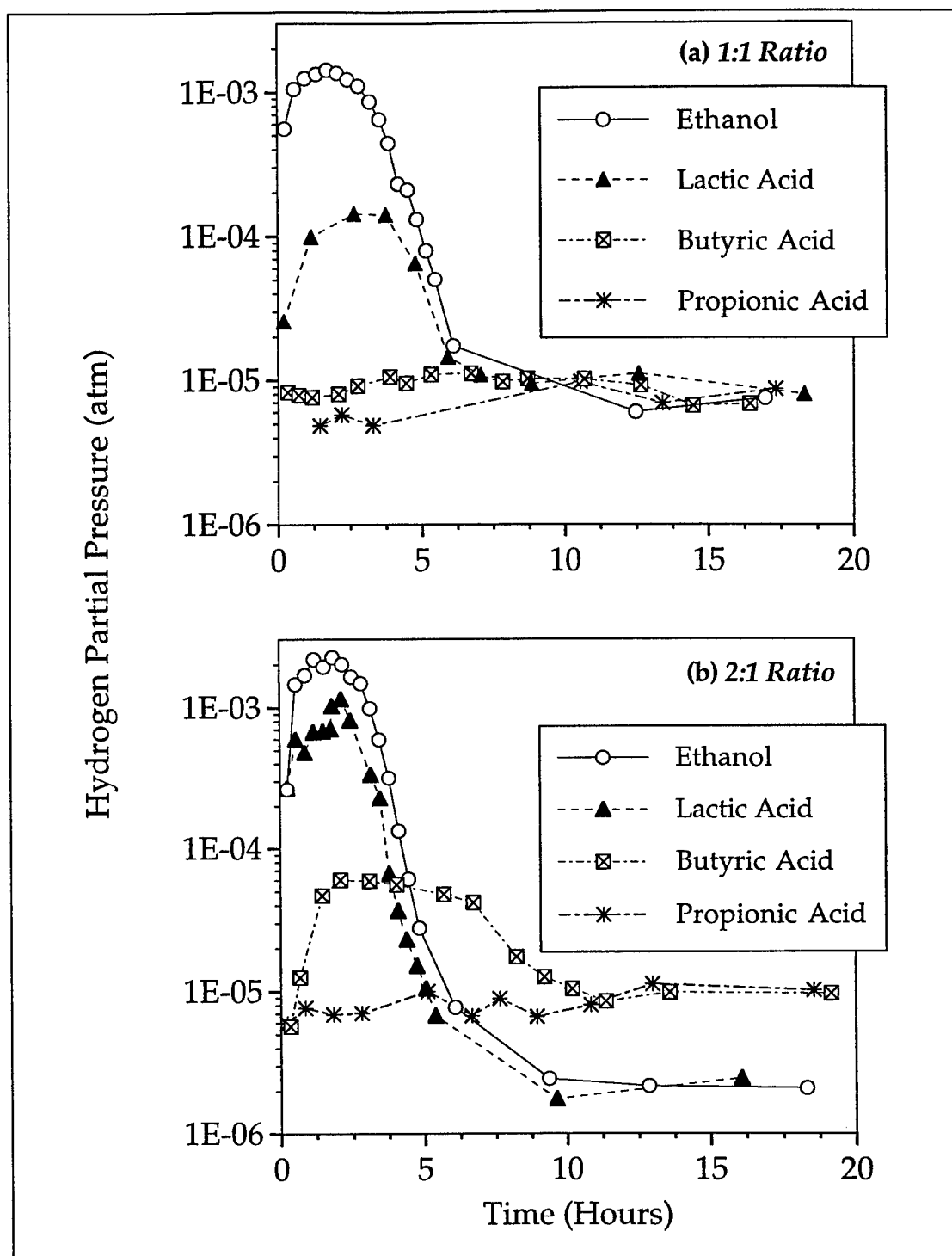


Figure 4.15. Comparison of hydrogen production from the fermentation of ethanol, lactic acid, butyric acid, and propionic acid at a (a) 1:1 donor to PCE ratio and (b) 2:1 donor to PCE ratio.

4.B. Time-Intensive Study of Ethanol Supplemented with FYE or SFYE

It was thought that the addition of FYE greatly influenced the outcome of the long-term tests, but it was not clear whether the difference was simply nutritional, or—the more likely explanation—that a fraction of the FYE served as slowly available electron donor which fueled dechlorination after the initial burst from primary donor degradation. This effectively masked the expected differences between the more slowly degraded H₂ sources which produce low levels of H₂ and those which are degraded more rapidly and produce higher H₂ levels.

This issue was explored in a series of TISs using ethanol, which generated a high-level H₂-production pattern that resulted in incomplete dechlorination during TISs. TISs were performed with duplicate cultures amended with ethanol only, ethanol with FYE, or ethanol with a surrogate FYE (SFYE, a blend of VFAs expected to contribute reducing equivalents comparable to FYE, but without FYE's micronutrient contribution).

Ten bottles were prepared with a 20 percent dilution of the high-PCE/methanol culture as described in Section 3.B.1. At set-up and then two days later, cultures were fed 11 µmol PCE, 88 µmol H₂, and FYE to ensure healthy cultures, and to determine that both dechlorinators and methanogens were active. After 4 days the experiment was begun (defined as Day 0). Thereafter, the cultures were fed (according to the long-term protocol in Table 4.1) PCE (11 µmol), ethanol (44 µmol), 40 µL FYE, and vitamins every fourth day. The bottles were operated for 30 days before the TISs were performed. FYE was routinely added to all the cultures as a

nutritional supplement during long-term operation, but during the TISs, either no supplement, FYE or SFYE was added as an amendment.

The results of the 40-day operational period at a 2:1 ethanol to PCE ratio was similar for all the bottles carried through the test. PCE was dechlorinated to VC and ETH as was observed in the earlier long-term operation of 2:1 ethanol to PCE cultures.

4.B.1. Results of Time-Intensive Tests with Ethanol Plus Supplements

Each ethanol plus supplement TIS yielded a similarly patterned TIS in terms of H_2 and CH_4 produced; however, different trace levels of VFAs were present depending upon the supplement added and thus different extents of dechlorination were obtained. A complete data set is shown in Figure 4.16 for one such TIS—an ethanol/SFYE-amended bottle. In nearly all respects the trend for the TIS is very much like that of EtOHTIS 4 shown in Figure 4.10. The difference is that the addition of SFYE also added significant quantities of VFAs (primarily butyric and propionic acids) which served as slowly available donors to fuel slower but complete dechlorination of PCE after ethanol was depleted. The duplicate test yielded very similar results.

Figure 4.17 presents a comparison of the results for the three different supplement conditions. H_2 is presented as a nmol/bottle quantity so that differences in the low levels can be seen more readily. In each case, the H_2 peaked at approximately 4000 nmol ($10^{-2.8}$ atm), but only the lower portion of the H_2 curve is shown. The VFAs butyric, propionic, isobutyric,

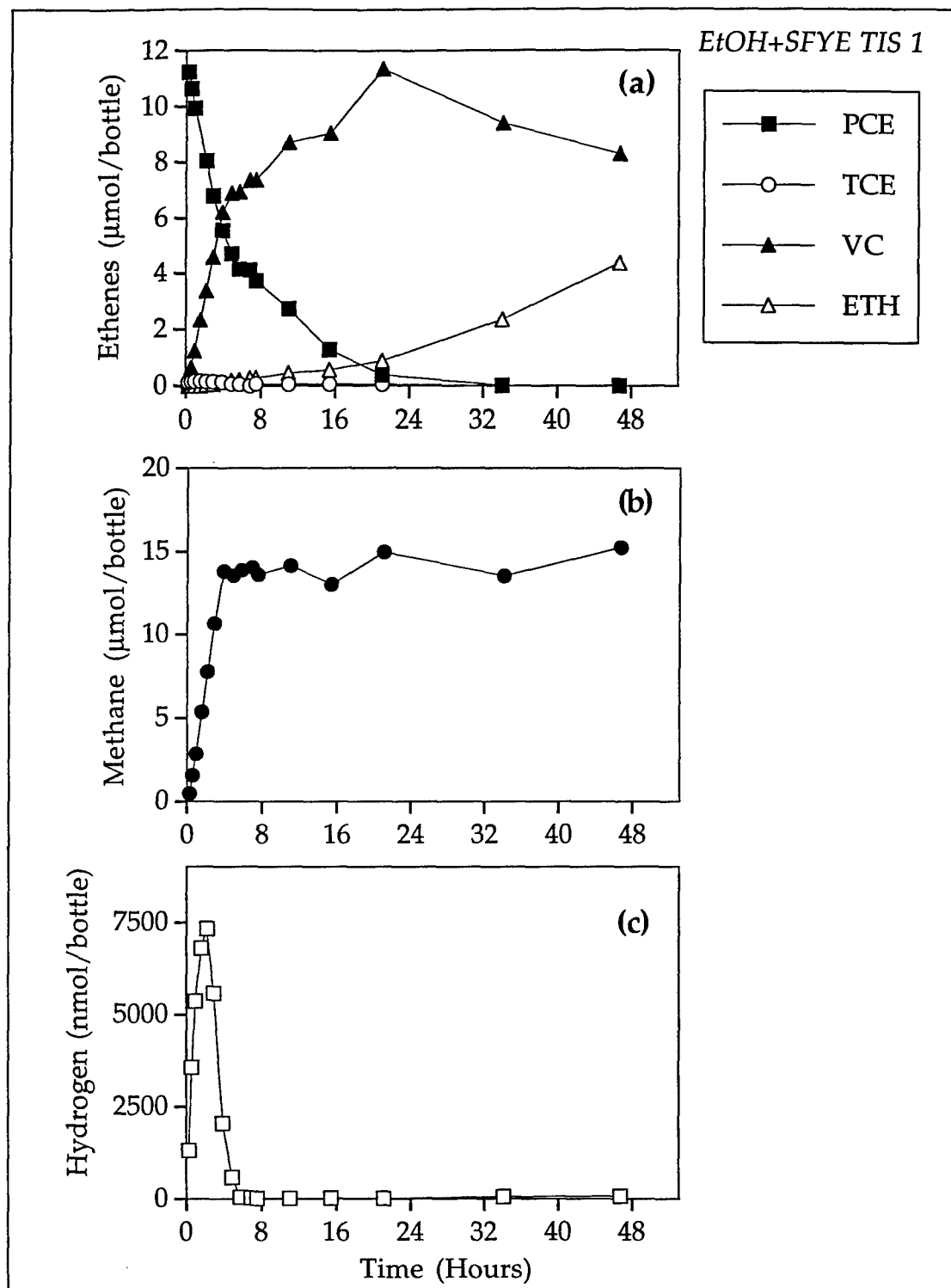
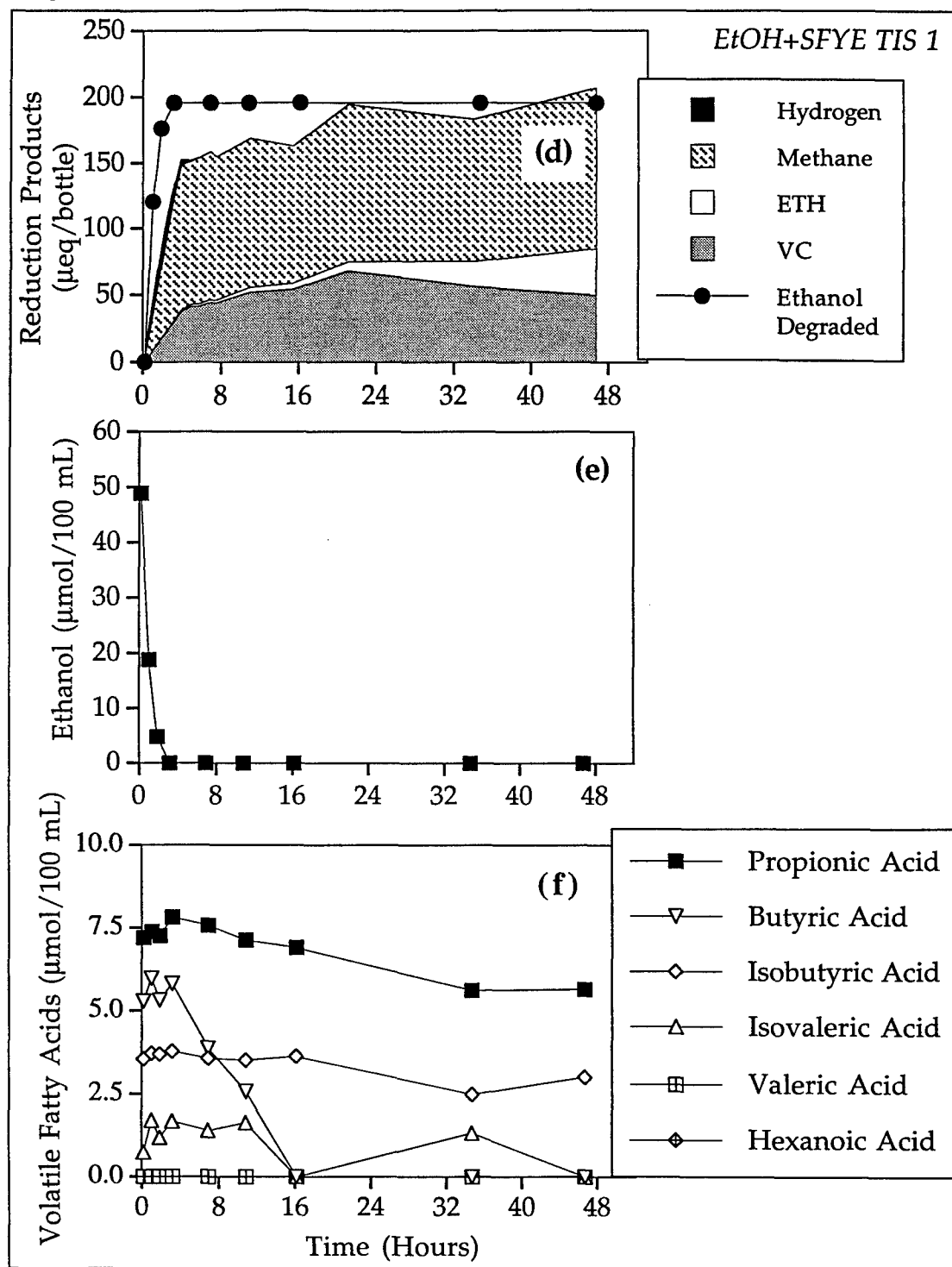


Figure 4.16. Time-intensive study of 2:1 ethanol to PCE ratio with SFYE: (a) dechlorination; (b) methane; (c) hydrogen; (d) reduction products; (e) ethanol; and (f) VFAs.

Figure 4.16 (Continued)



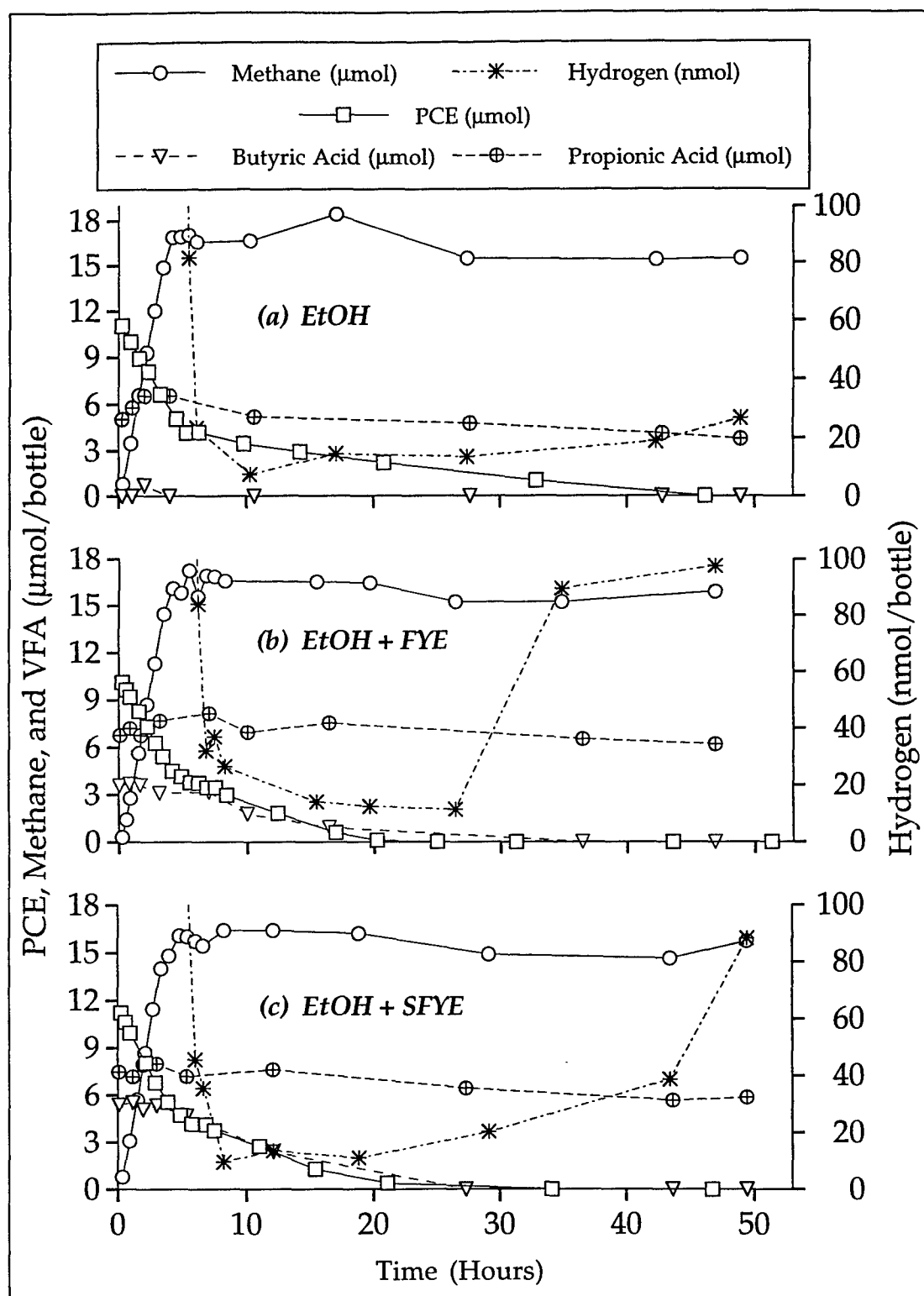


Figure 4.17. Time-intensive studies performed with ethanol-fed cultures at a 2:1 donor to PCE ratio with nutritional and electron donor supplements.

isovaleric, and hexanoic acids were quantified during these tests, however, only butyric and propionic acids were present in significant amounts.

When ethanol alone was added (Figure 4.17a), a typical response was observed—dechlorination and methanogenesis initially proceeded very rapidly, then methanogenesis ceased and dechlorination continued at a much slower rate as H_2 levels fell below the level that supported methanogenesis. Note that propionic acid—a potential electron donor—was present in the culture—as a residual of FYE amendment during the preceding 30 days, or perhaps produced during the degradation of ethanol. Note that as ethanol was depleted during the first 4 hr, the propionate did rise slightly. Its slow degradation after the H_2 levels fell below 20 nmol ($10^{-5.1}$ atm) continued to fuel dechlorination, though a sharp decrease in rate of dechlorination was observed.

Addition of FYE with the ethanol (Figure 4.17b) resulted in a less sharply delineated "break" in the rate of PCE dechlorination after the H_2 produced from ethanol degradation was depleted. The primary factor in this case was the presence of butyric acid (a constituent of FYE). Butyric acid was not degraded while H_2 was present at high levels, but when H_2 fell below about 30 nmol ($10^{-4.9}$ atm), butyric acid was degraded readily.

Addition of SFYE along with ethanol (Figure 4.17c) yielded results that were very similar to those of ethanol plus FYE—both butyric and propionic acids were degraded after ethanol was depleted—confirming the suspicion that FYE was serving as an important supplemental electron donor as well as nutritional supplement during the long-term tests.

4.C. *Butyric Acid Source Culture*

One of the purposes of this study was to demonstrate, over a period of years, that one of the low-H₂-producing donors could serve as an effective, stable stimulator of dechlorination even in the presence of competing methanogens at a noninhibitory, but significant intermediate PCE level. The high-PCE/methanol source culture [61] and H₂-amended purified-cultures of the dechlorinator [152] have been operated over long periods with sustained dechlorination. However, these cultures essentially operated in the absence of methanogens—through inhibition by high PCE in the case of the methanol cultures, and through purification in the case of the H₂-cultures. Thus, it was important to document dechlorination stability under conditions of a potentially functioning methanogenic population. Early experiments (data not shown) compared butyric acid as an H₂ donor at “intermediate”, noninhibitory PCE concentrations to methanol, ethanol, and lactic acid [78] and as a result of those experiments, a decision was made to further investigate the use of butyric acid over the long-term as a H₂ donor for PCE dechlorination at non-inhibitory PCE concentrations.

4.C.1. Start-Up Period

A 9-L source culture was started using a 10 percent dilution of the high-PCE/methanol culture as described in Section 3.A.3. The culture operational protocol is shown in Table 3.3. Prior to Day 125, no vitamin solution was added. Results of the operation of this culture are shown in Figure 4.18 on a per-100-mL basis so that comparisons to serum bottle studies are facilitated.

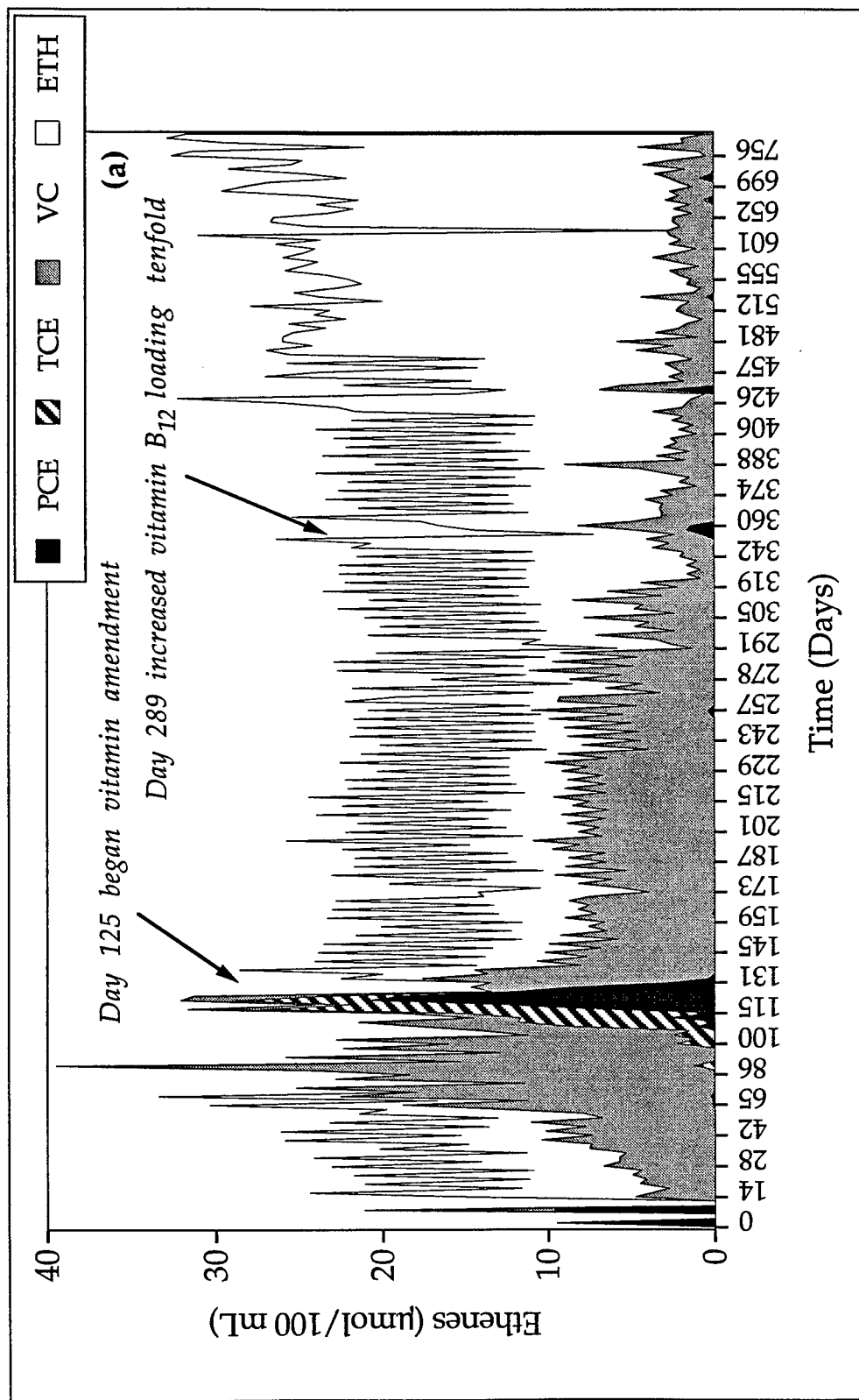
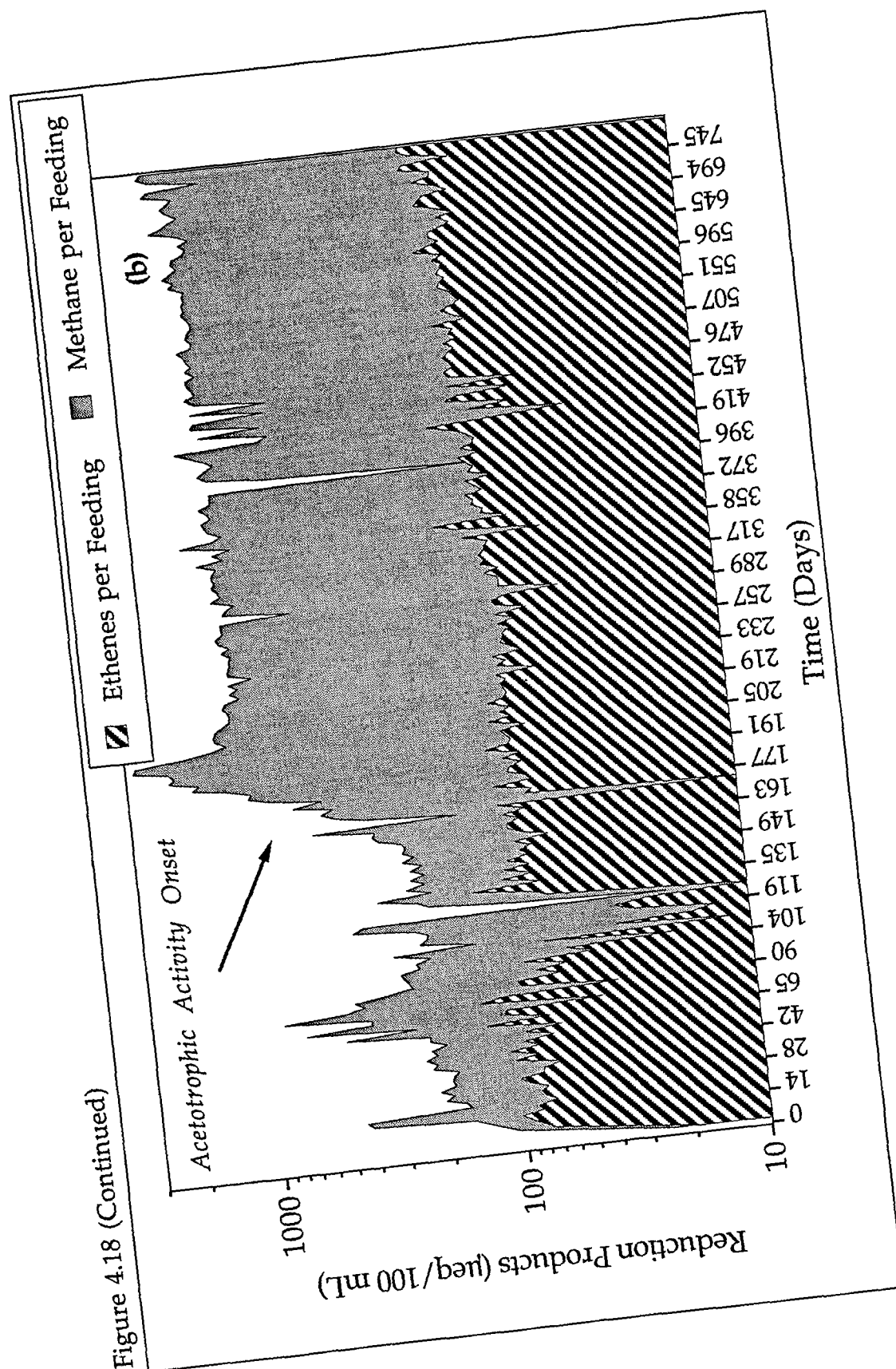
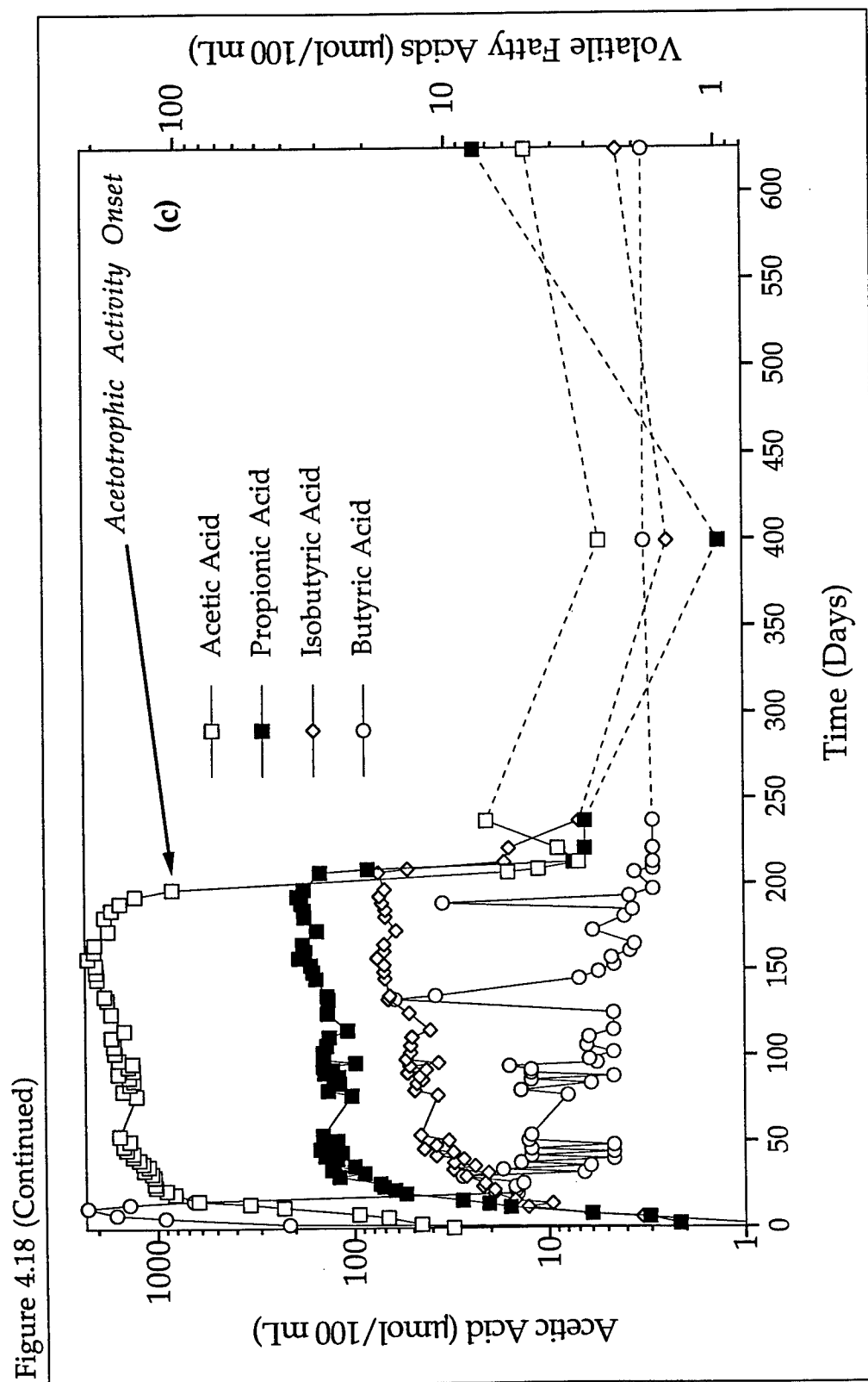


Figure 4.18. Low-PCE/butyric acid source culture performance over 2.2 years of operation:
 (a) dechlorination; (b) reduction products; and (c) VFAs.

Figure 4.18 (Continued)





A lag of 12 days was observed before the culture began to degrade butyric acid and dechlorinate PCE. Unfortunately, during the start-up period, the gas chromatography system was inoperable for several days and only two headspace analyses were performed during the first 12 days (Figure 4.18a and b). On Day 14 when headspace analysis was once again possible, all PCE had been dechlorinated to VC and ETH. Butyric acid was also not degraded substantially until after Day 12 (Figure 4.18c), and excess CH_4 was produced on Days 16, 18, and 20 from the accumulated donor (Figure 4.18b). Residual butyric acid concentrations after Day 18 were typically $7.5 \mu\text{mol}/100 \text{ mL}$. From undetectable concentrations at start up, propionic and isobutyric acids increased to 25 and $10 \mu\text{mol}/100 \text{ mL}$, respectively (Figure 4.18c). These amounts were somewhat higher than that expected from FYE addition alone (13 and $7.5 \mu\text{mol}/100 \text{ mL}$, respectively, see Section 4.A.2.f). After Day 18 when the remaining accumulated butyric acid was depleted, slightly more than the stoichiometric amount of reducing equivalents added as butyric acid ($176 \mu\text{eq}$ on a H_2 equivalent basis) were routinely accounted-for as CH_4 and ethenes (VC and ETH) (Figure 4.18b).

4.C.2. Vitamin Amendment

The culture performed well during the first 50 days, but beginning at about Day 65, culture performance began to deteriorate—less ETH was produced and more VC remained. After 100 days, TCE was a regular and increasing end product. After 115 days, PCE was detected and dechlorination eventually ceased completely (Figure 4.18a). While

dechlorination was failing, butyric acid continued to be fermented and the H_2 was converted to CH_4 (Figure 4.18b and c).

The failure of dechlorination was thought most likely to have been caused by a nutrient limitation. On Day 125, amendment with a vitamin solution that was routinely added to the purified cultures [152], but had never been added to the high-PCE/methanol source culture, was initiated. The initial formulation of the vitamin solution was as described in Table 3.7, except the vitamin B_{12} concentration was 1 mg/L solution. The entire culture volume, 5.69 L, was brought to the desired vitamin concentration by the addition of 28.5 mL of the vitamin solution. Thereafter, 2.85 mL of vitamin solution was added after basal medium exchange on every fourth day. On Day 125 when the vitamin solution was initially added, the butyric acid was inadvertently omitted at the time of feeding. Thus, on day 127, little dechlorination was observed. Despite this oversight, the effect of the addition of vitamin solution on dechlorination during subsequent days was dramatic. Eight days after the addition of the vitamins, PCE was once again being degraded primarily to VC and ETH. Maymó-Gatell *et al.* [78, 152] later determined that the crucial vitamin in the solution was vitamin B_{12} and that for optimizing dechlorination, the concentration should be increased 10-fold. Therefore, on Day 289, the vitamin B_{12} concentration in the culture was increased to 0.5 mg/L and the vitamin B_{12} of the vitamin solution was adjusted to 10 mg/L to supply this amount thereafter. After the change, dechlorination again improved over what had been observed previously. After Day 289, the culture dechlorinated about 83 percent of the added PCE to ETH and the remainder to VC in a very stable and continuous manner.

4.C.3. Biomass

The biomass content of the low-PCE/butyric acid culture is shown in Figure 4.19. Biomass is presented as mg VSS/L and was measured either through VSS determination or estimated from PON analysis. The biomass concentration eventually stabilized at approximately 70 mg VSS/L.

4.C.4. Effect of High PCE

An unsuccessful attempt was made to start a high-PCE/butyric acid source culture. An enrichment culture was started from inoculum (6.8 percent) from the low-PCE/butyric acid culture. The new culture was to operate on a high-PCE protocol (550 $\mu\text{mol/L}$ nominal PCE concentration) similar to that in the high-PCE/methanol cultures.

This culture did not start up successfully at the high PCE level. Butyric acid was not degraded and PCE was not dechlorinated. After Day 47, the PCE concentration was reduced to 110 $\mu\text{mol/L}$ and vitamin amendment, which had just been initiated in the original low-PCE/butyric acid culture, was initiated. Dechlorination began and the culture appeared to be recovering. As the PCE concentration was again increased stepwise to 550 $\mu\text{mol/L}$, dechlorination again failed. After Day 90, PCE was again reduced to 110 $\mu\text{mol/L}$ and the low-PCE protocol was used for the remainder of the study. This duplicate low-PCE/butyric acid culture yielded results that were similar to the results already presented for the original low-PCE/butyric acid culture.

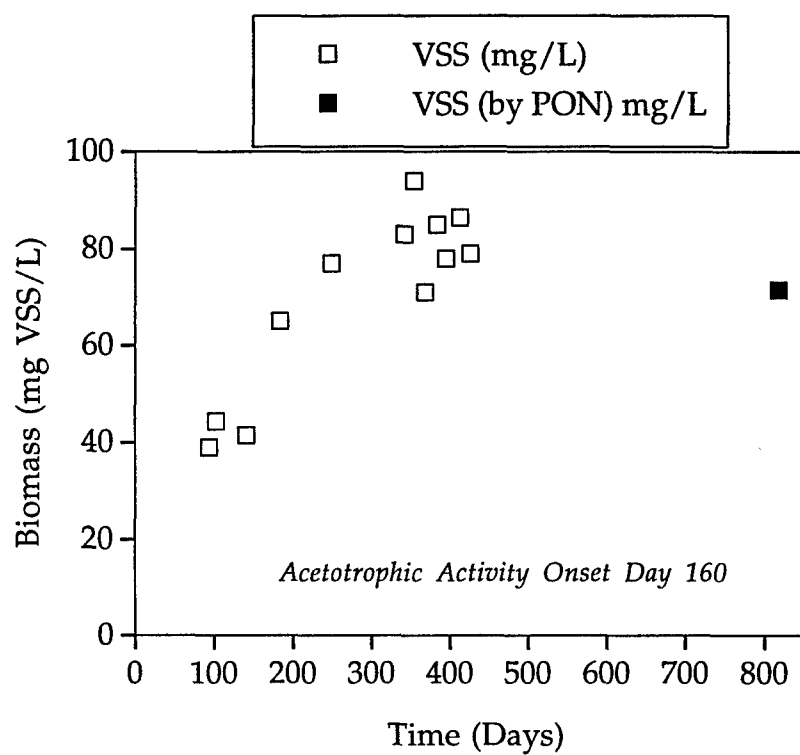


Figure 4.19. Biomass content of low PCE/butyric acid culture by VSS measurement and by PON analysis.

4.C.5. Time Intensive Studies of Low-PCE/Butyric Acid Source Culture

On Day 384, 100 percent culture from the low-PCE/butyric acid source culture was delivered directly from the source culture to two serum bottles as described in Section 3.B.1. The bottles were carried through the regular long-term low-PCE 2:1 ratio of butyric acid to PCE protocol as shown in Table 4.1 for 4 days to ensure a good transfer. After purge and basal medium exchange, the cultures were amended with 11 μmol of neat PCE and shaken for 4 hr on a wrist action shaker at 35°C to solubilize the PCE. After this equilibrium, the bottles were amended with 44 μmol neat butyric acid, then were carried through a TIS as described in Section 3.B.4. The duplicates behaved similarly and the results from one of the bottles are shown in Figure 4.20. This result compares well with the results of the 2:1 butyric-acid-amended serum bottle enrichment (Figure 4.8).

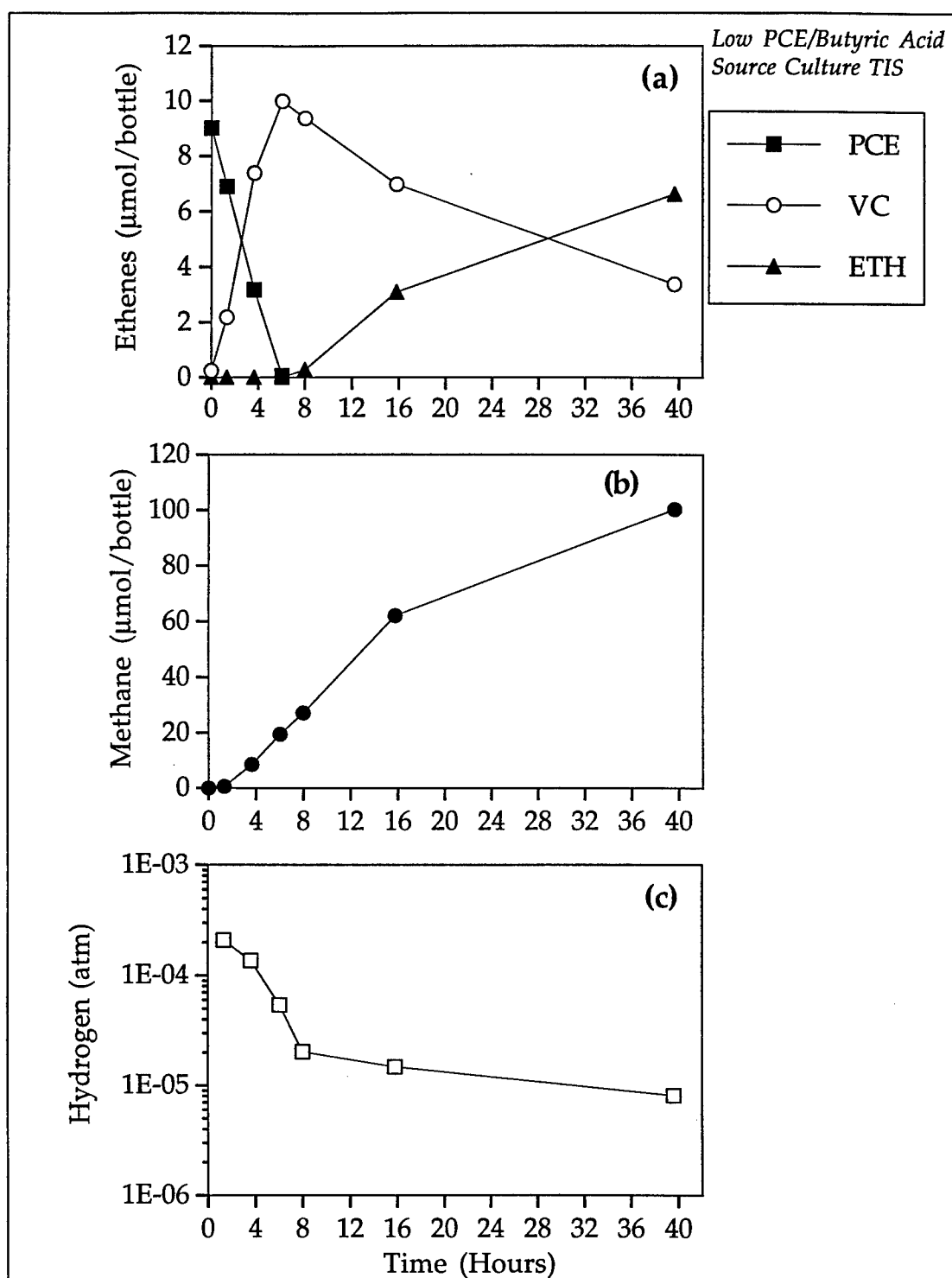
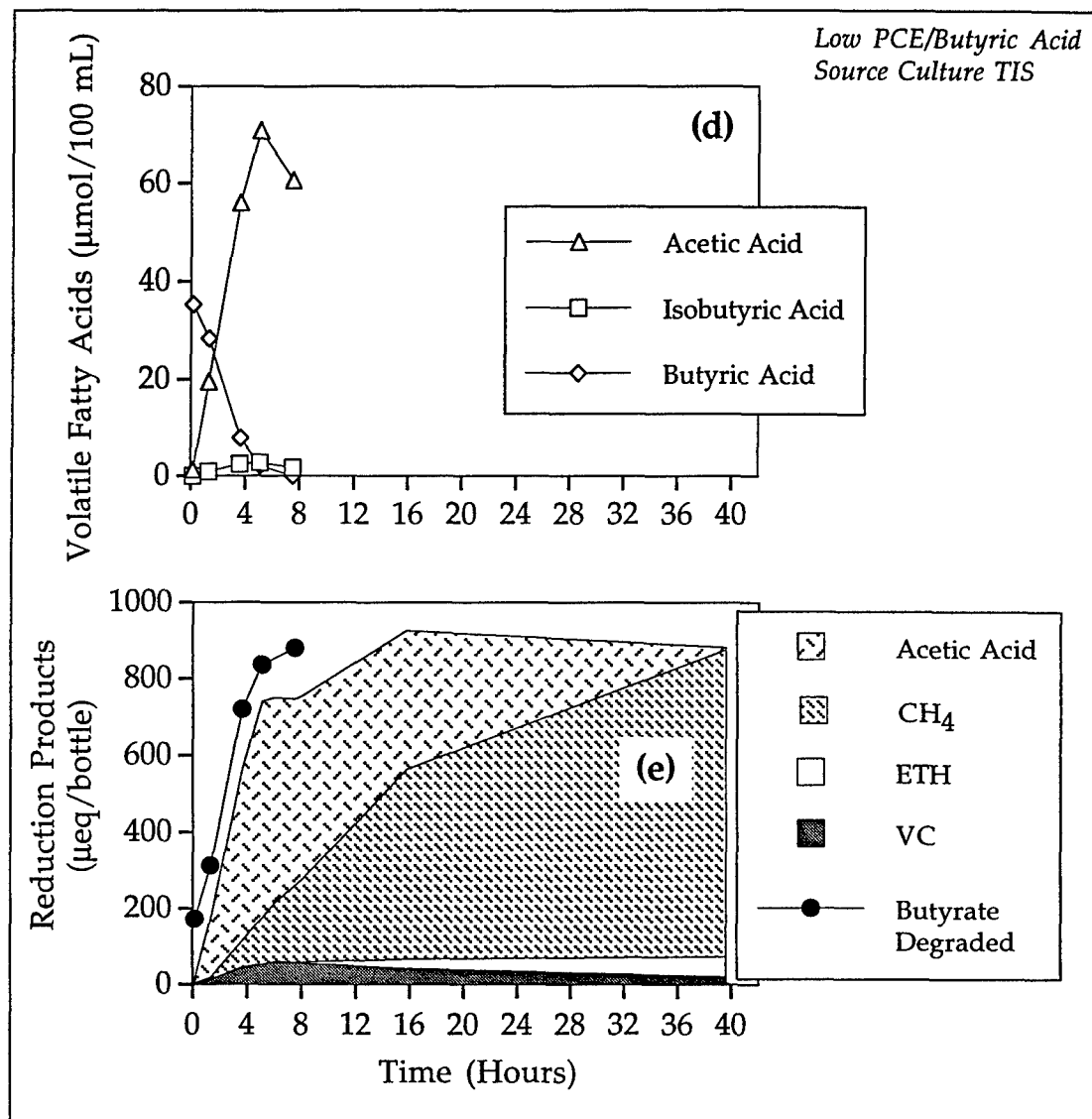


Figure 4.20. Time-intensive study of the low-PCE/butyric acid source culture on Day 384 of operation: (a) dechlorination; (b) methane; (c) hydrogen; (d) VFAs; and (e) reduction products.

Figure 4.20 (Continued)



CHAPTER FIVE

MODEL DEVELOPMENT

5.A. Modeling with STELLA Research®

A model—H₂CompPCE, Version 4.4.1—was developed using STELLA Research® Version 4.02 (High Performance Systems, Inc.). STELLA is an icon-driven, multi-level, hierarchical environment for constructing and interacting with finite-difference dynamic models. It allows visual, intuitive construction of complex models and sub-models and has two major layers that allow easy management of and interaction with highly complex models. A complete printout of H₂CompPCE, Version 4.4.1 is shown in Appendix III.

STELLA Research® was run on a Power Macintosh 7500. Data collected during simulations were automatically transferred to a worksheet in Microsoft® Excel 5.0 using Publish and Subscribe. After further manipulation in Excel 5.0, data were copied to CricketGraph III (Computer Associates) for graphical presentation.

5.A.1. Limitations of STELLA Research®

While STELLA Research® offers many advantages by allowing convenient and intuitive model construction, it does have serious limitations. For any one simulation, STELLA Research® allows only 32,672 time steps. Thus, the smaller the time step (dt) chosen, the shorter the maximum simulation time may be. For the dt used in this study, 0.03125 hr, the maximum simulation time was 1021 hr or 42 days. (Collection of data during a simulation of this length required massive

amounts of memory as STELLA inexplicably saves, in RAM, all data collected during a simulation run.) Long-term runs of 100+ days were carried out by running shorter simulations of 8 days in series and using ending values from the previous run as starting values for the next run.

A related problem was encountered in modeling the gaseous components H_2 and CH_4 . Modeling the transport of H_2 and CH_4 between the gas and aqueous phases required a dt as small as 0.005 hr to avoid instabilities in the model. H_2 was especially problematic since the aqueous H_2 pool is very small. The very small dt was required to avoid transporting excessively large "packets" of H_2 between the gas and liquid phases. The transport of "packets" that were too large resulted in the gaseous and aqueous phase H_2 stocks oscillating between zero and a finite value.

Thus, to avoid instability and to allow relatively long simulations, a compromise dt of 0.03125 hr was used and equilibrium was assumed between the gaseous and aqueous phases for H_2 and CH_4 . It was assumed that, given such extremely small dt values necessary to capture the dynamics of gas/liquid exchange, then such exchange must—almost by definition—be sufficiently rapid to justify an equilibrium assumption. The equilibrium assumption was validated by running a fuller version of the model that included a non-equilibrium module for H_2 (see Appendix IV).

Another limitation of STELLA Research® is that while data may be collected for any time increment greater than or equal to the dt , the increment of data collection is a global command. If one wishes to collect data for a certain parameter every 0.5 hr, but need data for another parameter for only 4-hr increments, it is not possible to separate these two

collection increments; the smaller value must be chosen for the entire model. Thus, in general, large quantities of data must be collected.

5.B. Timed Events for Simulating Serum Bottle Operation

The model was intended to simulate the operation of 160-mL serum bottles containing 100 mL of enrichment cultures as described in Chapter 3. The donors, acetate, and biomass were modeled with aqueous stocks (Mt) based on total μmol (substrates) or total mg VSS (biomass) in the bottle. Modeling the volatile compounds required consideration of aqueous-phase and gaseous-phase partitioning and in the case of the chloroethenes, stocks for both aqueous and gaseous-phases were utilized (Section 5.C).

5.B.1. Pulse Feedings

The every-second-day pulsed feedings of PCE, donor, and FYE were simulated using a PULSE function. In STELLA®, the PULSE function takes the form:

PULSE (<volume>[,<first pulse>, <interval>])

Where: *volume* is the size of the input, *first pulse* is the time at which the first pulse occurs, and *interval* is the time interval between subsequent pulses. The times that governed the pulse functions are shown in Table 5.1.

5.B.2. Waste and Purge Events

The every-fourth-day wasting events for exchange of basal medium and purging of the volatile compounds from the gaseous and aqueous phases were also simulated using a PULSE function. Wasting resulted in the dilution of the aqueous-phase, nonvolatile components (donors,

acetate, and biomass) by 10 percent at the appropriate time (just prior to feeding events). Purging the volatile compounds (the chloroethenes, H_2 , and CH_4) was simulated by using a PULSE function to empty appropriate gaseous or aqueous stocks at the appropriate time (just prior to feeding events). An IF...THEN... function was used to zero all volatile compound flows at the same instant. See Table 5.1 for relevant parameters.

Table 5.1. Model parameters governing feeding, wasting, and purging events.

Parameter	Meaning	Value
Feed Pulse Time PCE	time of first PCE pulse event	0 hr
Feed Pulse Time Donor	time of first donor pulse event	0 hr
Feed Increment Time	time interval between successive pulse feedings of PCE and donor	48 hr
Liquid Waste Rate	dilution upon basal medium exchange	0.1
Waste Pulse Time	time of first wasting/purging event	96-dt hr
Waste Increment Time	time interval between waste/purge events	96 hr

5.C. Modeling Volatile Compounds: Gas/Liquid Transfer

5.C.1. Chlorinated Ethenes

The chlorinated ethenes were modeled by giving each compound in the series an aqueous stock (M_w) representing the total μmol in the aqueous phase and a gaseous stock (M_g) representing the total μmol in the gaseous phase. For the aqueous PCE stock, the input flow was a pulse input feeding of PCE which simulated culture amendment, and the outputs were

the dechlorination of PCE to TCE, the volatilization/dissolution biflow explained below, and the purge pulse outflow. For each of the other chlorinated ethenes, the input to the aqueous phase was its production from the dechlorination of the next-higher chlorinated compound of the series, and its outputs were its dechlorination to the next-lower chlorinated compound in the series, the volatilization/dissolution biflow, and the purge outflow.

Each aqueous stock was connected to its gaseous counterpart by a volatilization/dissolution biflow that simulated the exchange of the compound between the gaseous and aqueous phases. When the flow was in the direction from the gaseous phase to the aqueous phase, dissolution was simulated, and when the flow was in the direction from the aqueous phase to the gaseous phase, volatilization was simulated. The volatilization/dissolution model was of the form shown in Equation 5.1. The difference in concentration between the two phases at each time step determined the direction of the flow—either from the aqueous phase to the gaseous phase or vice-versa.

$$\text{Volatilization/Dissolution} = V_w \times K_{la} \times \left(\frac{C_g}{H_c} - C_w \right) \quad (5.1)$$

Where:

- V_w = volume of the liquid portion of the bottle (L);
- K_{la} = mass transfer coefficient (hr^{-1});
- C_g = concentration of the compound in the gaseous phase ($\mu\text{mol/L}$);
- C_w = concentration of the compound in the aqueous phase ($\mu\text{mol/L}$); and

H_c = pseudo-dimensionless Henry's constant for the compound of interest.

See Table 5.2 for a list of model parameters related to gas-liquid transfer.

5.C.2. Methane

The model was constructed to allow tracking of the CH_4 pool generated from H_2 use separately from the CH_4 pool generated from acetate. Each CH_4 pool was modeled as an aqueous stock (M_t) representing the total μmol in the bottle. Equilibrium between the gaseous and aqueous phases was assumed. Separate gaseous- and aqueous-phase stocks with the volatilization/dissolution biflow were not included as they were for the chloroethenes because of the excessively small dt values required to avoid model instability (see Section 5.A.1). Aqueous- and gaseous-phase concentrations were calculated from the total stock using Equations 5.2 and 5.3. See Table 5.2 for a list of model parameters related to gas-liquid transfer.

$$C_{wCH_4} = \frac{M_{tCH_4}}{V_w + (H_{cCH_4} \times V_g)} \quad (5.2)$$

$$C_{gCH_4} = \frac{M_{tCH_4}}{\left(\frac{V_w}{H_{cCH_4}} \right) + V_g} \quad (5.3)$$

Where:

V_w = volume of the liquid portion of the bottle (L);

V_g = volume of the gas portion of the bottle (L);

Mt_{CH_4} = total CH_4 in the bottle (μmol);

Cg_{CH_4} = concentration of CH_4 in the gaseous phase ($\mu\text{mol/L}$);

Cw_{CH_4} = concentration of CH_4 in the aqueous phase ($\mu\text{mol/L}$); and

Hc_{CH_4} = pseudo-dimensionless Henry's constant for CH_4 .

5.C.3. Hydrogen

H_2 , like CH_4 , was modeled as a total aqueous stock (Mt) representing the total μmol in the bottle with equilibrium between the gaseous and liquid phases assumed (as explained in Section 5.A.1). Additionally, the H_2 model also included the formate/ H_2 lyase system, with equilibrium between H_2 and formate in the aqueous phase assumed: $HCOO^-(aq) + H_2O \rightarrow HCO_3^- + H_2(aq)$. In essence, formate was considered a non-volatile part of the total H_2 pool.

The free energy of this reaction at 35°C (see Appendix VI) is 19.03 kJ/mol formate. The ratio of formate (aq) to H_2 (aq)—assuming equilibrium—was calculated to be 121.8. Since no formate measurements were made during this study, there are no experimental data with which to compare this value. However, the value is of similar magnitude to those previously reported (50 to 350) for our enrichment cultures in a separate study [210]. The model included the calculation of this ratio so that the ratio would reflect the ionic strength and bicarbonate concentration which could be entered as model parameters.

Aqueous- and gaseous-phase concentrations of H_2 were calculated from the total stock (Mt) using Equations 5.4 and 5.5. See Table 5.2 for a list of model parameters related to gas-liquid transfer.

$$C_{W_{H_2}} = \frac{M_{t_{H_2}}}{V_w + (H_{c_{H_2}} \times V_g) + (\text{Formate}/H_2 \text{ ratio} \times V_w)} \quad (5.4)$$

$$C_{g_{H_2}} = \frac{M_{t_{H_2}}}{\left(\frac{V_w}{H_{c_{H_2}}}\right) + V_g + \left(\text{Formate}/H_2 \text{ ratio} \times \frac{V_w}{H_{c_{H_2}}}\right)} \quad (5.5)$$

Where:

$M_{t_{H_2}}$ = total H_2 in the bottle (μmol);

$C_{g_{H_2}}$ = H_2 gaseous-phase concentration ($\mu\text{mol/L}$);

$C_{W_{H_2}}$ = H_2 aqueous-phase concentration ($\mu\text{mol/L}$);

Formate/ H_2 ratio = ratio of formate (aq) to H_2 (aq) assuming equilibrium; and

$H_{c_{H_2}}$ = pseudo-dimensionless Henry's constant for H_2 .

Table 5.2. Model parameters for volatile compound partitioning (35°C).

Compound	Kla (hr^{-1})	Hc
PCE	25 ^a	1.116 ^c
TCE	36 ^b	0.591 ^c
cDCE	38.2 ^b	0.216 ^c
VC	40 ^a	1.42 ^c
ETH	60 ^a	9 ^c
CH_4	50 ^a	33.1 ^d
H_2	69.3 ^a	52.7 ^e

^a [208]; ^b estimated from [208] and [198]; ^c [93]; ^d [59]; ^e [272].

5.D. Modeling Primary Donor Fermentation

Fermentations of the H_2 donors used in this study proceed only when the reaction is thermodynamically favorable—i.e. when the Gibbs free energy for the reaction, ΔG_{rxn} , acquires a negative value. Furthermore,

since the physiological requirements of microorganisms dictate that a finite amount of free energy must be conserved before the organism can produce ATP, the free energy required for conservation of energy may be a set maximum value— $\Delta G_{\text{critical}}$ —which has been described in the literature and has been reported to be from -10 to -20 kJ per mol of donor for butyrate- or ethanol-oxidizing syntrophic bacteria [67, 190, 201, 202, 235]. For this reason, modeling was based on the premise that a specific amount of free energy must be available before the organism can conserve energy.

For a reaction such as $aA + bB \rightarrow cC + dD$, occurring under nonstandard culture conditions, ΔG_{rxn} is calculated using the Nernst equation, Equation 5.6.

$$\Delta G_{\text{rxn}} = \Delta G_{35^\circ}^{\circ} + RT \ln \left\{ \frac{\gamma_c [C]^c \gamma_d [D]^d}{\gamma_a [A]^a \gamma_b [B]^b} \right\} \quad (5.6)$$

Where:

$\Delta G_{35^\circ}^{\circ}$ is defined as the standard free energy (kJ/mol) at 35°C, with all solutes, including H^+ and H_2 (as an aqueous component rather than a gaseous one) at unit activity; R is the gas constant; T is the temperature ($^{\circ}K$); A , B , etc. are actual measured culture concentrations (mol/L); a , b , etc. are the stoichiometric coefficients for the reaction; and γ_a , γ_b , etc. are the activity coefficients for the respective constituents (see Appendix V).

Thus, the favorability of the reaction depends in great part on the relative concentrations of the products versus the reactants. Product accumulation can “shut down” a given fermentation by making it unfavorable for the organism. The primary product of concern in this

study was H_2 , however acetate exerts a similar influence and must also be considered.

5.D.1. Kinetic Model for Donor Fermentation

The model developed to describe the degradation of the H_2 donors is in the form of Michaelis-Menten kinetics, but with the inclusion of the effects of the formation of the products acetate and H_2 on the overall kinetics of the fermentation.

The model is an empirical one and the general form is shown in Equation 5.7:

$$\frac{dMt_{\text{Donor}}}{dt} = \frac{-k_{\text{Donor}}X_{\text{Donor}}(S - S^*)}{K_{S(\text{Donor})} + S} \quad (5.7)$$

Where:

Mt_{Donor} = total amount of substrate (donor) in the bottle (μmol);

k_{Donor} = maximum specific rate of donor degradation ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$);

X_{Donor} = donor-fermenting biomass in the serum bottle (mg VSS);

$K_{S(\text{Donor})}$ = half-velocity coefficient for the donor ($\mu\text{mol}/\text{L}$);

S = substrate (donor) concentration ($\mu\text{mol}/\text{L}$); and

S^* = hypothetical value of substrate (donor) concentration that, under the instantaneous culture conditions, would result in $\Delta G_{\text{rxn}} = \Delta G_{\text{critical}}$, given the concentrations of all the other reactants and products at that instant.

S^* is the "equilibrium" concentration of S and it is related to $\Delta G_{\text{critical}}$ as follows:

$$\Delta G_{\text{critical}} = \Delta G_{35^{\circ}\text{C}}^{\circ} + RT \ln \left(\frac{[\text{products}]}{S^* [\text{other reactants}]} \right)$$

Similarly, S is related to ΔG_{rxn} :

$$\Delta G_{\text{rxn}} = \Delta G_{35^{\circ}\text{C}}^{\circ} + RT \ln \left(\frac{[\text{products}]}{S [\text{other reactants}]} \right)$$

Subtracting and simplifying,

$$\frac{\Delta G_{\text{rxn}} - \Delta G_{\text{critical}}}{RT} = \ln \frac{S^*}{S}$$

$$\exp \left(\frac{\Delta G_{\text{rxn}} - \Delta G_{\text{critical}}}{RT} \right) = \frac{S^*}{S}$$

$$1 - \exp \left(\frac{\Delta G_{\text{rxn}} - \Delta G_{\text{critical}}}{RT} \right) = 1 - \frac{S^*}{S} = \frac{S - S^*}{S}$$

$$S - S^* = S \times \left[1 - \exp \left(\frac{\Delta G_{\text{rxn}} - \Delta G_{\text{critical}}}{RT} \right) \right] = S \times \Phi \quad (5.8)$$

where, Φ = the “thermodynamic factor”,

$$\Phi = 1 - \exp \left(\frac{\Delta G_{\text{rxn}} - \Delta G_{\text{critical}}}{RT} \right) \quad (5.9)$$

Thus, $(S - S^*)$ in Equation 5.7 is replaced by $S\Phi$ as per Equation 5.8, with the thermodynamic factor calculated using Equation 5.9, giving the form—Equation 5.10—found in the STELLA model.

$$\frac{dMt_{\text{Donor}}}{dt} = \frac{-k_{\text{Donor}} X_{\text{Donor}} S\Phi}{K_{S(\text{Donor})} + S} \quad (5.10)$$

Where:

Φ = the thermodynamic factor, "thermo factor", ranging from one (when there is no thermodynamic limitation on donor fermentation) to zero when $\Delta G_{\text{rxn}} \geq \Delta G_{\text{critical}}$.

Thus, for each time increment of the model, the ΔG_{rxn} for the donor fermentation reaction was calculated using the instantaneous aqueous-phase concentrations (C_w) of the pertinent compounds for a given fermentation. From ΔG_{rxn} and $\Delta G_{\text{critical}}$, Φ was calculated via Equation 5.9.

Φ is a measure of the distance of the reaction from thermodynamic equilibrium. The further from equilibrium that the fermentation is (i.e. the donor concentration is high relative to the concentration of the products of the reaction, H_2 and acetate), the more driving force there is and the more rapidly the fermentation will proceed. Far from equilibrium, Φ has a value approaching one and the fermentation reaction is limited primarily by kinetics. As the reaction approaches equilibrium (i.e. donor concentration has decreased and the products of the fermentation, H_2 and acetate, have increased), Φ approaches zero and the fermentation proceeds more slowly and is limited primarily by the thermodynamic situation. It

was determined that substituting $S-S^*$ for S in the denominator had little impact on the fit of the model.

The fermentation reactions included in the model and their respective $\Delta G^{\circ}_{35^{\circ}}$ and $\Delta G_{\text{critical}}$ values are shown in Table 5.3. Calculations for determining $\Delta G^{\circ}_{35^{\circ}}$ are shown in Appendix VI. $\Delta G_{\text{critical}}$ was determined for each donor from analysis of experimental data (see Section 4.A.3). In that section, it was shown that in many cases, the ΔG_{rxn} remained below -20 kJ/mol substrate fermented. Therefore, a value of -19 kJ/mol donor was chosen for use in the model.

The kinetic parameters, k and K_S , used in the donor fermentation kinetic model are shown in Table 5.4. These values were estimated from non-linear regression analysis of the experimental data, the measured biomass, and the estimated fraction of relevant biomass (see Appendix VII).

Table 5.3. Fermentation reactions for hydrogen donors examined during this study.

Fermentation to Acetate and H_2	$\Delta G^{\circ}_{35^{\circ}}$ (kJ/mol)	$\Delta G_{\text{critical}}$ (kJ/mol)
$\text{Butyrate}^- + 2 \text{H}_2\text{O} \rightarrow 2 \text{Acetate}^- + \text{H}^+ + 2 \text{H}_2$	123.16	-19
$\text{Ethanol} + \text{H}_2\text{O} \rightarrow \text{Acetate}^- + \text{H}^+ + 2 \text{H}_2$	84.85	-19
$\text{Lactate}^- + 2 \text{H}_2\text{O} \rightarrow \text{Acetate}^- + \text{HCO}_3^- + \text{H}^+ + 2 \text{H}_2$	71.01	-19
$\text{Propionate}^- + 3 \text{H}_2\text{O} \rightarrow \text{Acetate}^- + \text{HCO}_3^- + \text{H}^+ + 3 \text{H}_2$	166.9	-19
Fermentation to Propionate and Acetate	$\Delta G^{\circ}_{35^{\circ}}$ (kJ/mol)	$\Delta G_{\text{critical}}$ (kJ/mol)
$1 \text{ Ethanol} + 2/3 \text{HCO}_3^- \rightarrow$ $2/3 \text{Propionate}^- + 1/3 \text{Acetate}^- + 1/3 \text{H}^+ + 1 \text{H}_2\text{O}$	-26.41	-19
$1 \text{ Lactate}^- \rightarrow 1/3 \text{Acetate}^- + 2/3 \text{Propionate}^- +$ $1/3 \text{HCO}_3^- + 1/3 \text{H}^+$	-40.26	-19

Table 5.4. Kinetic parameters for donor fermentation assuming $\Delta G_{\text{critical}}$ is -19 kJ/mol donor (35°C).

Fermentation	k ($\mu\text{mol/mg VSS-hr}$)	K_S ($\mu\text{mol/L}$)
Butyrate ⁻ → Acetate ⁻ + H ₂	4.9	34.3
Ethanol → Acetate ⁻ + H ₂	21.9	17
Lactate ⁻ → Acetate ⁻ + H ₂	8.6	2.5
Propionate ⁻ → Acetate ⁻ + H ₂	2.2	11.3
Ethanol → Propionate ⁻ + Acetate ⁻	21.9	17
Lactate ⁻ → Propionate ⁻ + Acetate ⁻	8.6	2.5

5.E. Kinetic Models for Dechlorination

Kinetics of dechlorination (Equations 5.11 to 5.15) were described by Michaelis-Menten kinetics wherein the rate of dechlorination is described not only by the chloroethene concentration, but also by the H₂ concentration. Separate modules were incorporated for each of the chloroethenes, although TCE and DCE were not frequently detected species and were modeled so that they were degraded about as rapidly as they were produced. The DCE isomer of primary interest was *cis*-1,2-DCE and the DCE module included only constants for *cis*-1,2-DCE.

$$\left(\frac{dM_{wPCE}}{dt} \right)_{\text{degradation}} = - \frac{k_{PCE} X_{\text{Dechlor}} C_{wPCE}}{K_{S(PCE)} + C_{wPCE}} \times \frac{(C_{wH_2} - H_2 \text{ Threshold}_{\text{dechlor}})}{K_{S(H_2)\text{dechlor}} + (C_{wH_2} - H_2 \text{ Threshold}_{\text{dechlor}})} \quad (5.11)$$

$$\left(\frac{dMw_{TCE}}{dt} \right)_{\text{degradation}} = - \frac{k_{TCE} X_{\text{Dechlor}} Cw_{TCE}}{K_{S(TCE)} + Cw_{TCE}} \times \frac{(Cw_{H_2} - H_2 \text{ Threshold}_{\text{dechlor}})}{K_{S(H_2)_{\text{dechlor}}} + (Cw_{H_2} - H_2 \text{ Threshold}_{\text{dechlor}})} \quad (5.12)$$

$$\left(\frac{dMw_{cDCE}}{dt} \right)_{\text{degradation}} = - \frac{k_{cDCE} X_{\text{Dechlor}} Cw_{cDCE}}{K_{S(cDCE)} + Cw_{cDCE}} \times \frac{(Cw_{H_2} - H_2 \text{ Threshold}_{\text{dechlor}})}{K_{S(H_2)_{\text{dechlor}}} + (Cw_{H_2} - H_2 \text{ Threshold}_{\text{dechlor}})} \quad (5.13)$$

$$\left(\frac{dMw_{VC}}{dt} \right)_{\text{degradation}} = - \frac{k_{VC} X_{\text{Dechlor}} Cw_{VC}}{K_{S(VC)} + Cw_{VC}} \times \frac{(Cw_{H_2} - H_2 \text{ Threshold}_{\text{dechlor}})}{K_{S(H_2)_{\text{dechlor}}} + (Cw_{H_2} - H_2 \text{ Threshold}_{\text{dechlor}})} \quad (5.14)$$

Where, using the PCE equation as an example:

Mw_{PCE} = total amount of PCE in the aqueous phase (μmol);

k_{PCE} = maximum specific rate of the PCE utilization
($\mu\text{mol}/\text{mg VSS-hr}$);

X_{Dechlor} = dechlorinator biomass contained in the serum bottle
(mg VSS);

Cw_{PCE} = aqueous PCE concentration ($\mu\text{mol}/\text{L}$);

$K_{S(PCE)}$ = half-velocity coefficient for PCE use ($\mu\text{mol}/\text{L}$);

Cw_{H_2} = aqueous H_2 concentration ($\mu\text{mol}/\text{L}$);

$K_{S(H_2)dechlor}$ = half-velocity coefficient for H_2 use by dechlorinators ($\mu\text{mol/L}$); and

$H_2 \text{ Threshold}_{dechlor}$
= threshold for H_2 use by dechlorinators ($\mu\text{mol/L}$).

It was assumed that the same biomass, $X_{Dechlor}$, was responsible for each step of the dechlorination. The parameters used in the kinetic model for chloroethene and H_2 use by dechlorinators were taken or estimated from previous studies [208, 229] with "*D. ethenogenes*" and are shown in Table 5.5.

Table 5.5. Kinetic parameters for dechlorination (35°C).

Compound	$k(\text{Chloroethene})$ ($\mu\text{mol/mg VSS-hr}$)	$K_S(\text{Chloroethene})$ ($\mu\text{mol/L}$)	H_2 $\text{Threshold}_{dechlor}$ ($\mu\text{mol/L}$)
PCE	1.8 ^a	0.54 ^c	--
TCE	3 ^b	0.54 ^c	--
cDCE	3 ^b	0.54 ^c	--
VC	3 ^c	290 ^c	--
H_2	--	0.1 ^c	0.0015 ^d

^a [151]; ^b estimated [229]; ^c [208]; ^d observation from experimental data this study.

5.F. Kinetic Model for Hydrogenotrophic Methanogenesis

Methanogenesis from H_2 was also modeled using a Michaelis-Menten-type kinetic equation which incorporated the threshold for H_2 use by methanogens.

$$\left(\frac{dMt_{CH_4 \text{ from } H_2}}{dt} \right)_{\text{production}} = \frac{1}{4} k_{(H_2)\text{meth}} X_{\text{Hydrogenotroph}} \times \frac{(C_{W_{H_2}} - H_2 \text{ Threshold}_{\text{meth}})}{K_{s(H_2)\text{meth}} + (C_{W_{H_2}} - H_2 \text{ Threshold}_{\text{meth}})} \quad (5.15)$$

Where:

- $Mt_{CH_4 \text{ from } H_2}$ = total CH_4 produced via hydrogenotrophs (μmol);
- $k_{(H_2)\text{meth}}$ = maximum rate of H_2 utilization ($\mu\text{mol}/\text{mg VSS-hr}$);
- $X_{\text{Hydrogenotroph}}$ = hydrogenotrophic methanogenic biomass contained in the bottle (mg VSS);
- $C_{W_{H_2}}$ = aqueous hydrogen concentration ($\mu\text{mol}/\text{L}$);
- $K_{s(H_2)\text{meth}}$ = half-velocity coefficient for H_2 use by hydrogenotrophic methanogens ($\mu\text{mol}/\text{L}$); and
- $H_2 \text{ Threshold}_{\text{meth}}$ = threshold for H_2 use by hydrogenotrophic methanogens ($\mu\text{mol}/\text{L}$).

Kinetic parameters for H_2 use by methanogens that were used in the model are shown in Table 5.6. The maximum specific rate of H_2 use, $k_{(H_2)\text{meth}}$, was estimated from data in this study and from Smatlak [208] and Young [273]. Rates of CH_4 formation in bottles that exhibited no acetotrophic methanogenesis were estimated directly from plots of CH_4 formation, and by stoichiometric conversion the rate of H_2 use ($\mu\text{mol}/\text{hr}$) was determined. This value was then divided by the estimated H_2 -using methanogenic biomass in the bottle to obtain a rate in $\mu\text{mol } H_2/\text{mg VSS-hr}$. The estimated biomass (mg VSS) was determined for the Smatlak and

Young data by multiplying the expected fraction of H_2 -using methanogens in a butyric-acid-enrichment culture fed a 2:1 ratio of donor to PCE—the culture used for those experiments (Table 5.14)—by the total amount of biomass in the bottle (*circa* 70 mg/L). Data from this study were analyzed in the same way and appropriate biomass fractions and contents were applied. The observed estimated maximum specific rates ranged from 5 to 80 $\mu\text{mol } H_2/\text{mg VSS-hr}$. A rate of 40 $\mu\text{mol } H_2/\text{mg VSS-hr}$ was applied in the model. The $k_{(H_2)\text{meth}}$ shown in Table 5.6 is higher than those reported in the literature for pure cultures of methanogens using H_2 . For example, maximum specific rates of H_2 use by various methanogens of 0.6 to 10.3 $\mu\text{mol } H_2/\text{mg VSS-hr}$ were reported by Robinson and Tiedje [180] (see Appendix I, Table A1.5).

The H_2 Threshold_{meth} was determined from data collected during this study. Butyric-acid-amended bottles containing approximately 25 nmol H_2 exhibited CH_4 formation (see Section 4.A.3). This corresponded to an aqueous H_2 concentration of *circa* 0.008 μM . The threshold value for methanogenesis from H_2 observed in this study is somewhat lower than values (0.021 to 0.075 μM) cited in the literature (see examples in Table A1.5).

$K_{S(H_2)\text{meth}}$ values range from 0.0076 to 13 μM in the literature (see Table A1.5). Smatlak *et al.* [210] reported an average value of 0.96 ± 0.18 μM . A value of 0.5 μM was incorporated into the model (Table 5.6).

Table 5.6. Kinetic parameters for H₂ use by methanogens (35°C).

Compound	$k_{(H_2)_{meth}}$ ($\mu\text{mol}/\text{mg VSS-hr}$)	$K_{S(H_2)_{meth}}$ ($\mu\text{mol}/\text{L}$)	H ₂ Threshold _{meth} ($\mu\text{mol}/\text{L}$)
H ₂	40 ^a	0.5 ^b	0.008 ^c

^a Estimate, this study, Young [273], and Smatlak [208]; ^b within range from reported values Table A1.5, Appendix I; ^c observation from this study.

5.G. Kinetic Model for Acetotrophic Methanogenesis

Acetotrophic methanogenesis was modeled using Michaelis-Menten-type kinetics.

$$\left(\frac{dM_{t_{Acetate}}}{dt} \right)_{\text{degradation}} = - \frac{k_{Acetate} X_{Acetotroph} C_{W_{Acetate}}}{K_{S(Acetate)} + C_{W_{Acetate}}} \quad (5.16)$$

Where:

- $M_{t_{Acetate}}$ = total amount of acetate in the bottle (μmol);
- $k_{Acetate}$ = maximum specific rate of acetate utilization ($\mu\text{mol}/\text{mg VSS-hr}$);
- $X_{Acetotroph}$ = acetotrophic methanogenic biomass contained in the bottle (mg VSS);
- $C_{W_{Acetate}}$ = aqueous acetate concentration ($\mu\text{mol}/\text{L}$); and
- $K_{S(Acetate)}$ = half-velocity coefficient for acetate use by acetotrophic methanogens ($\mu\text{mol}/\text{L}$).

Kinetic parameters for acetate use by methanogens that were used in the model were taken from literature values (see Table A1.6) and are shown in Table 5.7.

Table 5.7. Kinetic parameters for acetate use by methanogens (35°C).

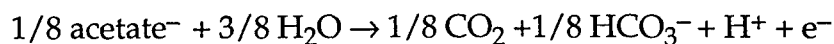
Compound	k ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)	K_s ($\mu\text{mol}/\text{L}$)
Acetate	5.65 ^a	1000 ^a

^a[169], [274].

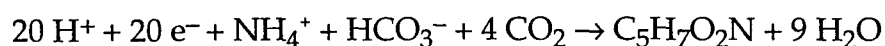
5.H. Product Formation From Substrate Degradation: Adjustment for Energy to Biosynthesis

The fermentation of the donors to acetate and H_2 (or to acetate and propionate in the case of some lactic acid and ethanol fermentations); the conversion of acetate and H_2 to CH_4 ; and the use of H_2 for dechlorination were the reactions of interest for the modeling effort. A fraction of the converted substrate in all these cases was used for the synthesis of new biomass (f_s) and a fraction was used for energy generation (f_e). To accurately estimate the amount of product formation, f_s and f_e were estimated for each of the energy-yielding reactions.

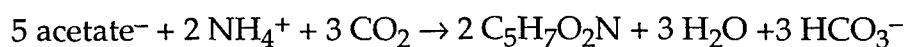
f_s and f_e were estimated from experimentally reported yield values. To determine the stoichiometry of substrate conversion to biomass, a half reaction for complete substrate degradation to CO_2 and e^- was coupled with a half reaction for production of biomass, modeled as $\text{C}_5\text{H}_7\text{O}_2\text{N}$ [153], from basic components. An example of the calculation—for acetate—is shown. The half-reaction for acetate:



was balanced with the half reaction for biomass formation:



and when balanced, this yielded:



or, 5/2 moles acetate⁻ to synthesis yields 1 mole (113 g) C₅H₇O₂N.

Calculations were performed in a similar manner for butyrate, lactate, propionate, and ethanol. The fraction of substrate to synthesis, f_s , was calculated via Equation 5.17.

$$f_s = Y \times \left(\frac{\text{mol substrate to synthesis}}{113 \text{ g C}_5\text{H}_7\text{O}_2\text{N formed}} \right) \quad (5.17)$$

$$\text{where } Y = \left(\frac{\text{g VSS (C}_5\text{H}_7\text{O}_2\text{N) formed}}{\text{total mol substrate used}} \right).$$

Equation 5.18 was used to calculate the fraction of substrate to energy generation, f_e .

$$f_e = 1 - f_s \quad (5.18)$$

Values for each substrate are shown in Table 5.8.

Table 5.8. Literature yield (Y) values and calculated values for f_s and f_e for butyrate, ethanol, lactate, propionate, and acetate.

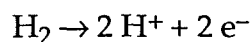
Substrate	Y (g VSS/mol substrate used)	mol substrate to synthesis/mol C ₅ H ₇ O ₂ N formed	f_s	f_e
Butyrate	2.79 ^a	1	0.0247	0.9753
Ethanol	1.98 ^b	5/3	0.0292	0.9708
Lactate	3.51 ^c	5/3	0.0518	0.9482
Propionate	1.44 ^d	10/7	0.0182	0.9818
Acetate	1.89 ^e	5/2	0.0418	0.9582

^a [4]; ^b [202]; ^c [256]; ^d [256]; ^e [51].

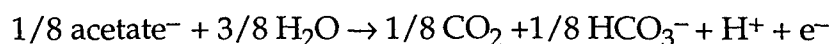
For H_2 use by dechlorinators and methanogens, the reported "yields" (in the literature) were based not on observation of H_2 consumption, but were instead reported on the basis of product formation, which is generally easier to quantify. Here, yields reported on the basis of g VSS formed per mole of substrate that is channeled to energy formation will be referred to as Y_{energy} while those reported for the total amount of substrate used, i.e., both for energy formation and synthesis will be referred to as Y .

For "*D. ethenogenes*", it was reported that 4.8 g protein was formed/mol Cl^- released during dechlorination [151]. Assuming the organism is 70 percent protein and the dry matter is 90 percent volatile, then this may also be expressed as 18.36 g VSS formed/mol PCE dechlorinated to VC. For each mol of PCE dechlorinated to VC, 3 mol H_2 are consumed (for energy). When then based on H_2 use, this value becomes 6.12 g VSS formed per mol H_2 used for energy production (Y_{energy}). Note that while the organism gains energy from dechlorination of PCE to TCE, TCE to DCE, and DCE to VC; it does not apparently gain energy from the dechlorination of VC to ETH. Also, while the organism uses H_2 as an electron donor and chloroethenes as the electron acceptor, acetate serves as the carbon source and thus must be included in the synthesis reactions [151].

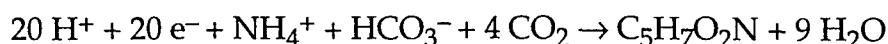
The half-reaction for the energy source, H_2 :



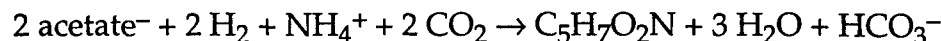
and for the carbon source, acetate,



were balanced with the half reaction for biomass formation:



and when balanced, this yielded:



or, 2 moles H_2 to synthesis yields 1 mole (113 g) $\text{C}_5\text{H}_7\text{O}_2\text{N}$.

Since,

$$Y = Y_{\text{energy}} \left(\frac{\text{g VSS (C}_5\text{H}_7\text{O}_2\text{N) formed}}{\text{mol H}_2 \text{ used for energy}} \right) \times f_e \left(\frac{\text{mol H}_2 \text{ used for energy}}{\text{total mol H}_2 \text{ used}} \right)$$

and $Y_{\text{energy}} = 6.12$, f_s could be solved for in terms of f_e ,

$$f_s = (6.12 \times f_e) \left(\frac{\text{g VSS (C}_5\text{H}_7\text{O}_2\text{N) formed}}{\text{total mol H}_2 \text{ used}} \right) \times \left(\frac{2 \text{ mol H}_2 \text{ to synthesis}}{113 \text{ g C}_5\text{H}_7\text{O}_2\text{N formed}} \right)$$

or, $f_s = 0.1083 \times f_e$

and since $f_e = 1 - f_s$,

$$f_s = 0.1083 \times (1 - f_s)$$

then solving, $f_s = 0.0977$, and $f_e = 0.9023$.

A similar calculation was performed for H_2 use by methanogens.

The results of this analysis for dechlorination and methanogenesis are shown in Table 5.9.

Table 5.9. f_s and f_e values for dechlorination and methanogenesis.

Bioprocess	Y_{energy} (g VSS formed per mol H_2 to energy)	Y (g VSS/mol H_2 used)	mol H_2 to synth/mol $\text{C}_5\text{H}_7\text{O}_2\text{N}$ formed	f_s	f_e
Methane Formation	1.43 ^a	1.27 ^c	10	0.11	0.89
PCE to VC Dechlorination	6.12 ^b	5.52 ^c	2	0.098	0.902

^a[261]; ^b [151]; ^c computed from $Y_{\text{energy}} * f_e$.

For determination of product formation, the rate of substrate utilization for a particular compound was multiplied by a molar conversion factor applicable to the energy reaction (mol product formed/mol of donor used for energy) *and* by f_e to reflect the fact that some of the substrate was channeled to biomass production. An example is shown for acetate formation during the fermentation of butyrate:

$$\frac{d Mt_{\text{Acetate}}}{dt} = -\frac{d Mt_{\text{Butyrate}}}{dt} \times \frac{2 \text{ mol Acetate}^- \text{ formed}}{\text{mol Butyrate}^- \text{ used for energy}} \times f_e$$

5.1. Kinetic Model for Biomass Growth

Biomass growth was modeled separately for each distinct group of organisms in the mixed culture using Equation 5.19.

$$\frac{dX}{dt} = Y \left(\frac{-dMt}{dt} \right) - k_d X \quad (5.19)$$

Where:

Y = organism yield (from literature values) on the substrate (mg VSS/ μ mol substrate used);

$\frac{dMt}{dt}$ = change in substrate of interest over the time increment (μ mol/hr);

X = biomass of the specific organism group contained in the bottle (mg VSS); and

k_d = decay coefficient for the organism group (hr^{-1}).

Yield values used for all aspects of the modeling were shown in Table 5.10. A k_d value of 0.001 hr^{-1} was assumed for all microbial groups.

Table 5.10. Literature yield values for bioprocesses examined in this study.

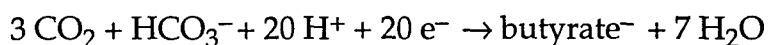
Bioprocess	Y (mg VSS/ total μmol substrate used)	Reference
Butyrate Fermentation	0.00279	[4]
Ethanol Fermentation (to acetate)	0.00198	[202]
Ethanol Fermentation (to propionate)	0.00297	[240]
Lactate Fermentation (to acetate)	0.00351	[256]
Lactate Fermentation (to propionate)	0.00563	[187]
Propionate Fermentation	0.00144	[256]
Acetotrophic Methanogenesis	0.00189	[52]
Bioprocess	Y_{energy} (mg VSS/ μmol H_2 used for energy)	Reference
Dechlorination	0.00612	[151]
Hydrogenotrophic Methanogenesis	0.00143	[261]

5.J. Modeling Organism Decay as a Source of Reductant

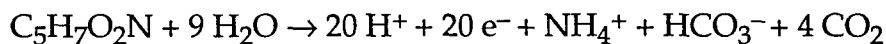
From time-intensive analysis of enrichment cultures amended with no donor or FYE, it was determined that endogenous decay contributed to the reducing equivalents pool. The quantification of this reductant source was described in Appendix II. For modeling purposes it was necessary to

calculate the quantity of organism decay on a reducing equivalents basis and to decide how these reducing equivalents would manifest themselves in a real system. Since it is likely that initial products of fermentation decay would be complex and slowly available—and would likely be subject to the same thermodynamic limitations as the other donors—the decay of biomass was modeled as a contribution to the butyric acid pool. Butyric acid in this case serves as a “model” slowly-available compound. One could argue that modeling decay as a contribution to propionic acid would be more suitable, however, it was demonstrated in at least some of the control bottles that propionic acid persisted while endogenous decay was on-going—an indication that in whatever form they actually were—decay equivalents were more thermodynamically available than propionic acid. The conversion of biomass, $C_5H_7O_2N$, to butyrate occurs according to the following coupled half-reactions [153].

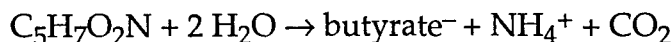
The half-reaction for butyrate formation:



was balanced with the half reaction for biomass, modeled as $C_5H_7O_2N$, breakdown:



and this yielded:



or, 1 mole (113 g VSS) $C_5H_7O_2N$ decaying, yields 1 mole butyrate⁻.

Thus, decay from each of the biomass pools was channeled on a 1:1 basis as butyrate directly into the butyrate pool.

5.K. Estimation of Biomass for Modeling Purposes

5.K.1. General Approach

Appropriate starting biomass values for modeling purposes were estimated from literature yield values (Table 5.10) and application of stoichiometric considerations to the semi-continuously operated bottles.

Equation 5.20 was used to calculate steady-state biomass concentration in a continuous-flow system.

$$X_{\text{est}} = \frac{Y(S_o - S_e)}{1 + k_d \theta_c} \quad (5.20)$$

Where:

X_{est} = biomass concentration of a particular microbial group (mg VSS/L);

Y = yield of that microbial group (mg VSS/ μmol substrate);

S_o = influent substrate concentration ($\mu\text{mol/L}$);

S_e = effluent substrate concentration ($\mu\text{mol/L}$);

k_d = decay coefficient (d^{-1}); and

θ_c = solids retention time (d).

The solids retention time, θ_c , was 40 days, the decay coefficient, k_d , was 0.024 d^{-1} , and the yield coefficients, Y (mg VSS/ μmol substrate), for each organism type are shown in Table 5.10.

S_o was calculated from the basal medium exchange rate, and the amount of primary electron donor added to the bottle as a pulse feeding at the time of basal medium exchange as shown in Figure 5.1.

Thus, the calculation of S_o for an administered donor was by Equation 5.21:

$$S_o = \frac{\text{Donor Added } (\mu\text{mol/d})}{\text{Basal Medium Exchange (L/d)}} \quad (5.21)$$

S_o for substrates that were formed from the fermentation of the added electron donor was calculated from the stoichiometry of the fermentation and the expected fraction of the donor that would be used for energy, f_e , from Tables 5.8 and 5.9. For example for acetate, Equation 5.22 was used:

$$S_o = \frac{\text{Added Donor Fermented } (\mu\text{mol/d})}{\text{Basal Medium Exchanged (L/d)}} \times \frac{\mu\text{mol Acetate Formed}}{\mu\text{mol Donor Fermented}} \times f_e \quad (5.22)$$

For H_2 , S_o was determined from the stoichiometry of the fermentation of the added donor and the fraction (see Table 5.11) of the H_2 channeled to the particular biological process—i.e. dechlorination or methanogenesis. These values were averaged from the results of the long-term operation of enrichment cultures, (slightly different than what was observed during the TISs, Table 4.2). An example calculation of S_o for H_2 used for dechlorination is shown in Equation 5.23:

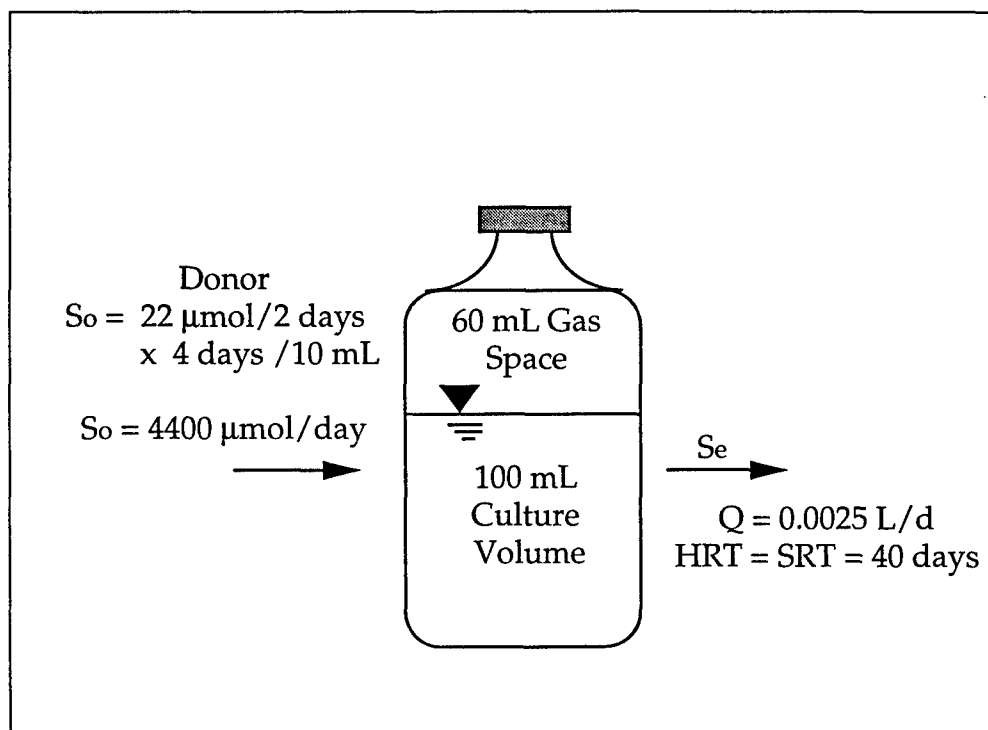


Figure 5.1. Semi-continuous operation of serum bottle. Example of S_o calculation for a 1:1 donor to PCE ratio.

$$S_o = \frac{\text{Added Donor Fermented } (\mu\text{mol/d})}{\text{Basal Medium Exchanged (L/d)}} \times \frac{\mu\text{mol H}_2 \text{ Formed}}{\mu\text{mol Donor Fermented}} \quad (5.23)$$

$$\times f_e \times \frac{\mu\text{mol H}_2 \text{ Used For Dechlorination}}{\mu\text{mol H}_2 \text{ Used}}$$

During "steady-state" operation of the bottles, all of the added electron donor (except for propionic acid, see Table 5.11), was fermented and remaining S_e for the donor was a very small value or zero. The biomass for acetotrophs was assumed to be zero when no acetotrophic activity was occurring (early in the 1:1 donor amendment runs). For some of the intermediate data from long-term runs, however, S_e for acetate was non-zero and active acetotrophic activity was on-going. In this case the biomass value was calculated using an average acetate effluent concentration which was determined by averaging the effluent values from the time of apparent onset of acetotrophism, when the concentration was high, until the end of a run when the concentration had (in some enrichments) reached undetectable levels. For all cases, S_e for H_2 was a very small (nM) value and was set to zero.

Table 5.11. Fraction of hydrogen channeled to methanogenesis and dechlorination and effluent acetic and propionic acids concentrations in enrichment cultures as determined from average results from long-term operation.

Enrichment Culture	Fraction of H ₂ to CH ₄	Fraction of H ₂ to Dechlorination	S _e Acetic Acid (μmol/L)	S _e Propionic Acid (μmol/L)
Butyrate (1:1) (no acetotrophism)	0.15	0.85	3567	64
Butyrate (2:1) (with acetotrophism)	0.55	0.45	3240	76
Ethanol (1:1) (no acetotrophism)	0.40	0.60	2249	36
Ethanol (2:1) (with acetotrophism)	0.65	0.35	3560	102
Lactate (1:1) (no acetotrophism)	0.20	0.80	2044	164
Lactate (2:1) (with acetotrophism)	0.70	0.30	4068	116
Propionate (1:1) (no acetotrophism)	0.02	0.98	1414	114
Propionate (2:1) (with acetotrophism)	0.20	0.80	3023	239

5.K.2. Fermented Yeast Extract Contribution to Available Reducing Equivalents

The concentrations of VFAs in FYE were measured for several different batches and are shown in Table 5.12.

Table 5.12. Measured VFA content of FYE.

FYE Batch	Acetic Acid ($\mu\text{mol/L}$)	Prop. Acid ($\mu\text{mol/L}$)	Butyric Acid ($\mu\text{mol/L}$)	Isobut. Acid ($\mu\text{mol/L}$)	Valeric Acid ($\mu\text{mol/L}$)	Isoval. Acid ($\mu\text{mol/L}$)	Hex. Acid ($\mu\text{mol/L}$)
Nov 96	70000	9500	51400	5400	1200	8500	7500
Sept 95	71300	20600	80800	9600	nd	12900	8800
Mar 95	65400	17800	67400	12300	nd	11300	5600

nd = not detected

At each feeding, 40 μL of FYE was added to cultures amended with a 2:1 donor to PCE ratio and 20 μL was added to bottles amended with a 1:1 donor to PCE ratio. For a 20 μL addition, the amounts of the VFAs contributed to a culture are shown in Table 5.13. It was determined from measuring the amount of methanogenesis and dechlorination that occurred (in excess of that occurring because of endogenous decay) that approximately 31.3 μeq of products were formed with each 40 μL addition of FYE (see Appendix II). Thus, if it is assumed that 10 percent of the reduction equivalents provided were channeled to synthesis, then approximately 34.8 μeq reducing equivalents per 40 μL (17.4 $\mu\text{eq}/20 \mu\text{L}$) must have been available from the FYE. The VFA content accounted for about 60 percent of the total amount of reducing equivalents provided by FYE (see Figure 5.2).

To simplify the biomass contribution from FYE, contributions from acetic, propionic, and butyric acids (as measured) were added to their respective S_o values and all remaining contributed reducing equivalents (17.4 μeq minus equivalents contributed by propionic and butyric acids) were added as a contribution to the butyric acid pool. This was a simpler

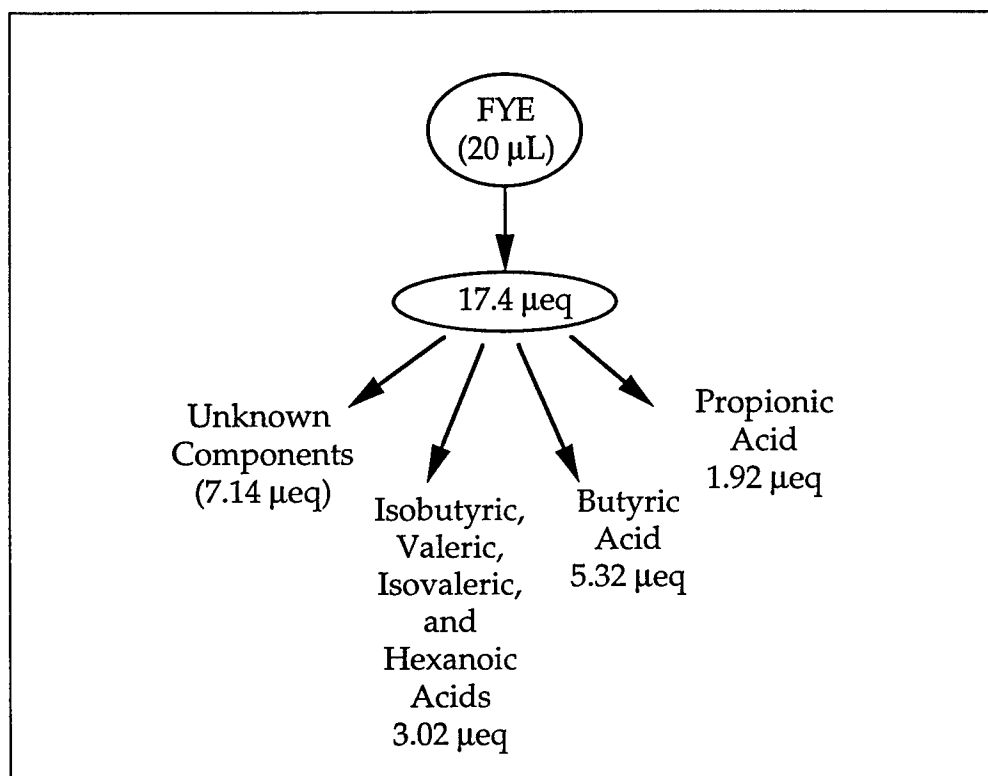


Figure 5.2. Estimated contribution of reducing equivalents by FYE.

approach than attempting to account for longer-chain fatty acids and other unknown contributors. It could be argued based on literature reports of the substrate specificity of many of the syntrophic butyrate oxidizers that the active butyrate-oxidizer biomass probably is to a great extent responsible for the degradation of these higher fatty acids [155, 156, 182, 222]. Some of the reducing equivalents are certainly contributed by the fermentation of other compounds by other types of organisms—for example carbohydrates or proteins. These contributions were probably small, relatively speaking.

Using the method outlined above and handling FYE as a substrate contribution to acetic, propionic, and butyric acids (as measured) and remaining reducing equivalents through the butyric acid pathway, biomass estimates were made for conditions observed during this study.

Additionally, for the model, FYE addition to cultures was simulated in the same way.

Table 5.13. VFA contribution of FYE by a 20 μL volume addition.

FYE Batch	Acetic Acid (μmol)	Prop. Acid (μmol)	Butyric Acid (μmol)	Isobutyric Acid (μmol)	Valeric Acid (μmol)	Isovaleric Acid (μmol)	Hex. Acid (μmol)
Nov 96	1.4	0.19	1.03	0.11	0.02	0.17	0.15
Sept 95	1.4	0.41	1.62	0.19	nd	0.26	0.18
Mar 95	1.3	0.36	1.35	0.25	nd	0.23	0.11
Ave (μmol)	1.37	0.32	1.33	0.18	--	0.22	0.15
Ave (μeq)	--	1.92	5.32	0.72	--	1.1	1.2

nd = not detected

5.K.3. Summary of Estimated Biomass Concentrations

Estimated biomass levels for different microbial groups for some enrichments are shown in Table 5.14. A comparison of estimated biomass values and values actually measured for the same types of enrichment cultures is shown in Table 5.15.

Table 5.14 Biomass estimates for selected enrichment culture types.

Donor (Ratio) [Pathway]	Aceto- trophic Activity	Donor Degrader Biomass (mg/L)	Butyrate Degrader Biomass (mg/L)	Propionate Degrader Biomass (mg/L)	Aceto- trophic Biomass (mg/L)	H ₂ -Using Methanogen Biomass (mg/L)	Dechlor- inator Biomass (mg/L)	Total Biomass (mg/L)
Butyrate (1:1)	no	7.37	(see donor)	0	0	1.11	26.79	35.26
Butyrate (2:1)	no	14.73	(see donor)	0.0385	0	8.18	28.58	51.53
Butyrate (1:1)	yes	7.37	(see donor)	0	6.56	1.11	26.79	41.82
Butyrate (2:1)	yes	14.73	(see donor)	0.0385	16.66	8.18	28.58	68.18
Ethanol (1:1) [to HAc]	no	4.44	1.10	0.0206	0	2.96	18.99	27.52
Ethanol (2:1) [to HAc]	no	8.89	2.20	0.0191	0	9.59	22.06	42.76
Ethanol (1:1) [to HAc]	yes	4.44	1.10	0.0206	3.70	2.96	18.99	31.22
Ethanol (2:1) [to HAc]	yes	8.89	2.20	0.0191	8.01	9.59	22.06	50.77

Table 5.14 (continued)

Donor (Ratio) [Pathway]	Aceto- trophic Activity	Donor Degrader Biomass (mg/L)	Butyrate Degrader Biomass (mg/L)	Propionate Degrader Biomass (mg/L)	Aceto- trophic Biomass (mg/L)	H ₂ -Using Methanogen Biomass (mg/L)	Dechlor- inator Biomass (mg/L)	Total Biomass (mg/L)
Ethanol (1:1) [to Prop]	no	6.67	1.10	2.11	0	2.92	18.70	31.50
Ethanol (2:1) [to Prop]	no	13.33	2.20	4.20	0	9.44	21.72	50.90
Ethanol (1:1) [to Prop]	yes	6.67	1.10	2.11	3.65	2.92	18.70	35.15
Ethanol (2:1) [to Prop]	yes	13.33	2.20	4.20	7.91	9.44	21.72	58.81

Table 5.14 (continued)

Donor (Ratio) [Pathway]	Aceto- trophic Activity	Donor Degrader Biomass (mg/L)	Butyrate Degrader Biomass (mg/L)	Propionate Degrader Biomass (mg/L)	Aceto- trophic Biomass (mg/L)	H ₂ -Using Methanogen Biomass (mg/L)	Dechlor- inator Biomass (mg/L)	Total Biomass (mg/L)
Lactate (1:1) [to HAc]	no	7.88	1.10	0	0	1.40	23.88	34.26
Lactate (2:1) [to HAc]	no	15.76	2.20	0.0088	0	10.10	18.49	46.57
Lactate (1:1) [to HAc]	yes	7.88	1.10	0	3.68	1.40	23.88	37.94
Lactate (2:1) [to HAc]	yes	15.76	2.20	0.0088	7.31	10.10	18.49	53.88
Lactate (1:1) [to Prop]	no	12.64	1.10	1.97	0	1.38	23.50	40.59
Lactate (2:1) [to Prop]	no	25.28	2.20	4.10	0	9.95	18.21	59.74
Lactate (1:1) [to Prop]	yes	12.64	1.10	1.97	3.63	1.38	23.50	44.22
Lactate (2:1) [to Prop]	yes	25.28	2.20	4.10	7.22	9.95	18.21	66.95

Table 5.14 (continued)

Donor (Ratio) [Pathway]	Aceto- trophic Activity	Donor Degrader Biomass (mg/L)	Butyrate Degrader Biomass (mg/L)	Propionate Degrader Biomass (mg/L)	Aceto- trophic Biomass (mg/L)	H ₂ -Using Methanogen Biomass (mg/L)	Dechlor- inator Biomass (mg/L)	Total Biomass (mg/L)
Lactate (1:1) [30% to Prop]	no	5.52 (to Hac) 3.79 (to Prop)	1.10	0.54	0	1.39	23.77	36.1
Lactate (2:1) [30% to Prop]	no	11 (to Hac) 7.58 (to Prop)	2.20	1.23	0	10.06	18.41	50.52
Lactate (1:1) [30% to Prop]	yes	5.52(to Hac) 3.79 (to Prop)	1.10	0.54	3.66	1.39	23.77	39.77
Lactate (2:1) [30% to Prop]	yes	11 (to Hac) 7.58 (to Prop)	2.20	1.23	7.28	10.06	18.41	57.80
Propionate (1:1)	no	2.12	1.10	(see donor)	0	0.15	30.67	34.04
Propionate (2:1)	no	4.24	2.20	(see donor)	0	2.93	49.99	59.36
Propionate (1:1)	yes	2.12	1.10	(see donor)	3.09	0.15	30.67	37.13
Propionate (2:1)	yes	4.24	2.20	(see donor)	5.72	2.93	49.99	65.08

Table 5.15. Comparison of measured and estimated biomass concentrations.

Enrichment Type	Measured Biomass (mg VSS/L) (by PON) ^a	Estimated Biomass (mg VSS/L)
Butyrate 1:1 (no acetotrophism)	36.82	35.26
Butyrate 2:1 (with acetotrophism)	69.86, 71.55	68.18
Ethanol 1:1 [to HAc] (no acetotrophism)	32.34 ^b	27.52
Ethanol 2:1 [to HAc] (with acetotrophism)	35.48 ^b	50.77
Ethanol 1:1 [half to Prop] (no acetotrophism)	--	29.51
Ethanol 2:1 [half to Prop] (with acetotrophism)	--	54.91
Lactate 1:1 [to HAc] (no acetotrophism)	35.10 ^b	34.26
Lactate 2:1 [to HAc] (with acetotrophism)	39.56 ^b	53.88
Lactate 1:1 [30% to Prop] (no acetotrophism)	--	36.10
Lactate 2:1 [30% to Prop] (with acetotrophism)	--	57.80
Propionate (1:1) (no acetotrophism)	22.78	34.04
Propionate (2:1) (with acetotrophism)	65.62	65.08

^a mg VSS/L = [mg organic-N/L sample] x [1 mg VSS/0.125 mg organic-N]

^b Ethanol- and lactate-enriched cultures exhibited propionate accumulation over that added by FYE. These enrichments may have had some combination of donor to H₂ and donor to propionate conversions, thus values are presented for comparison.

CHAPTER SIX

MODELING RESULTS

6.A. General Approach For Simulating Experimental Data

Simulations were performed to duplicate experimental data and to determine whether the model:

- (1) captured the dynamic patterns of dechlorination, H₂ production and consumption, and methanogenesis that were observed during the short-term TISs with each of the four H₂ donors;
- (2) predicted the same steady-state performance level as was observed during the long-term operation of the 2:1 low-PCE/butyric acid source culture; and
- (3) captured the short-term, TIS behavior of the 2:1 low-PCE/butyric acid source culture at the end of the long-term simulation.

After it was determined that the model did indeed adequately fit the experimental data, additional simulations were run with the following purposes:

- (4) to compare the electron donors ethanol and propionic acid *without* FYE addition during long-term operation—something that was not possible experimentally—and compare those results to the same simulations *with* FYE addition; and
- (5) to determine the hypothetical outcome of a 10:1 ethanol to PCE ratio scenario over the long term.

The parameters described in Chapter 5 were entered in the model; and, with the exception of initial biomass, amount and type of donor fed, amount of FYE added, and for some simulations of butyric acid and

propionic acid, the $\Delta G_{\text{critical}}$ value and k_{Butyrate} or $k_{\text{Propionate}}$, these remained fixed for all simulations.

To simulate specific experimental results, the amount of biomass for each relevant microorganism population was estimated from the total biomass content of the culture as determined by PON analysis (see Table 4.3 and Figure 4.19) and the relative fraction of biomass expected for each population type as determined by the steady-state biomass estimates (see Table 5.14). The donor amendment type and amount (μmol) and the PCE amendment (μmol) were adjusted to the same levels that were observed in actual tests. (For example, during the 1:1 butyric acid TIS, while the target PCE addition was 11 μmol , the actual amount added as determined from actual analysis was 12 μmol . Thus, for some of the TISs, the precise 1:1 or 2:1 ratios were not achieved, and the actual observed added amounts were used in models to simulate real data.) FYE was omitted or added as necessary. A specific simulation time was set, and the model was run with a dt of 0.03125 hr. For TISs, simulations were run for a maximum of 48 hr. For simulation of long-term operation, the model was run until biomass, dechlorination products, and VFA content stabilized. This required simulations of as long as 104 days and was performed by running in 8-day increments. Output was overlaid on experimental data to examine closeness of fit to experimental data.

6.B. Simulation of Time-Intensive Tests for Each H_2 Donor

The initial PCE and donor amounts used in each of the TIS simulations (set to duplicate actual TISs) are shown in Table 6.1 and the biomass settings for the simulations are shown in Table 6.2.

Table 6.1 Initial PCE and donor amounts (to mimic actual data) used for the time-intensive study simulations.

TIS	PCE (μmol)	Donor (μmol)
Butyric acid 1:1	12	18
Butyric acid 2:1	11	44
Ethanol 1:1	11	22
Ethanol 2:1	12	44
Lactic Acid 1:1	11	25
Lactic Acid 2:1	12	50
Propionic Acid 1:1	9	15
Propionic Acid 2:1	11	26

Table 6.2. Initial biomass (mg VSS) used for time-intensive study simulations.

TIS	Total	Donor Fermenter	Butyric Acid Fermenter	Prop. Acid Fermenter	Acetate-Using Methanogen	H ₂ -Using Methanogen	Dechlorinator
Butyric Acid 1:1	3.68	0.77	--	0	0	0.12	2.7
Butyric Acid 2:1	6.99	1.51		0.004	1.71	0.84	2.93
Ethanol 1:1	3.23	0.52	0.13	0.002	0	0.35	2.23
Ethanol 2:1	3.54	0.62	0.15	0.0014	0.56	0.67	1.54
Lactic Acid 1:1 ^a	3.51	0.53 (to HAc) 0.36 (to Prop)	0.12	0.052	0	0.14	2.31
Lactic Acid 2:1 ^a	3.96	0.755 (to HAc) 0.519 (to Prop)	0.15	0.085	0.5	0.69	1.26
Lactic Acid 2:1	3.96	1.16	0.16	0.0008	0.54	0.74	1.36
Propionic Acid 1:1	2.27	0.14	0.074	--	0	0.01	2.05
Propionic Acid 2:1 ^b	6.52	0.43	0.22	--	0.345	0.29	5.01

^a assumes that 30% of the lactate is fermented via the propionate fermentation pathway.

^b model used an acetotroph population of 60% of that predicted since the culture was clearly not at steady state with respect to acetogenesis.

6.B.1. Butyric Acid 1:1 Ratio to PCE

The model simulation of a 1:1 butyric-acid-amended culture overlaid on data from two TISs of the same is shown in Figure 6.1. The model predicted less rapid and complete dechlorination than actually occurred (Figure 6.1a). In the simulation, PCE persisted after 24 hours, while in reality it was depleted after about 20 hr. The predicted amount of CH_4 formed (Figure 6.1b) was less than that which was actually observed. The butyric acid was also depleted more rapidly than the model predicted (Figure 6.1c); however, H_2 level was duplicated well by the model (Figure 6.1d). Thus, while the overall shape and trend of the 1:1 butyric-acid data were captured very well by the model, a precise fit of the entire data set was not obtained.

The formation of more CH_4 in real cultures at those H_2 levels suggests that kinetics of CH_4 formation were more favorable at these H_2 levels than was expected.

6.B.2. Butyric Acid 2:1 Ratio to PCE

Figure 6.2 depicts a 2:1 butyric acid-amended culture simulation overlaid with data from HButTIS 4. The model predicted initially more rapid VC dechlorination to ETH than was observed experimentally (Figure 6.2a), then a gradual cessation as butyric acid and the resulting H_2 pool were depleted. Predictions of methanogenesis (Figure 6.2.b), butyric acid degradation, acetic acid formation and depletion (Figure 6.2c), and the ultimate H_2 level generated (Figure 6.2d) were fairly close to those actually observed. During the experiment, VC dechlorination continued slowly

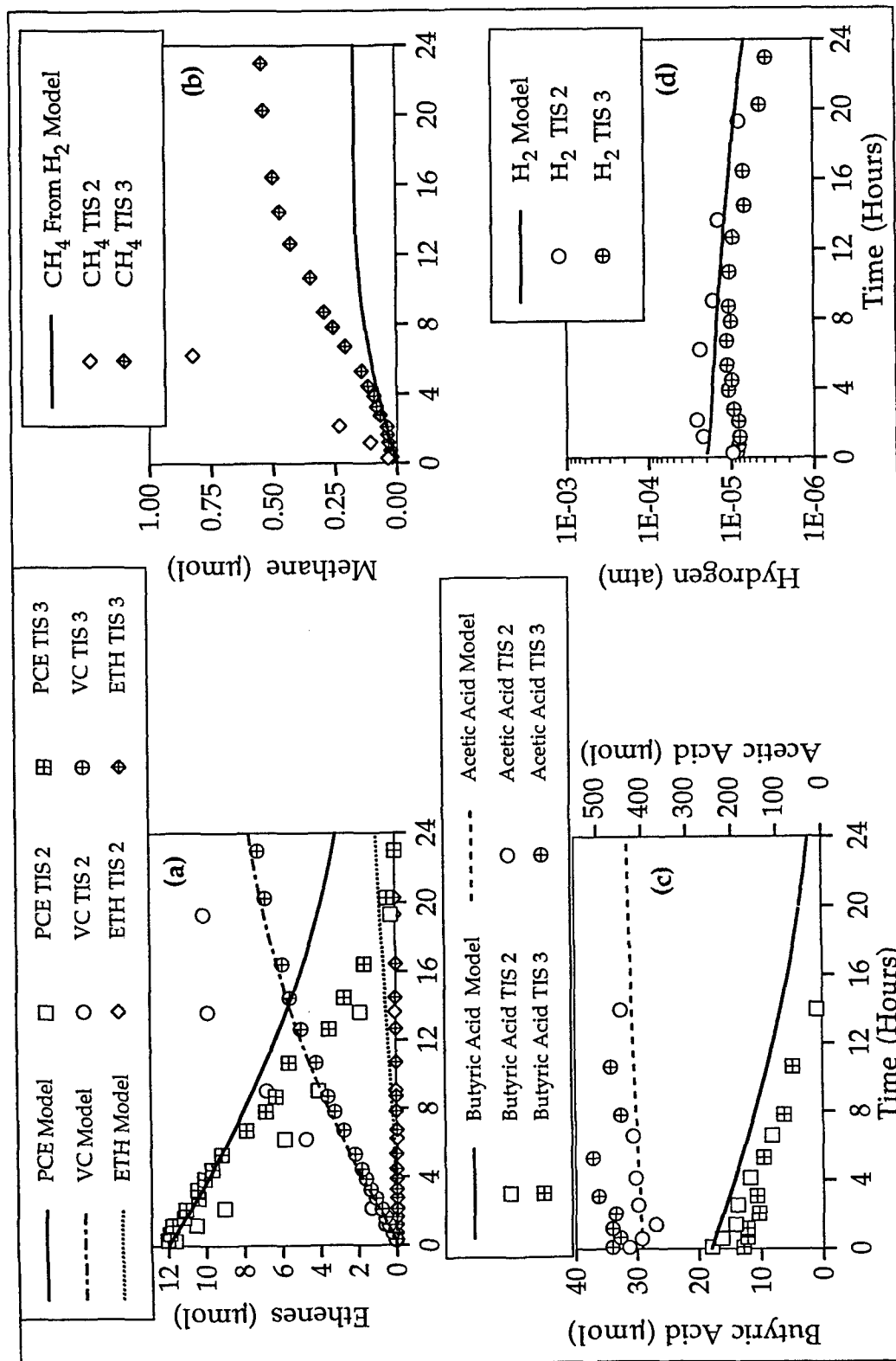


Figure 6.1. Comparison of butyric-acid-amended culture fed 1:1 donor to PCE ratio with model prediction. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

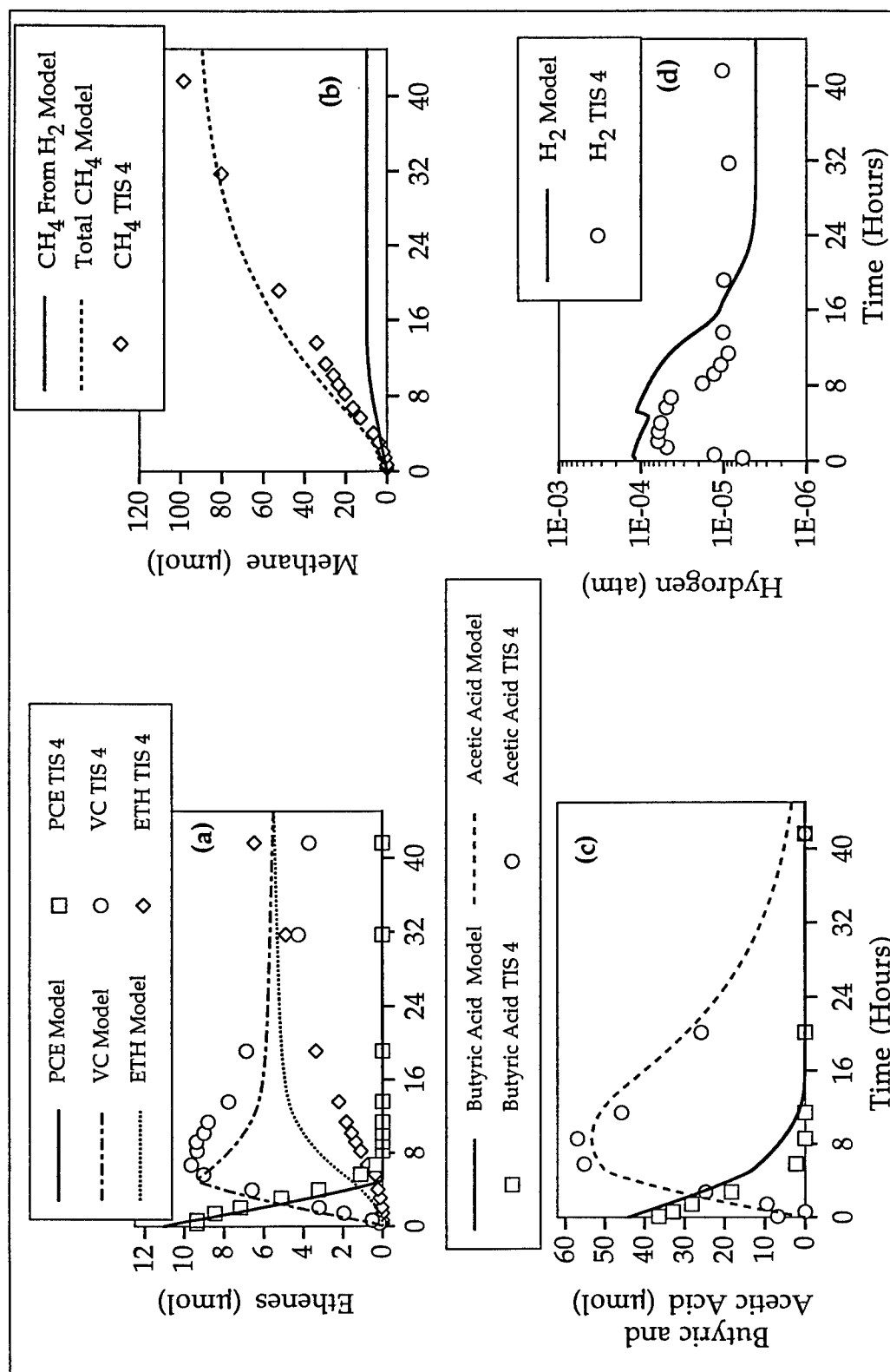


Figure 6.2. Comparison of butyric-acid-amended culture fed 2:1 donor to PCE ratio with model prediction. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

after butyric acid was depleted and the level of H_2 persisting in the bottle after butyric acid depletion was slightly higher than that predicted by the model. The slightly elevated H_2 level suggests that some other source of donor was available that was not reflected in the model. No propionic acid was present in the bottle and FYE was not added. One possibility is that the conversion of acetic acid to CH_4 , which occurred until nearly the end of the test, supplied reducing equivalents that were in turn being scavenged by the dechlorinators. Lovely and Ferry [141] reported that 1×10^{-4} to 9×10^{-4} atm of H_2 was maintained in methanogenic cultures metabolizing acetic acid. Krzycki reported that 5 to 20 nmol H_2 was generated per μmol CH_4 evolved by *Methanosarcina barkeri* [124]. This avenue of reducing equivalent generation, which was not included in the model, may be one explanation for the observed data.

6.B.3. Ethanol 1:1 Ratio to PCE

A fit of the model to data from 1:1 ethanol-amended cultures is shown in Figure 6.3. While the model captured the overall dynamic behavior of the TIS quite well, dechlorination (Figure 6.3a) was predicted to occur more rapidly than was actually observed, less CH_4 was predicted than was actually formed (Figure 6.3b) and ethanol disappeared slightly more rapidly than observed (Figure 6.3c). The H_2 level was predicted well; however, the persistence of H_2 was longer in actuality than the model predicted (Figure 6.3d).

6.B.4. Ethanol 2:1 Ratio to PCE

The fit of the model to a TIS of ethanol at a 2:1 ratio is shown in Figure 6.4. The model simulation fit the data from this study very well.

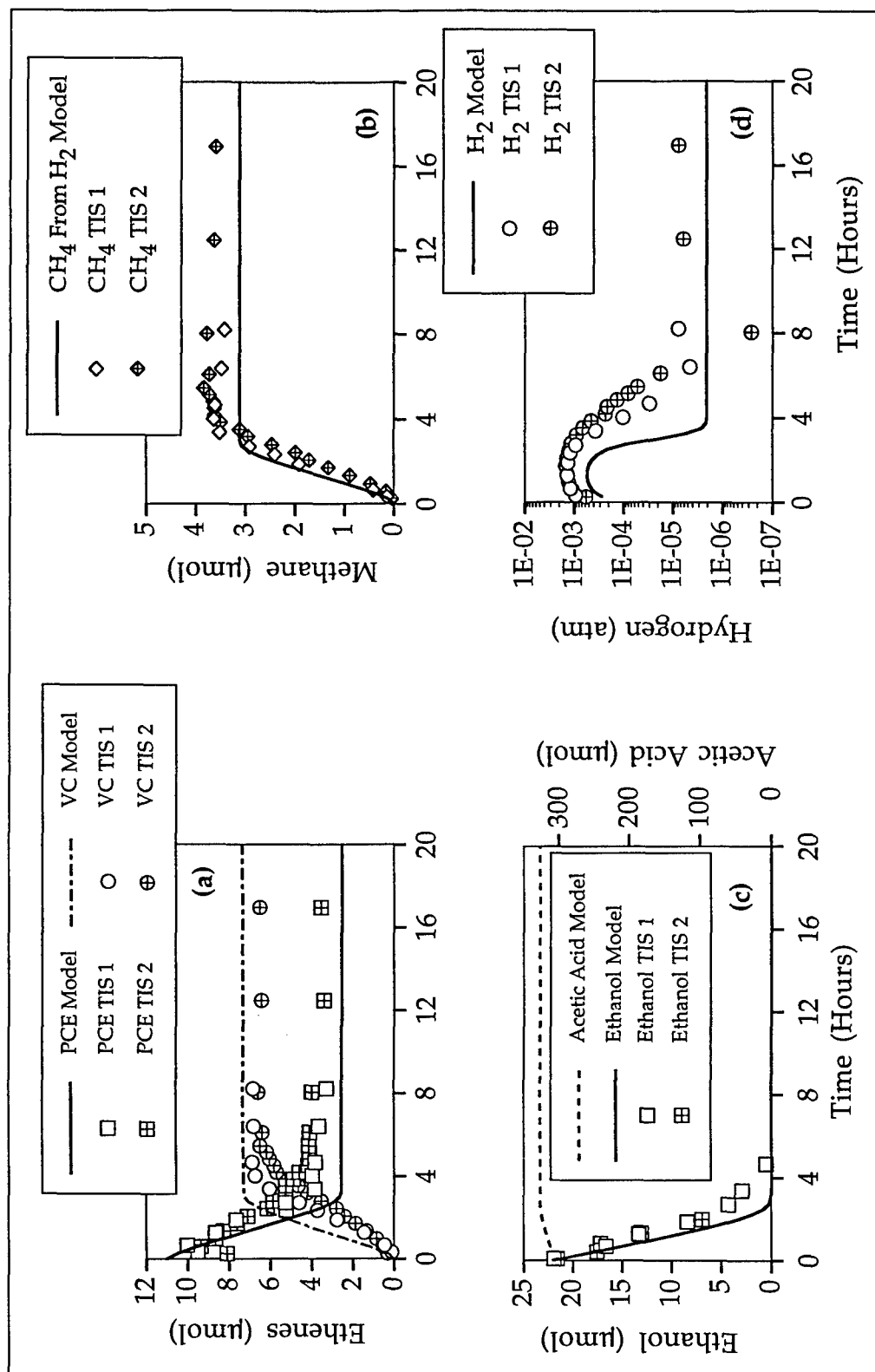


Figure 6.3. Comparison of ethanol-amended culture fed 1:1 donor to PCE ratio with model prediction. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) donor and VFAs; and (d) hydrogen.

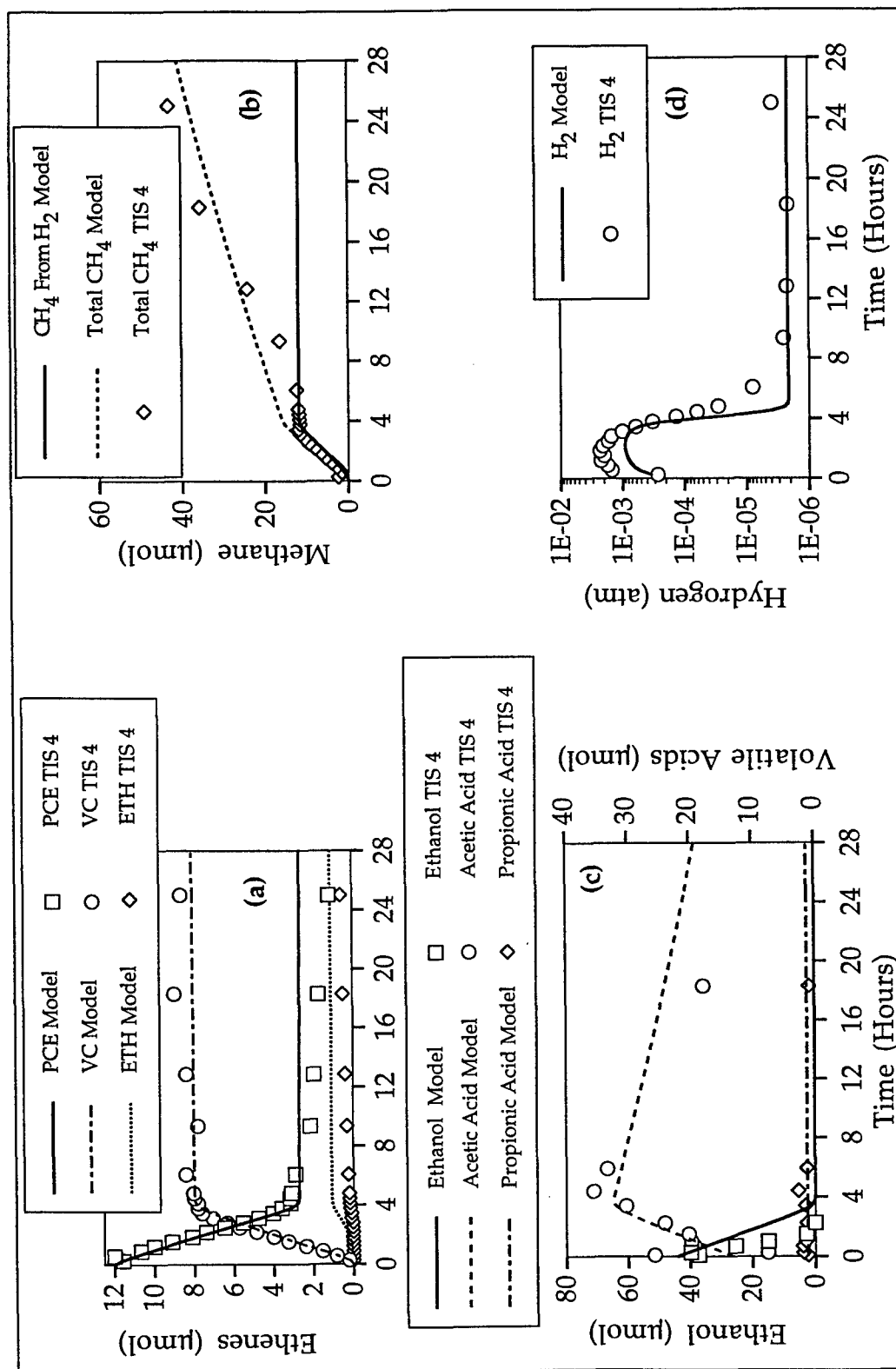


Figure 6.4. Comparison of ethanol-amended culture fed 2:1 donor to PCE ratio with model prediction. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) donor and VFA; and (d) hydrogen.

The one parameter that was not ideal was ethanol disappearance (Figure 6.4c). In the actual tests, ethanol was degraded more rapidly than the model predicted.

6.B.5. Lactic Acid 1:1 Ratio to PCE

Difficulty was encountered when attempting to model the lactic acid TISs. It appeared that some of the lactate was fermented to propionate in these cultures, but that was not always clearly demonstrated during the short-term TIS results.

The fit of a 1:1 lactic-acid-amended culture is shown in Figure 6.5. The fit was accomplished by assuming that 30 percent of the lactic acid was fermented to propionate—which was in turn fermented after the H_2 level had decreased to levels low enough for favorable energetics. While the dechlorination results for up to approximately 6 hr were matched well by the simulation (Figure 6.5a), the rate of PCE dechlorination that occurred after that time—after lactic acid was depleted—was observed to be more rapid in the culture than the model predicted. The dechlorination was presumably fueled by the significant pool of propionic acid present (Figure 6.5c). Propionic acid degradation was also apparently more rapid in the culture than predicted. Lactic acid (Figure 6.5c) and H_2 (Figure 6.5d) were adequately modeled, while methanogenesis (Figure 6.5b) was not. As with butyric acid, more CH_4 was produced than was predicted by the model.

6.B.6. Lactic Acid 2:1 Ratio to PCE

Two attempts at fitting the 2:1 lactic acid TIS data are shown here. In the first, Figure 6.6, it was assumed that 30 percent of the lactic acid was fermented to propionate. The fits for the dechlorination curves were

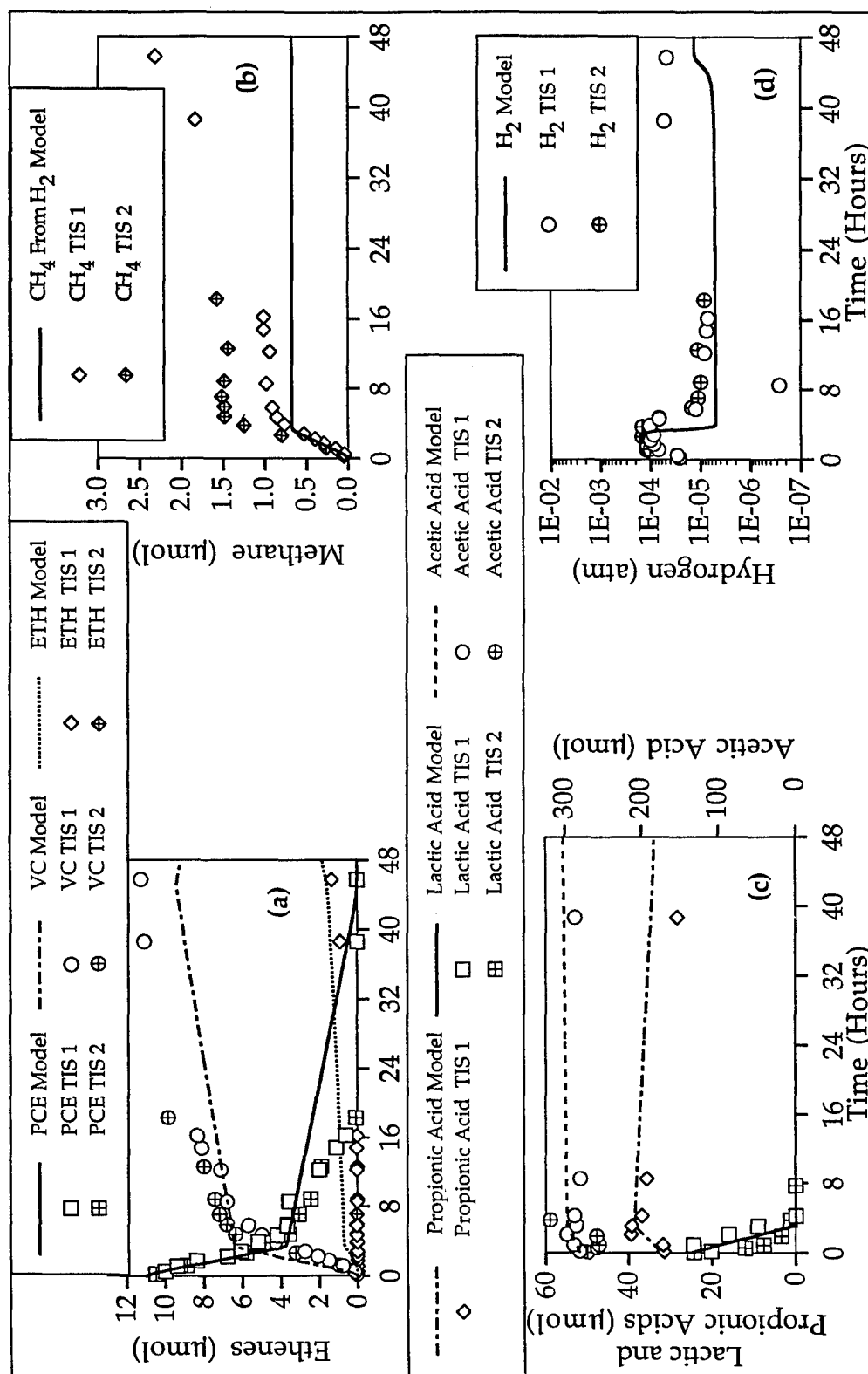


Figure 6.5. Comparison of lactic-acid-amended culture fed 1:1 donor to PCE ratio with model prediction, assuming 30% of lactate fermented to propionate. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) donor and VFAs; and (d) hydrogen.

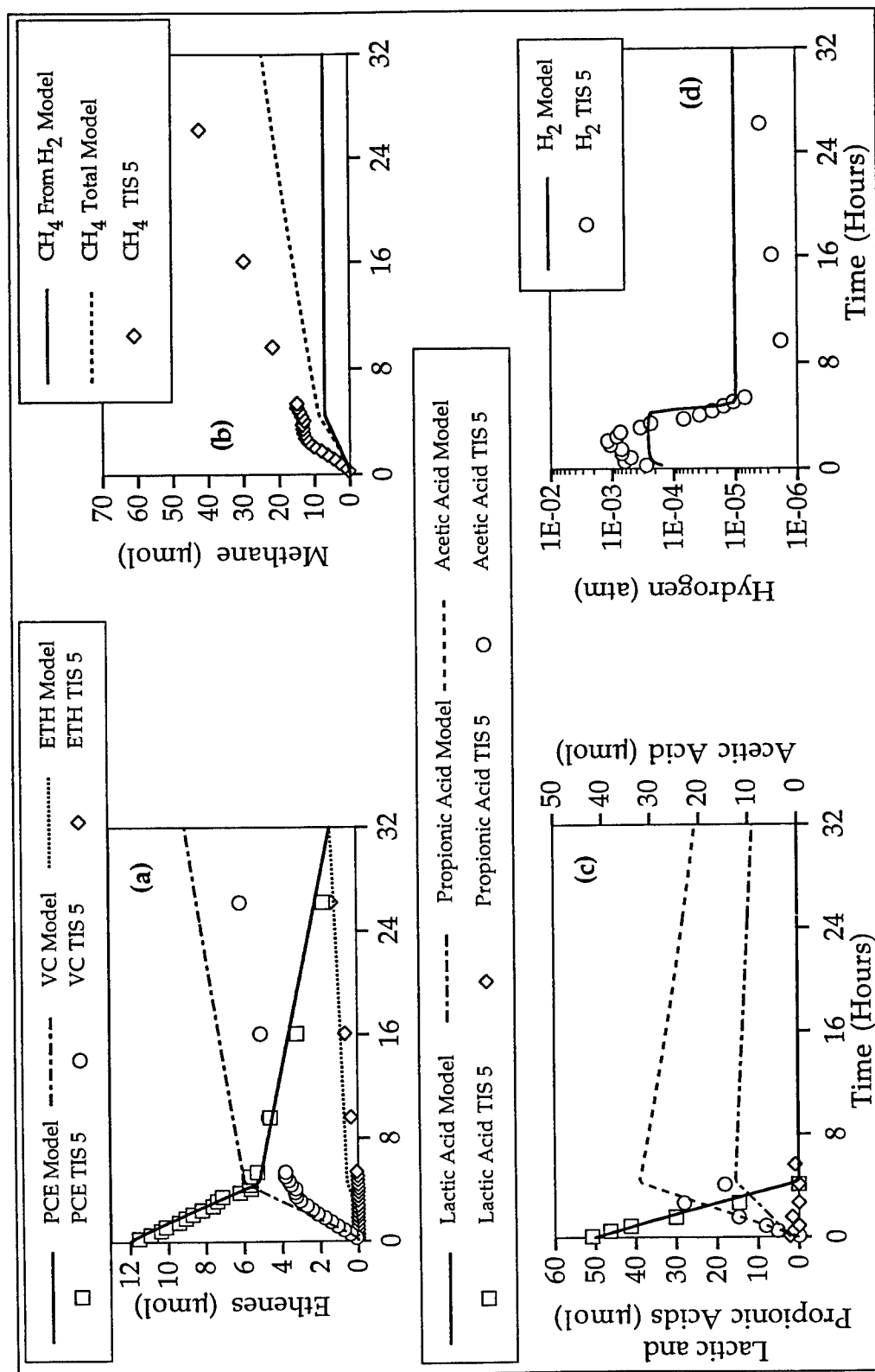


Figure 6.6. Comparison of lactic-acid-amended culture fed 2:1 donor to PCE ratio with model prediction assuming 30% of lactate fermented to propionate. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) donor and VFAs; and (d) hydrogen.

reasonable (Figure 6.6a), particularly the PCE disappearance curve. The lactic acid degradation curve also fit the data quite well (Figure 6.6c). The generation and consumption of acetic acid and propionic acid did not fit the actual measured data. There was, in fact, very little propionic acid detected in the culture during the test (by HPLC analysis) and the acetic acid level was also low and did not persist. Furthermore, the amounts of CH_4 and H_2 were under-predicted (Figures 6.6b and 6.6d).

In the second attempt to fit the experimental data (Figure 6.7), all of the lactic acid was assumed to be fermented to acetic acid and H_2 . In this case, only CH_4 and H_2 were predicted with any accuracy. Dechlorination was not fit well by that simulation.

This data set is highly suspect and it is likely that either something entirely different, physiologically, happened during the test that was not captured by the data set; or the acetic and propionic acid data determined by HPLC analyses were unreliable. Problems were encountered with HPLC measurements during the study. These problems may have resulted in poor analyses in some cases and that possibility cannot be ruled out here.

6.B.7. Propionic Acid 1:1 Ratio to PCE

Figure 6.8 depicts a model fit for 1:1 propionic-acid-amended TISs. The model fit this data quite well. The rate of dechlorination of PCE (Figure 6.8a) was, for TIS 1, slightly faster in the culture than the simulation predicted. Other trends were well-fitted by the model. While some trace amounts of CH_4 were detected in the cultures, the model predicted no methanogenesis (Figure 6.8b) at the low H_2 concentrations generated (Figure 6.8d).

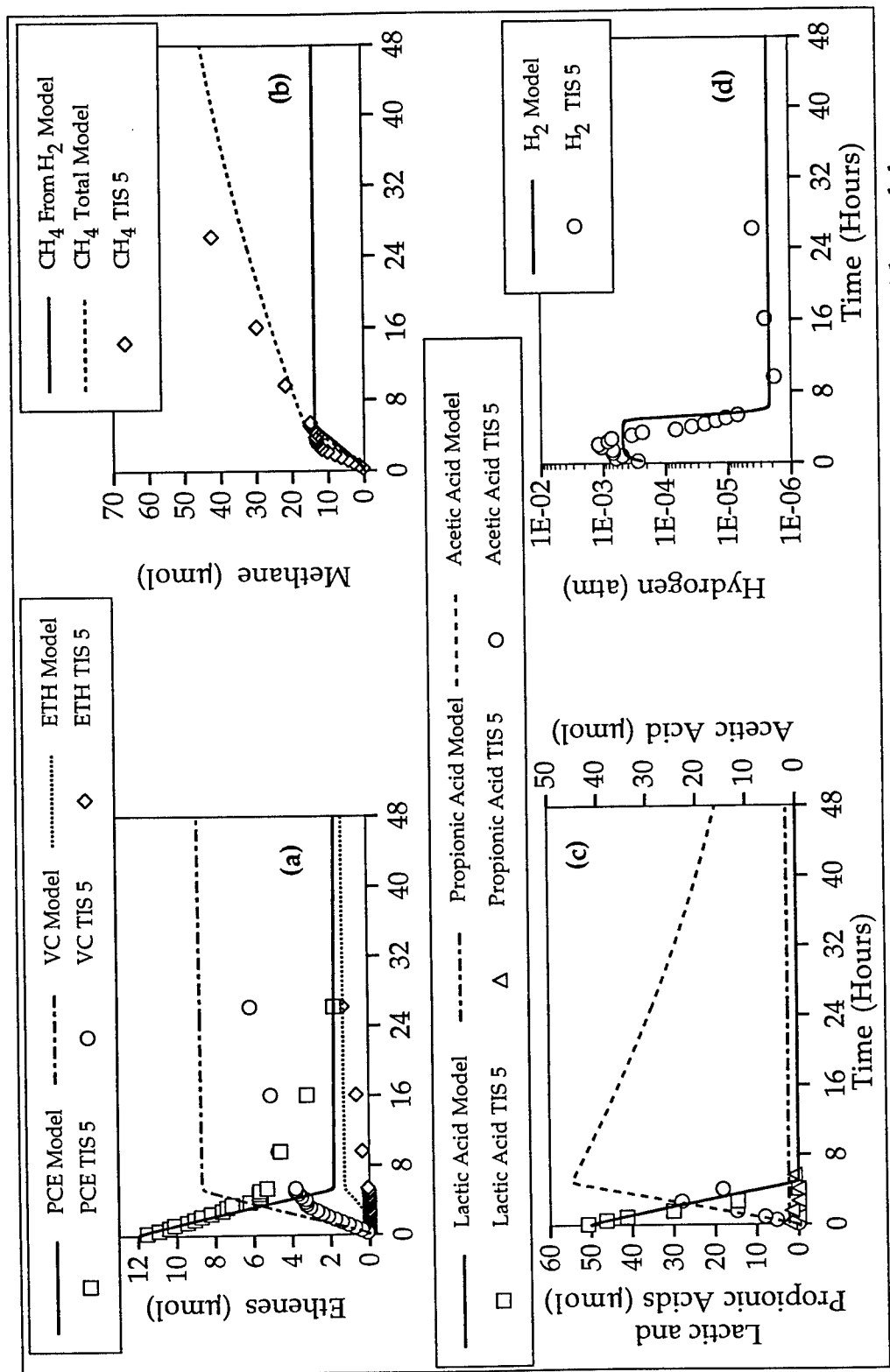


Figure 6.7. Comparison of lactic-acid-amended culture fed 2:1 donor to PCE ratio with model prediction, assuming all lactate is fermented to acetate and hydrogen. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) donor and VFAs; and (d) hydrogen.

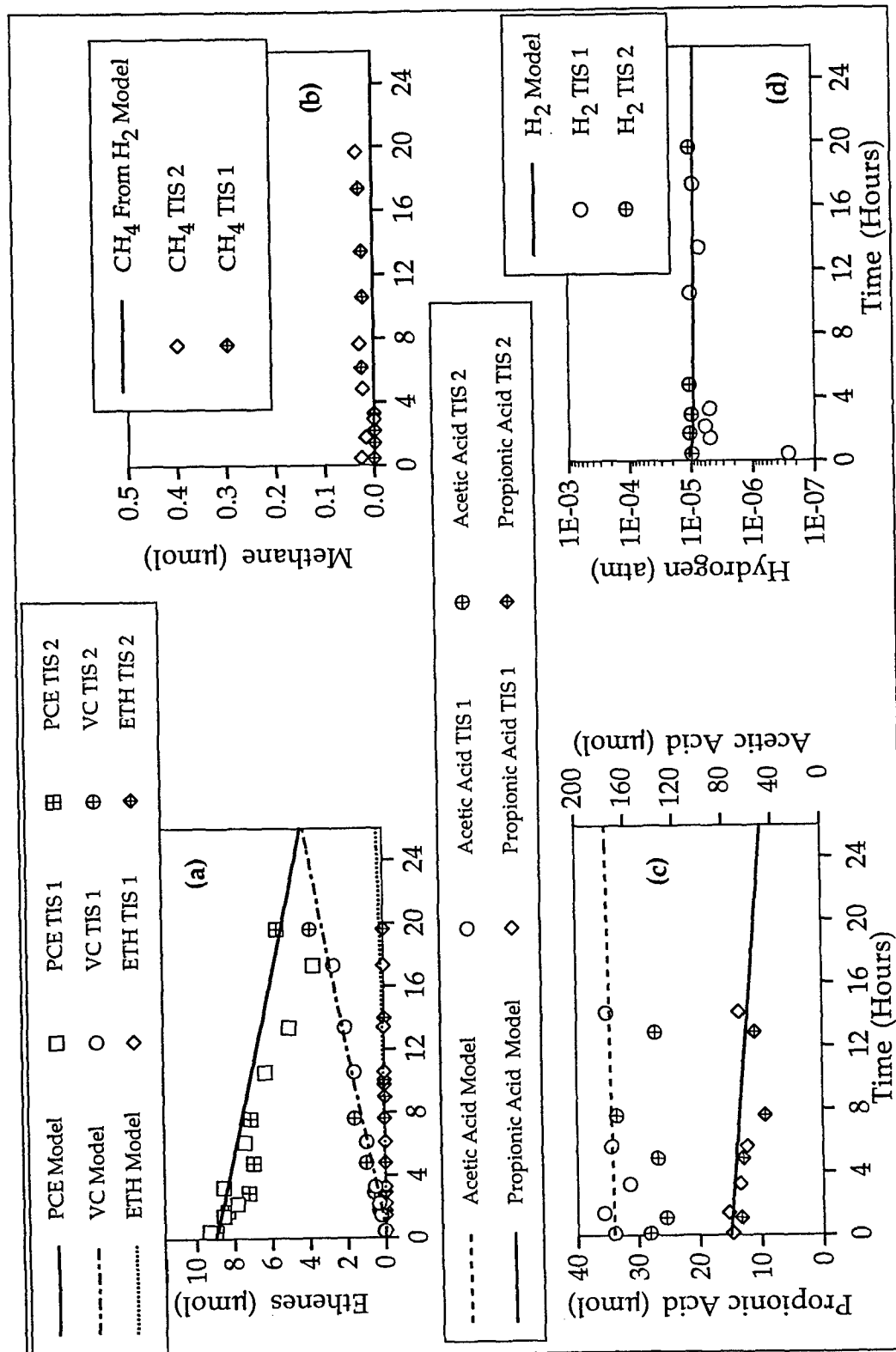


Figure 6.8. Comparison of propionic-acid-amended culture fed 1:1 donor to PCE ratio with model prediction. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

6.B.8. Propionic Acid 2:1 Ratio to PCE

The model fit to 2:1 propionic-acid-amended cultures is shown in Figure 6.9. This fit was accomplished by using all biomass predictions for a steady-state except that acetotrophic methanogens were assumed to be at only 60 percent of the level expected. The reduced population was used because the data were obtained when the culture was very obviously not at steady-state with respect to acetic acid use. Once again, the model fit this data set well and captured the overall shapes and trends. One exception was that propionic acid fermentation (Figure 6.9c) was more rapid and complete in reality than predicted by the model. The model predicted that when the primary H_2 sink—PCE dechlorination—was satisfied, the H_2 level increased somewhat and that increase caused propionic acid fermentation to slow dramatically. H_2 was then used by VC dechlorination but none was predicted to go to methanogenesis at the low H_2 level. In the actual data, the propionic acid fermentation proceeded apparently little affected by the disappearance of PCE and the slight increase in H_2 level after about 24 hr. Since acetotrophic activity was present, no separate determination of CH_4 production via H_2 could be made. Production of H_2 from the remaining propionic acid in the model simulation would have added approximately 10 $\mu\text{mol } CH_4$ to the total amount produced. The total amount in that case would still fit the actual data fairly well. It is possible that this was the fate of the remaining propionic acid.

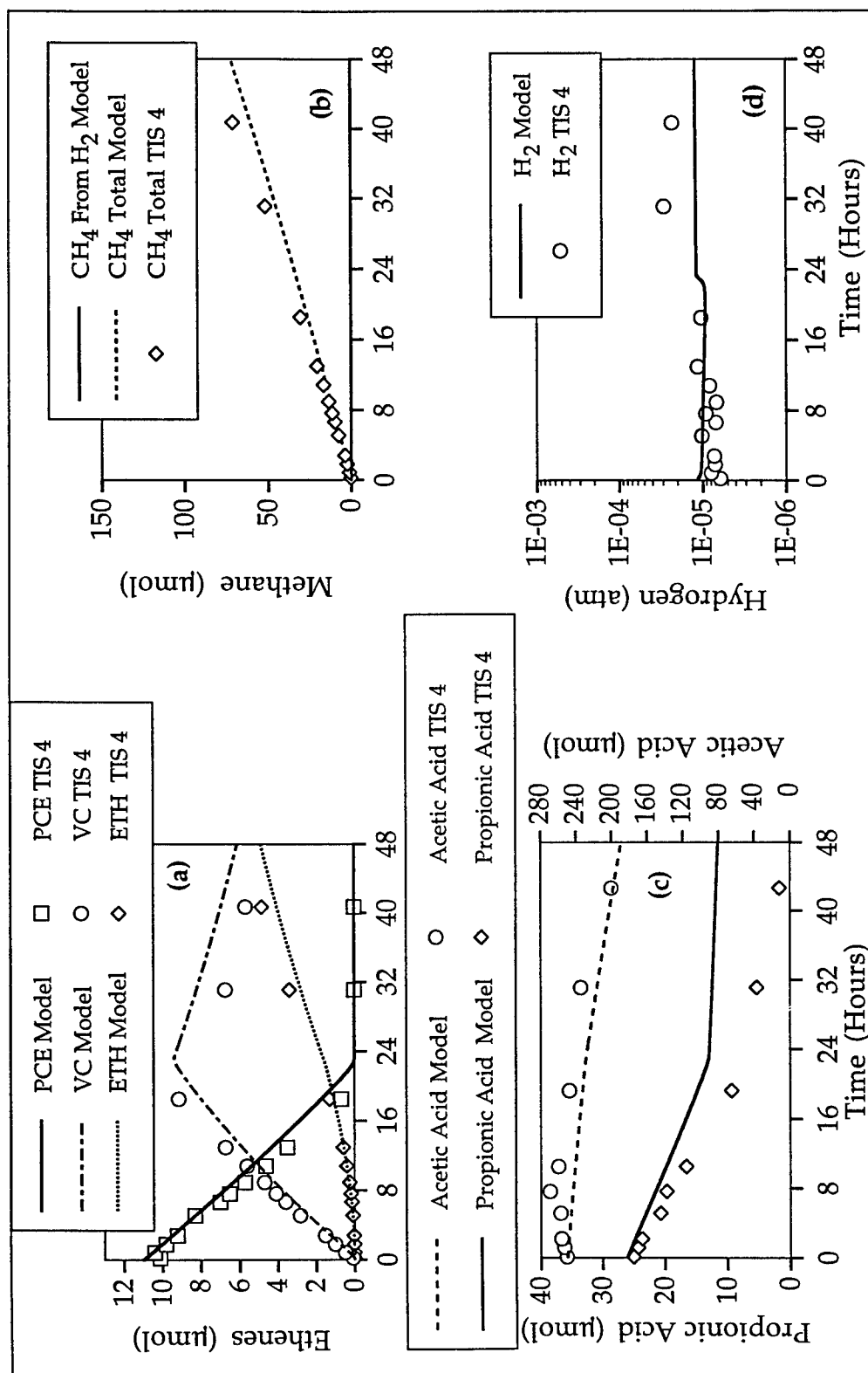


Figure 6.9. Comparison of propionic-acid-amended culture fed 2:1 donor to PCE ratio with model prediction, assuming 60% of the estimated steady-state acetotrophic activity.
 $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

6.C. *Alternative Simulations of Time-Intensive Studies*

As was noted in the previous section, simulations of butyric and propionic acid fermentation under (relatively) high acetic acid concentrations, while basically capturing the overall shapes and trends of the entire data set, did not fit the donor fermentation data as well as for some of the studies where the background acetic acid level was lower. Two alternative modeling approaches were explored in an attempt to improve the donor fermentation model fits.

6.C.1. Alteration of $\Delta G_{\text{critical}}$

In Section 4.A.3, free-energy analyses were presented for the TISs. From those analyses and from literature values, a $\Delta G_{\text{critical}}$ of -19 kJ/mol donor was chosen as a model input to govern donor fermentation (Table 5.3). In reality, ΔG_{rxn} during the butyric and propionic acids TISs sometimes approached -5 kJ/mol donor. Thus, entering a $\Delta G_{\text{critical}}$ of -19 kJ/mol as a limitation on butyric and propionic acid fermentations controlled the fermentations at a slower rate than was experimentally observed (Figure 6.1 and 6.9). For comparison, some simulations were repeated, using a $\Delta G_{\text{critical}}$ set 25 percent higher, i.e. -14.25 kJ/mol donor, and the k_{Butyrate} or $k_{\text{Propionate}}$ that corresponded to this $\Delta G_{\text{critical}}$ (see Appendix VII).

Such a simulation for 1:1 butyric acid is shown in Figure 6.10. The k_{Butyrate} used was 2.8 $\mu\text{mol/mg VSS-hr}$. This simulation was somewhat improved over the one using $\Delta G_{\text{critical}}$ of -19 kJ/mol (Figure 6.1), but still did not fully predict the extent of dechlorination, or the totality of CH_4

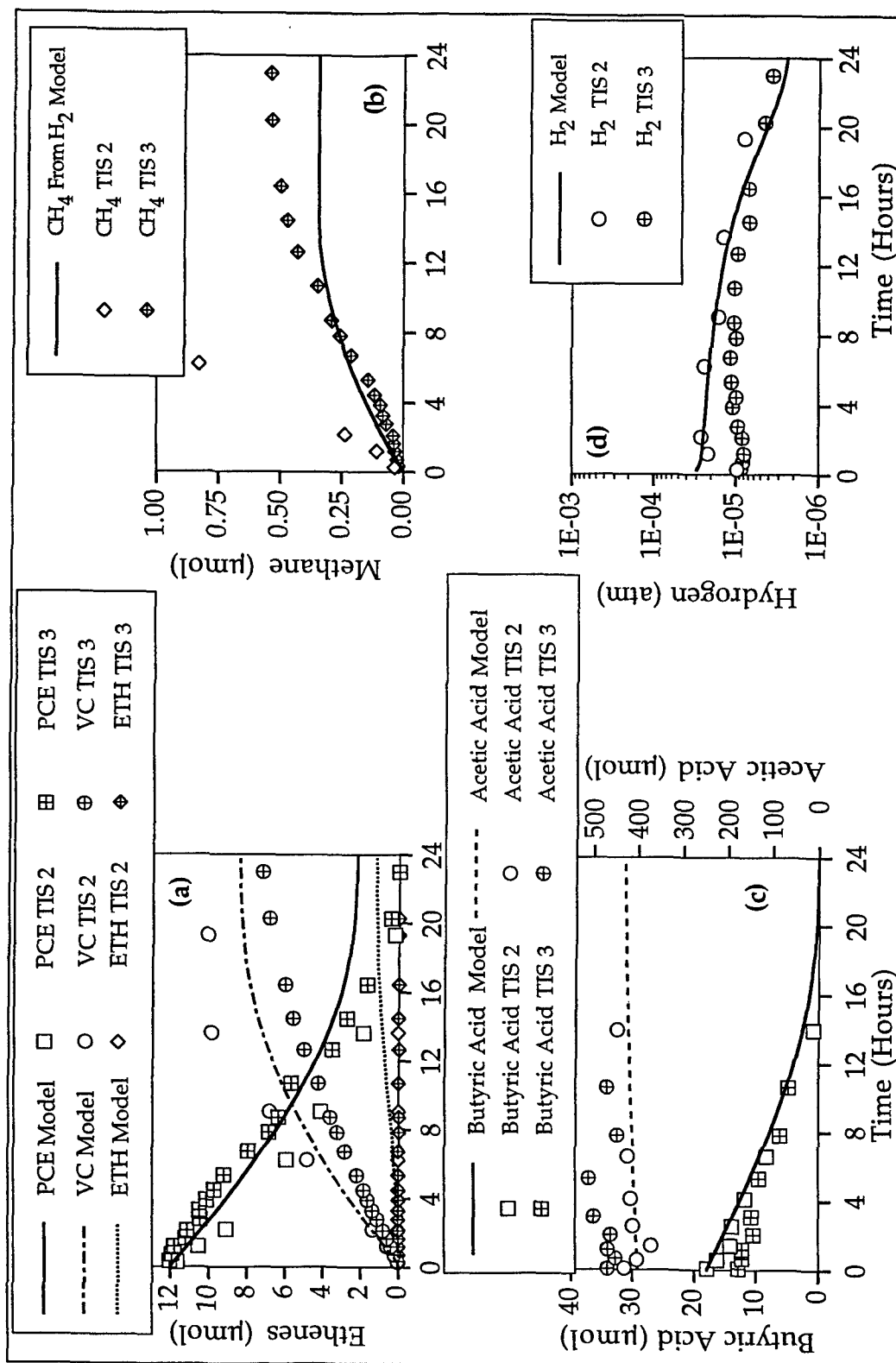


Figure 6.10. Comparison of butyric-acid-amended culture fed 1:1 donor to PCE ratio with model prediction. $\Delta G_{\text{critical}} = -14.25 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

formation. However, the H_2 level predicted was still within the levels observed during the TISs.

The 2:1 butyric acid model simulation was also re-run using the -14.25 kJ/mol donor $\Delta G_{\text{critical}}$ setting and the corresponding k_{Butyrate} . The result of the simulation, shown in Figure 6.11, did not fit the data as well as the simulation run with a $\Delta G_{\text{critical}}$ of -19 kJ/mol donor (Figure 6.2), especially in terms of how rapidly the butyric acid was degraded and the acetic acid and H_2 were formed. This comparison helps to demonstrate the difficulty of accurately modeling all the data collected during the experimental phase of the study.

The model simulation of 2:1 propionic acid was repeated with a $\Delta G_{\text{critical}}$ setting of -14.25 kJ/mol (Figure 6.12) and the corresponding $k_{\text{Propionate}}$ (1.5 $\mu\text{mol}/\text{mg VSS-hr}$). While the changes allowed a slight increase in the rate of propionic acid fermentation after 20 hr (when PCE was depleted) and resulted in an increased H_2 level, as was observed experimentally, the model still predicted very little CH_4 production from H_2 —which would have probably been the primary sink for the excess H_2 reducing equivalents at that time. The change in the $\Delta G_{\text{critical}}$ setting otherwise had very little overall effect on the fit.

6.C.2. Alteration of ΔG°_f for Acetate

Since the earlier model fits for butyric acid and propionic acid fermentation were more in error at (relatively) high acetic acid values than at low ones, an examination of ΔG°_f values for acetate was warranted. The second approach for attempting to improve model fits, therefore, involved examining the literature for other values of ΔG°_f for acetate.

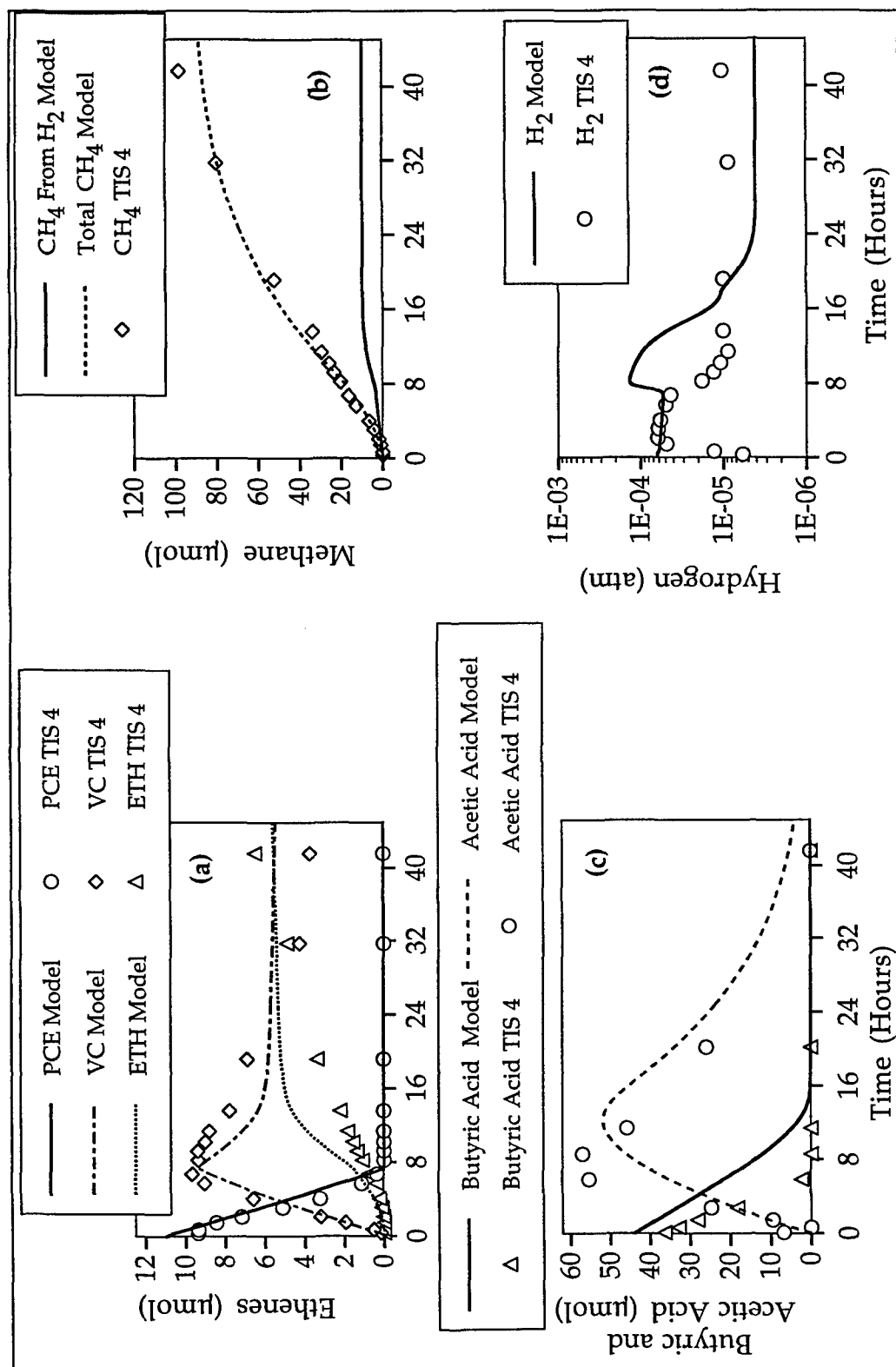


Figure 6.11. Comparison of butyric-acid-amended culture fed 2:1 donor to PCE ratio with model prediction. $\Delta G_{\text{critical}} = -14.25 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

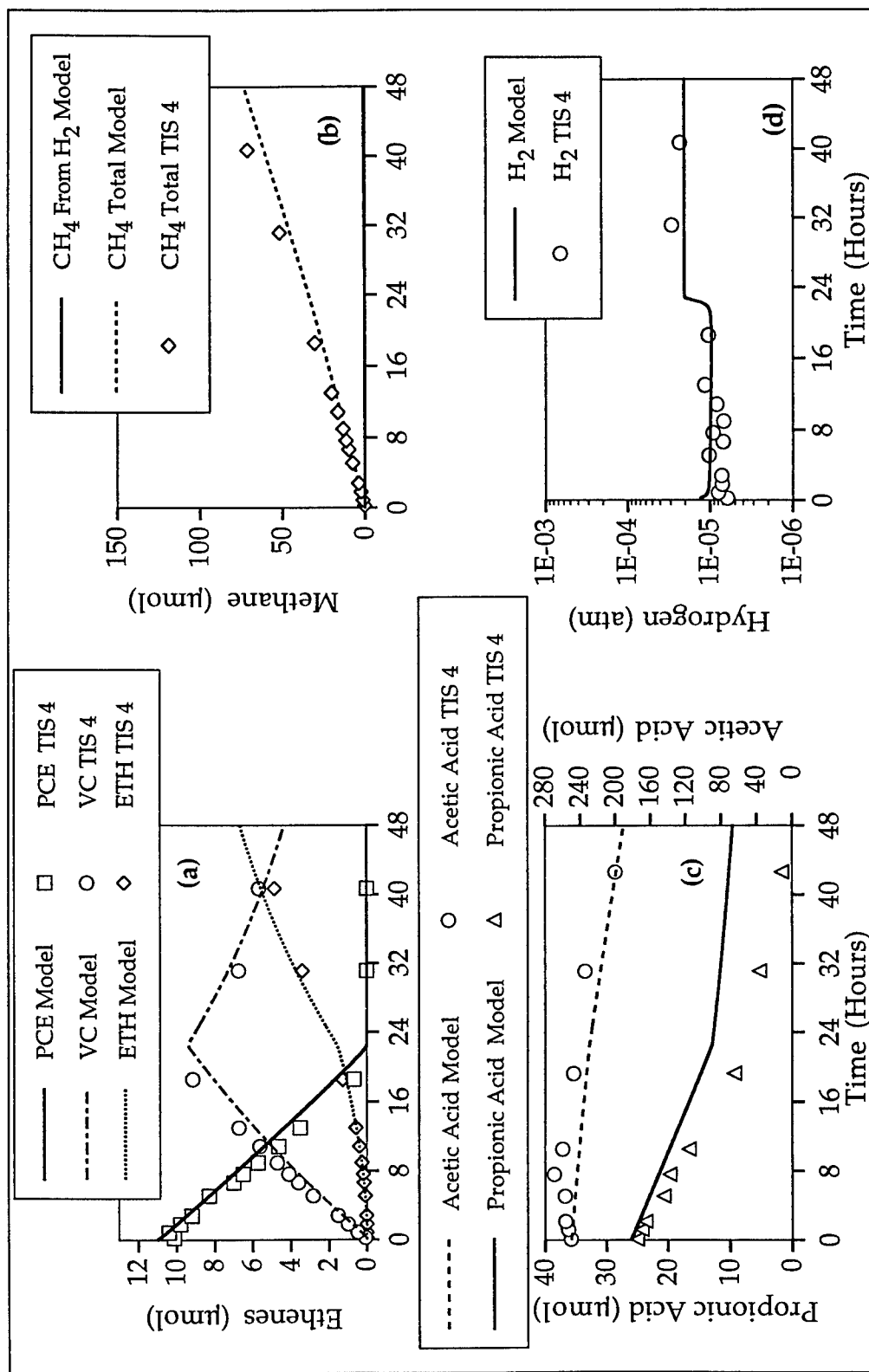


Figure 6.12. Comparison of propionic-acid-amended culture fed 2:1 donor to PCE ratio with model prediction, assuming 60% of the estimated steady-state acetotrophic activity. $\Delta G_{\text{critical}} = -14.25 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

Thermodynamic values from Thauer *et al.* [233] were used, where possible, for this study to be consistent with the literature—because these values are widely and almost exclusively used for these types of analyses. Most of Thauer *et al.*'s values are taken from a National Bureau of Standards compilation. Other values do exist, and there is, of course, some variation in the reported values for various substrates. If one chose the value of ΔG°_f for acetate reported by Wilhoit [266], -376.89 kJ/mol acetate, for example, results would be quite different since this value is significantly different than the one tabulated by Thauer *et al.*, -369.41 kJ/mol acetate. Using Wilhoit's value, a new $\Delta G^\circ_{35^\circ\text{C}}$ for butyric acid fermentation (107.7 kJ/mol butyrate) was calculated according to Appendix VI and a new k_{Butyrate} (2.7 $\mu\text{mol/mg VSS-hr}$) was calculated according to Appendix VII. Alternative simulations for 1:1 and 2:1 butyric acid were run using these new values and retaining the $\Delta G_{\text{critical}}$ setting of -19 kJ/mol donor. Results are shown in Figures 6.13 and 6.14.

In comparing the simulations for 1:1 butyric acid, Figures 6.1 and 6.13, it could be argued that using the different ΔG°_f for acetate improved the fit of the model to the 1:1 butyric acid data in some respects. Butyric acid fermentation was fit especially well. However, the simulation with the alternative ΔG°_f for acetate still did not fully capture the extent of dechlorination—PCE dechlorination leveled off in the simulation after 16 hr, while during the experiment, it continued.

The simulation using the alternative ΔG°_f for acetate for 2:1 butyric acid (Figure 6.14) again, fit the experimental data in some respects, but still predicted slower butyric acid fermentation than was observed, and a peak in H_2 (after PCE depletion) that was not observed experimentally. Overall,

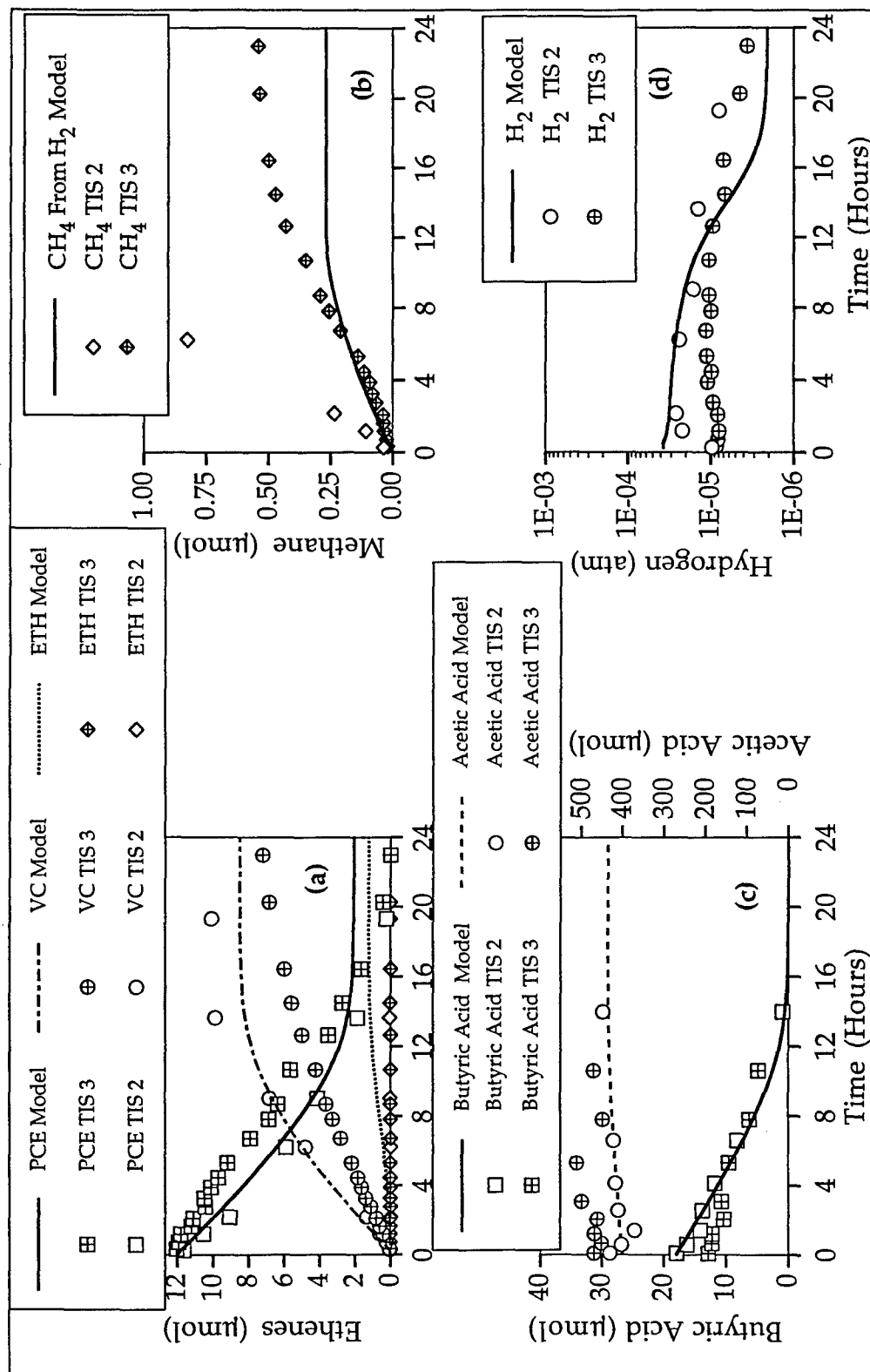


Figure 6.13. Comparison of butyric-acid-amended culture fed 1:1 donor to PCE ratio with model prediction, using alternate ΔG°_f value for acetate. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

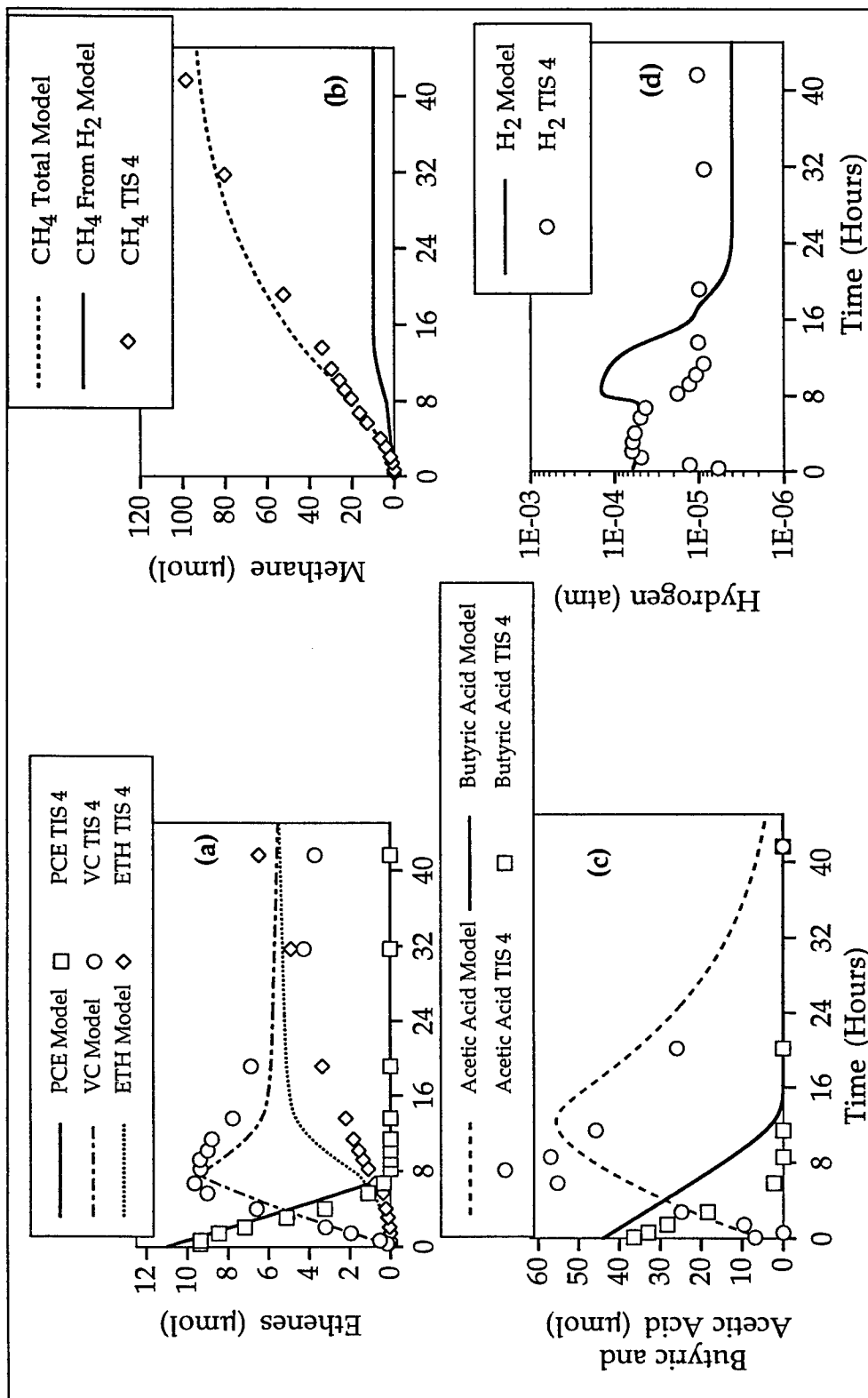


Figure 6.14. Comparison of butyric-acid-amended culture fed 2:1 donor to PCE ratio with model prediction, using alternate ΔG°_f for acetate. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

the simulation which incorporated the Thauer *et al.* ΔG°_f value for acetate (Figure 6.2) was a superior fit for this data set.

6.D. Simulation of the Low-PCE/Butyric-Acid-Amended Source Culture.

6.D.1. Simulation of the Long-Term Operation of the Low-PCE/Butyric Acid-Amended Culture

The model was used to simulate the long-term performance of the low-PCE/butyric acid source culture. These data were the most “steady-state” data available for comparison to simulation. The simulation was performed by starting with a somewhat arbitrarily chosen biomass content and then running the model for a 104-day simulation to observe where the simulation stabilized. The initial biomass content was based on the estimated biomass distribution for a 2:1 butyric-acid-fed system (see Table 6.2), except that the expected acetotrophic biomass was arbitrarily set at one-third of that expected. The initial biomass (mg VSS/100 mL) settings were 1.51 butyric acid degraders, 0.0042 propionic acid degraders, 0.57 acetotrophic methanogens, 0.84 hydrogenotrophic methanogens, and 2.93 dechlorinators. The arbitrarily chosen initial settings for VFAs, per 100 mL, were 1000 μmol acetic acid and 25 μmol propionic acid. The repetitive (every 48 hr) inputs were: PCE, 11 μmol ; butyric acid; 44 μmol ; and FYE 40 μL .

The model simulation is shown in Figure 6.15. This simulation may be compared with Figure 4.18, the data from the long-term operation of the source culture. The simulation predicted a higher VC residual (*circa* 5 μmol per feeding) than was actually observed in the culture (*circa* 2 μmol

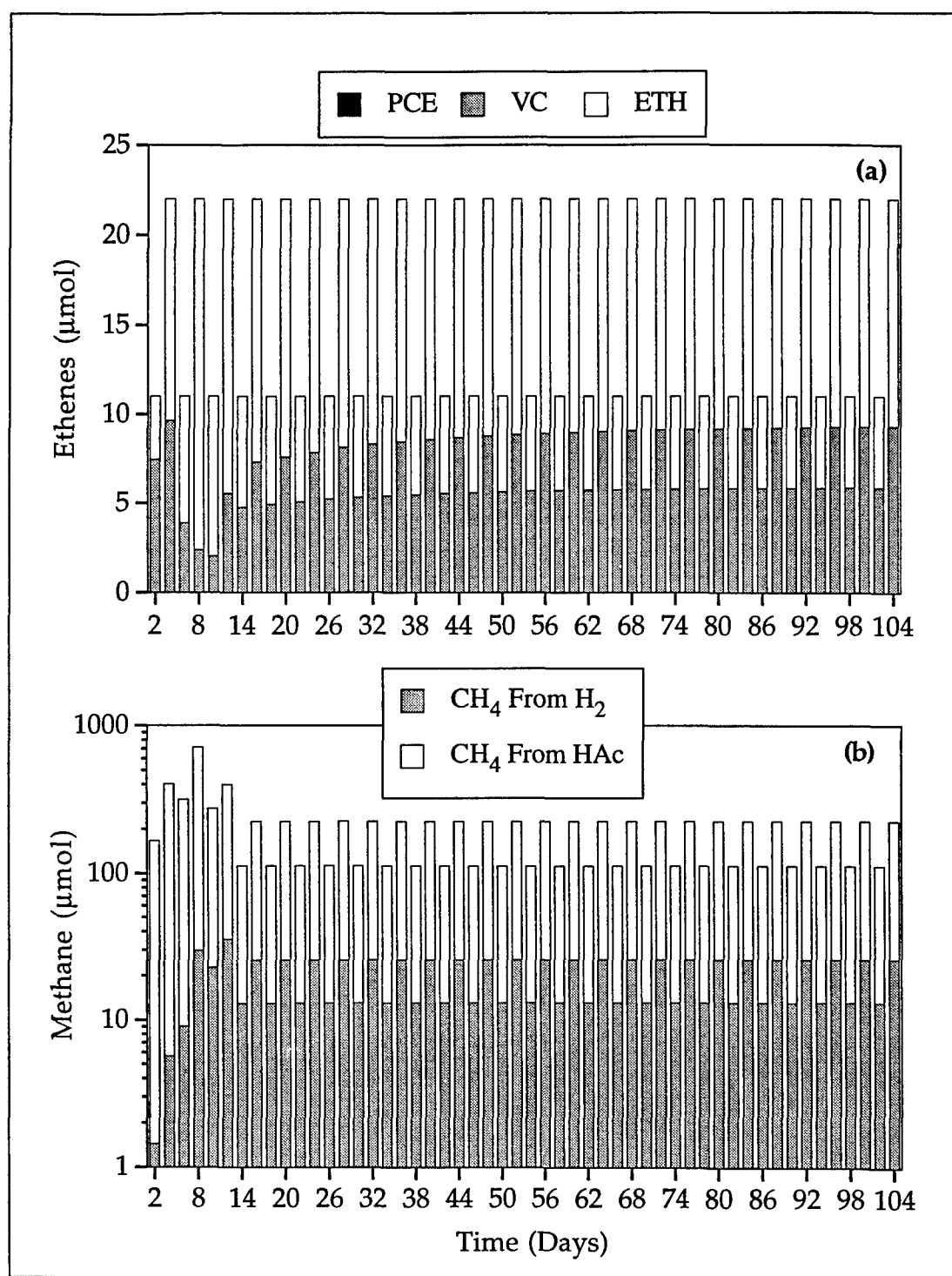
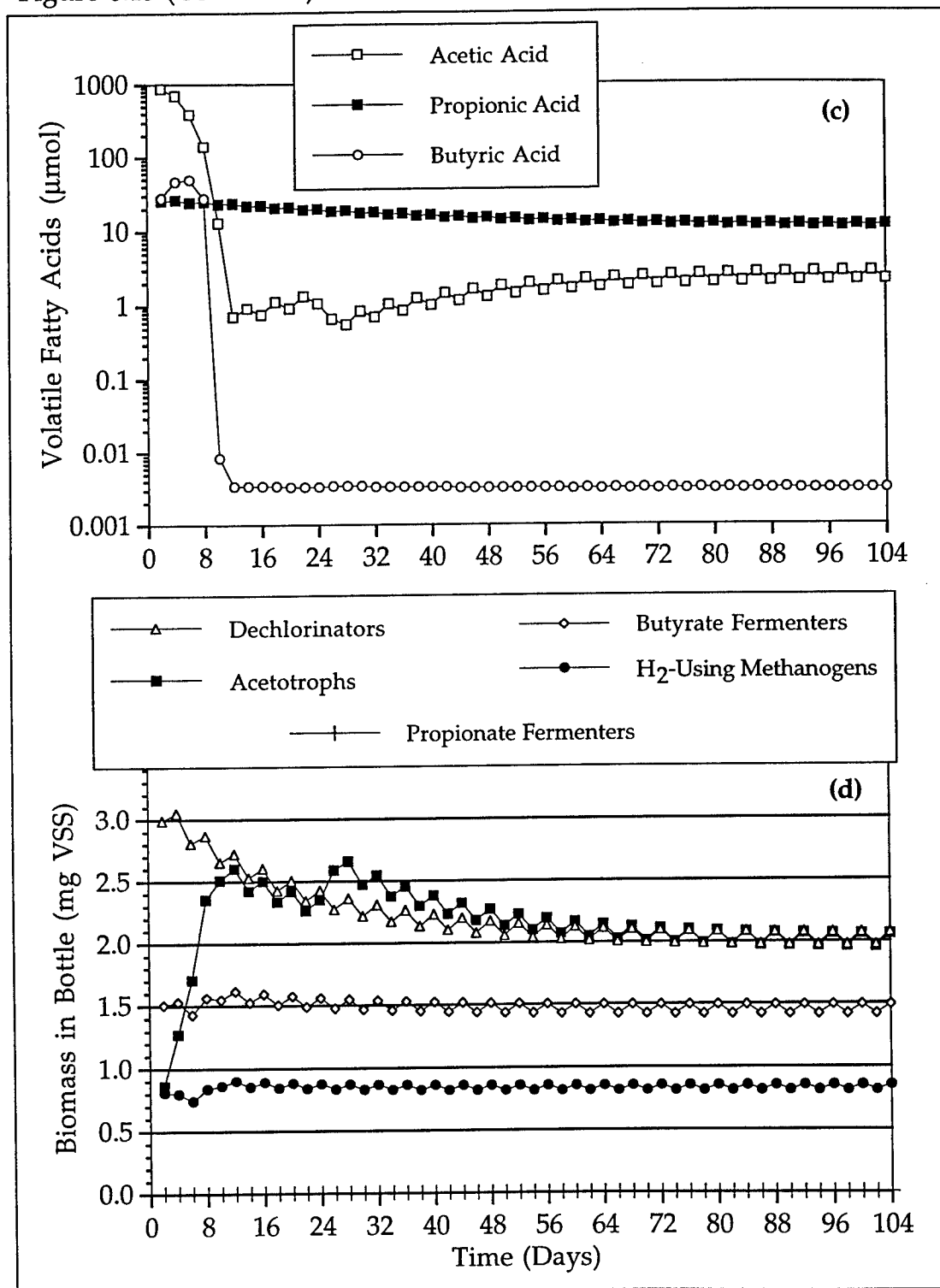


Figure 6.15. Simulation of culture amended with butyric acid at a 2:1 donor to PCE ratio with FYE amendment: (a) dechlorination; (b) methane from hydrogen; (c) VFAs; and (d) biomass.

Figure 6.15 (Continued)



per feeding), and less total CH_4 formation (*circa* 112 μmol per feeding) than was observed in the culture (*circa* 125 μmol per feeding).

After some initial flux during the first 8 days, butyric and acetic acids stabilized at 0.003 $\mu\text{mol}/100\text{ mL}$ and 3 $\mu\text{mol}/100\text{ mL}$, respectively. In the culture, butyric acid was measured at 2 to 3 $\mu\text{mol}/100\text{ mL}$, while acetic acid (after acetotrophic activity was established) ranged from 10 to 20 $\mu\text{mol}/100\text{ mL}$. The simulated propionic acid concentration approached a steady-state value of 10 $\mu\text{mol}/100\text{ mL}$; while in the culture, propionic acid was measured at 3 to 7 $\mu\text{mol}/100\text{ mL}$ residual (after acetotrophic activity was established).

Simulated biomass levels (Figure 6.15d) stabilized after about 64 days. The dechlorinator biomass shifted significantly from the 3 $\text{mg}/100\text{ mL}$ value, used as a model input, to 2 $\text{mg}/100\text{ mL}$ at the end of simulation. Acetotrophic biomass also shifted, but that was expected since the initial amount entered was only one-third of the expected steady-state amount. Nonetheless, it stabilized at near 2 $\text{mg}/100\text{ mL}$ —slightly higher than that expected at steady state from the biomass estimates (1.71 $\text{mg}/100\text{ mL}$). The final total biomass for the simulation was 6.48 $\text{mg}/100\text{ mL}$, comparable to the actual content of the butyric acid source culture—6.5 to 7.2 $\text{mg VSS}/100\text{ mL}$ —estimated via PON analysis (see Table 5.15).

The reduction product accumulation (on a CO_2 equivalents basis) in the low-PCE/butyric acid culture and generated by the simulation are displayed in Figure 6.16. The solid line on each graph depicts the amount of reducing equivalents added on a CO_2 equivalents basis in the form of added butyric acid (880 μeq) and added FYE (160 μeq).

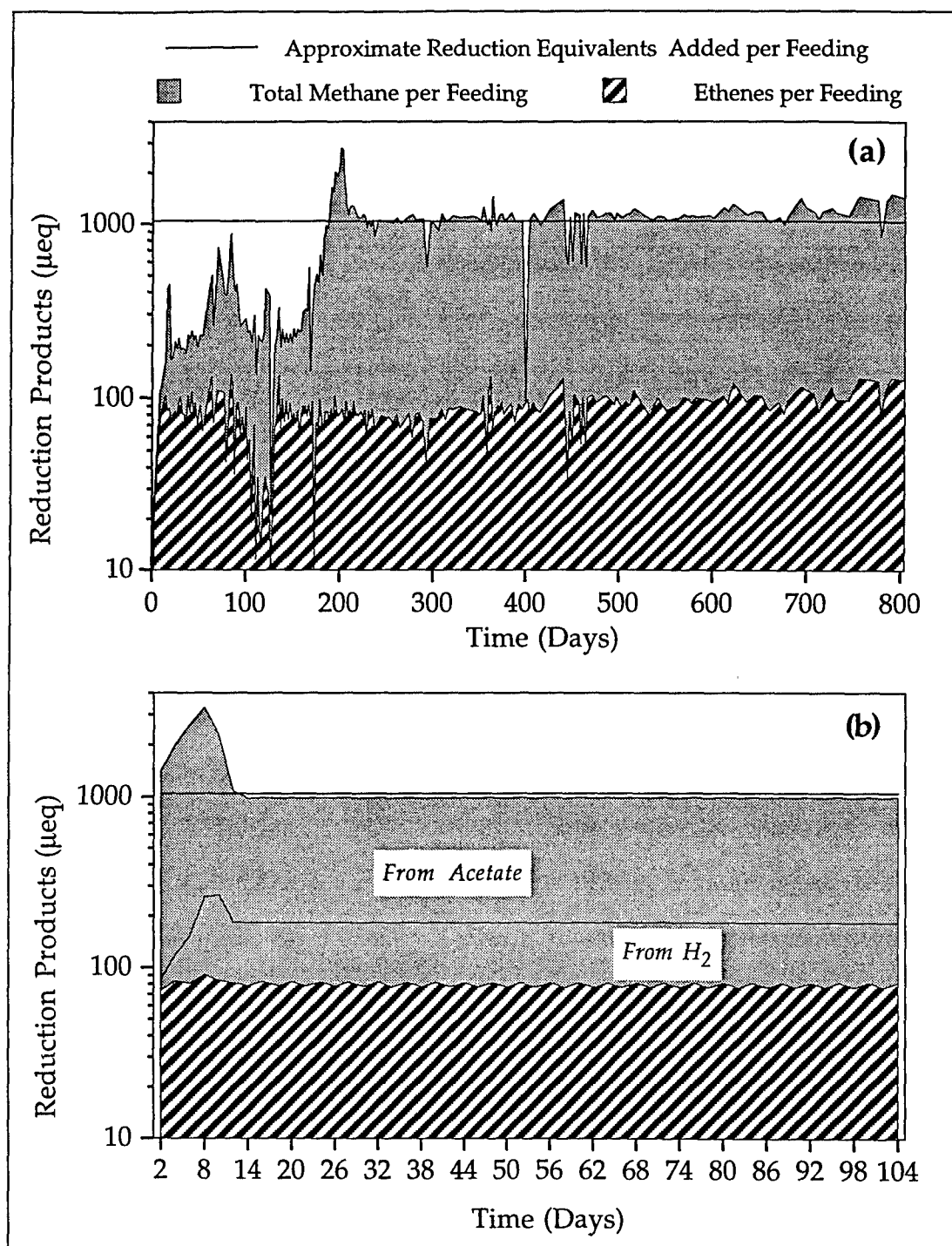


Figure 6.16. Reduction products formed with a 2:1 butyric acid to PCE ratio plus FYE: (a) low-PCE/butyric acid source culture and (b) model simulation.

If the steady-state portions of each graph (Days 400 to 800, Figure 6.16a; Days 62 to 104 Figure 6.16b) are examined, it is observed that approximately 12 percent more reducing equivalents (dechlorination products plus CH_4) were formed (1105 μeq) in the culture than were predicted by the model (986 μeq). Also, the sum of the dechlorination products in the source culture was 12 to 15 μmol (96 to 120 μeq per feeding) rather than the expected 11 μmol (88 μeq per feeding), which was used for modeling. This difference alone would account for an excess of 8 to 32 μeq of reduction products (per feeding) in the source culture over that predicted by the model. These differences are most likely explained by the fact that the large, 9-L source culture bottles were purged for only 2 to 5 min every 4 days to remove the accumulated CH_4 and dechlorination products. It is very likely that this purging did not adequately remove all the accumulated products from the bottle. These remaining products would then have contributed to the total amount of dechlorination products and CH_4 detected at the subsequent measurement. The detection of excess dechlorination products, especially, suggests that this explanation is valid.

Thus, while some differences do exist (most probably caused by inadequate purging) the model predicts the steady-state behavior of the long-term operation of the butyric acid source culture very well.

6.D.2. Simulation of Time-Intensive Study of the Low-PCE/Butyric Acid-Amended Culture

At the end of the 104-day simulation, ending values for biomass and VFAs were entered as beginning values and a 48-hr TIS simulation was

run. The results of the simulation were overlaid over actual data for a TIS that was run with source culture on Day 384 of operation (see Section 4.C.3) (Figure 6.17). The model, while capturing the overall shape and trend of the data fairly well, did not predict the same amount of ETH formation, and butyric acid fermentation was more rapid experimentally than predicted. The CH_4 and H_2 levels were predicted well.

6.E. Simulations of Long-Term Operation of Propionic-Acid- and Ethanol-Amended Cultures

Long-term simulations were run for propionic acid and ethanol—the two donors that yielded the most contrasting data sets. The simulations were run both with and without FYE addition—the latter being something that was not possible for the actual experiments. The simulations were performed for the 1:1 donor to PCE ratio with no acetotrophic activity since these data sets provided the sharpest contrast. Long-term simulations were set up using the biomass inputs for propionic acid and ethanol at 1:1 ratios as shown in Table 6.2. The repetitive amendment amounts were 15 μmol propionic acid or 22 μmol ethanol, and 11 μmol PCE. FYE, when added, was set at 20 μL . The initial 48 hr of the simulations run with FYE are shown to compare with the TIS for each donor without FYE which were already shown in Section 6.B.

6.E.1. Simulations of Propionic Acid at a 1:1 Ratio

The initial 48 hr of the long-term simulation of a 1:1 propionic-acid-amended culture with FYE added is shown in Figure 6.18. This simulation can be roughly compared to Figure 6.8 which depicts a model simulation of actual 1:1 propionic acid TISs that did not include FYE addition. The

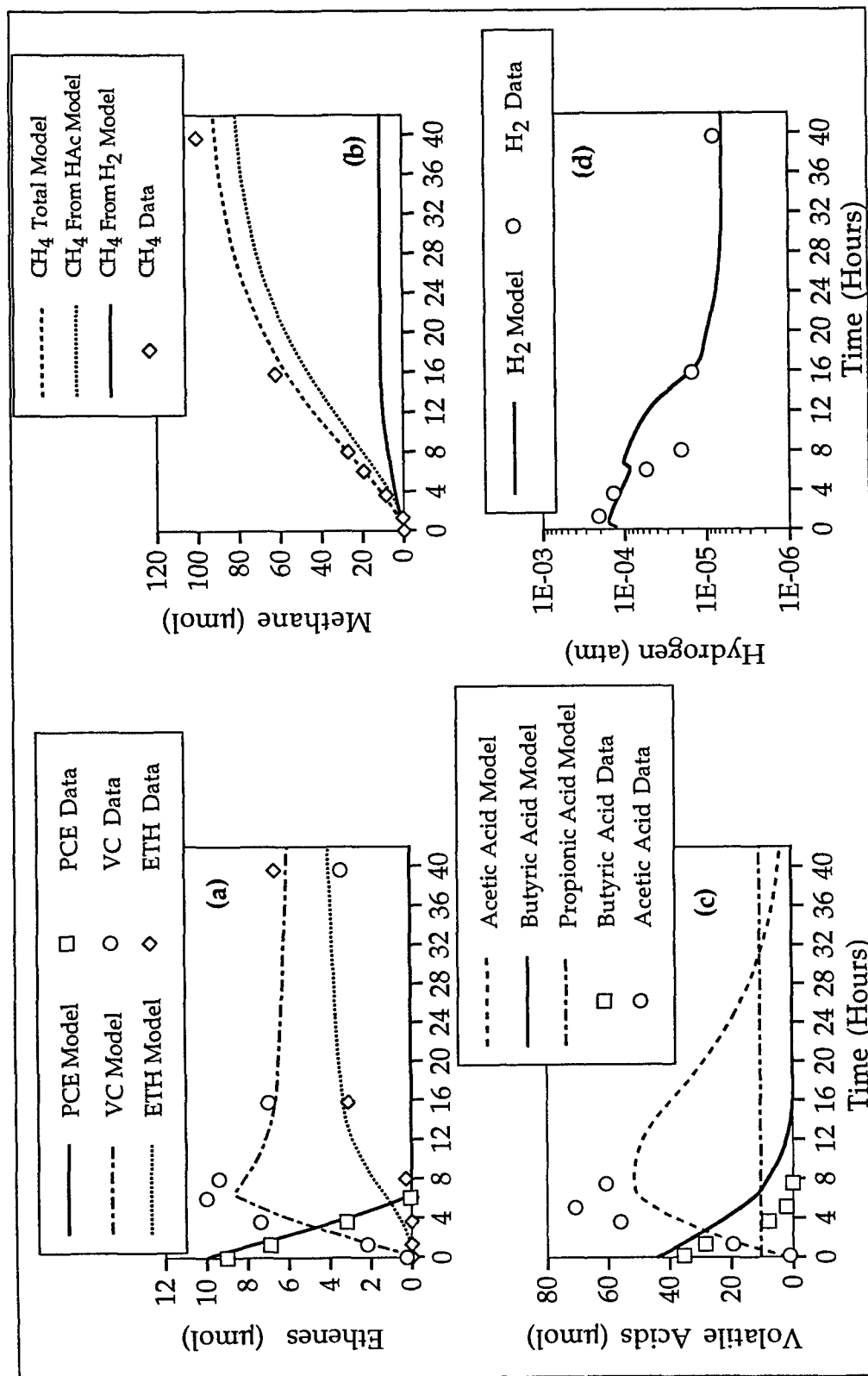


Figure 6.17. Comparison of a time-intensive study of the low-PCE/butyric acid source culture on Day 384 of operation with model simulation: (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

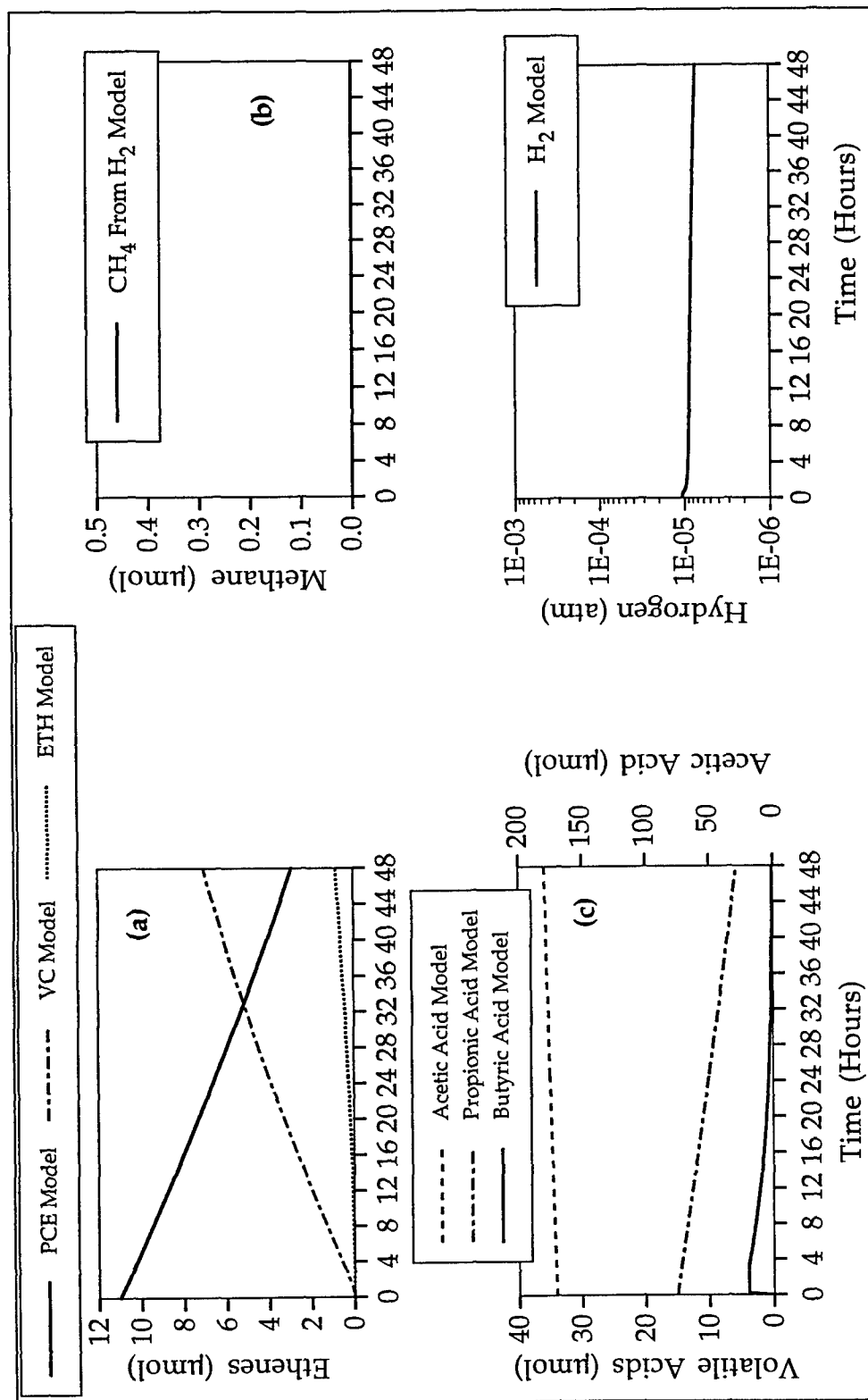


Figure 6.18. Short-term simulation of propionic-acid-amended culture fed 1:1 donor to PCE ratio and FYE. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) VFAs; and (d) hydrogen.

simulation with FYE (Figure 6.18) used a different starting value of PCE (11 μmol), and was run for 48 hr (as opposed to 26 hr for Figure 6.8). The pattern generated is similar to Figure 6.8, except for the presence of the more readily degraded butyric acid that was contributed by the FYE.

The long-term simulation of propionic acid without FYE is shown in Figure 6.19. Note that while, initially, PCE remained as a residual in the culture, dechlorination eventually became more complete (Figure 6.19a). The reason that PCE dechlorination was incomplete in the beginning was that propionic acid fermentation was unfavorable and not enough was degraded to fully supply all the reducing equivalents needed for dechlorination. The relatively high background acetic acid level (170 μmol) contributed to the unfavorability of the fermentation. Only a trace of CH_4 was formed in these systems (Figure 6.19b). As propionic acid accumulated in the system (Figure 6.19c), the thermodynamics of the system shifted and more fermentation occurred. The same accumulation in propionic-acid-amended systems was observed in the experiments. Occasionally the propionic acid was withheld during experiments to ensure that it did not vastly exceed the desired donor to PCE ratio. If the simulation were run with a different $\Delta G_{\text{critical}}$, the fermentation would be able to proceed further at a same propionic acid and acetic acid levels. Again, the appropriate setting for the $\Delta G_{\text{critical}}$ was not entirely clear from the experimental data, and some uncertainty exists as to what the appropriate setting should be. The final biomass content of the system was 2.3 mg/100 mL.

The long-term simulation of propionic acid with FYE added is shown in Figure 6.20. This simulation can be compared to the actual data

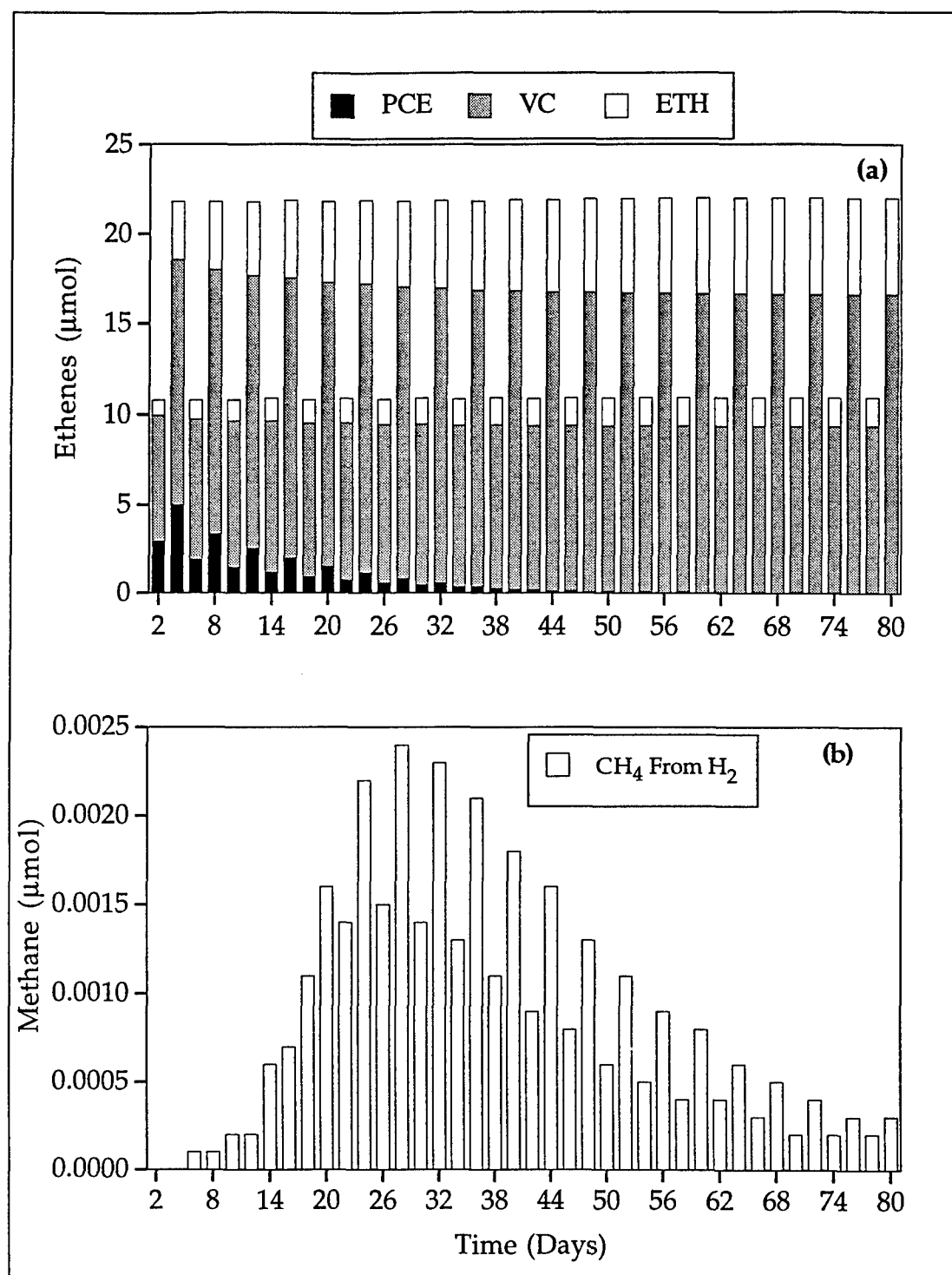
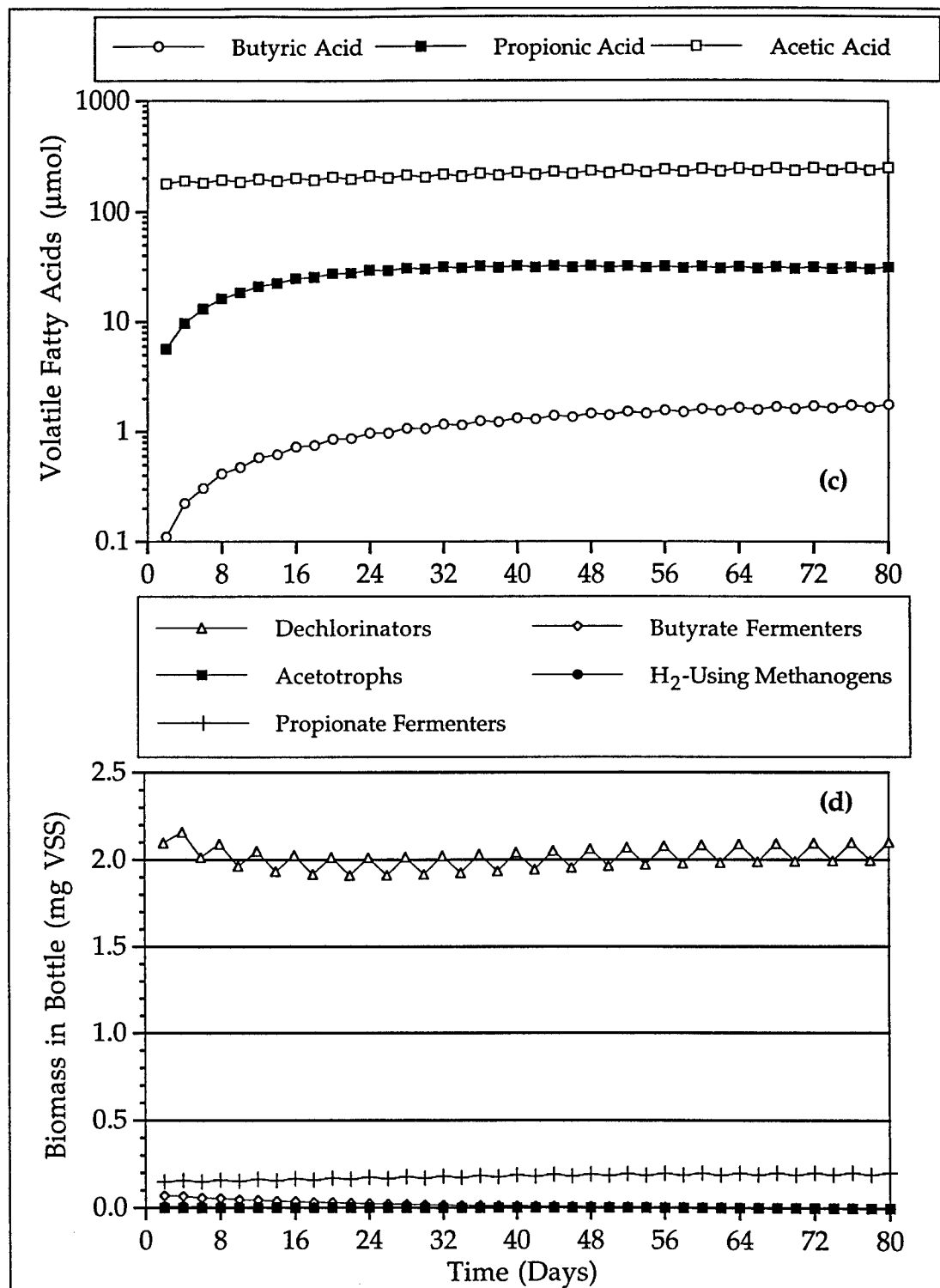


Figure 6.19. Long-term simulation of propionic-acid-amended culture fed 1:1 donor to PCE ratio without FYE amendment: (a) dechlorination; (b) methane from hydrogen; (c) VFAs; and (d) biomass.

Figure 6.19 (Continued)



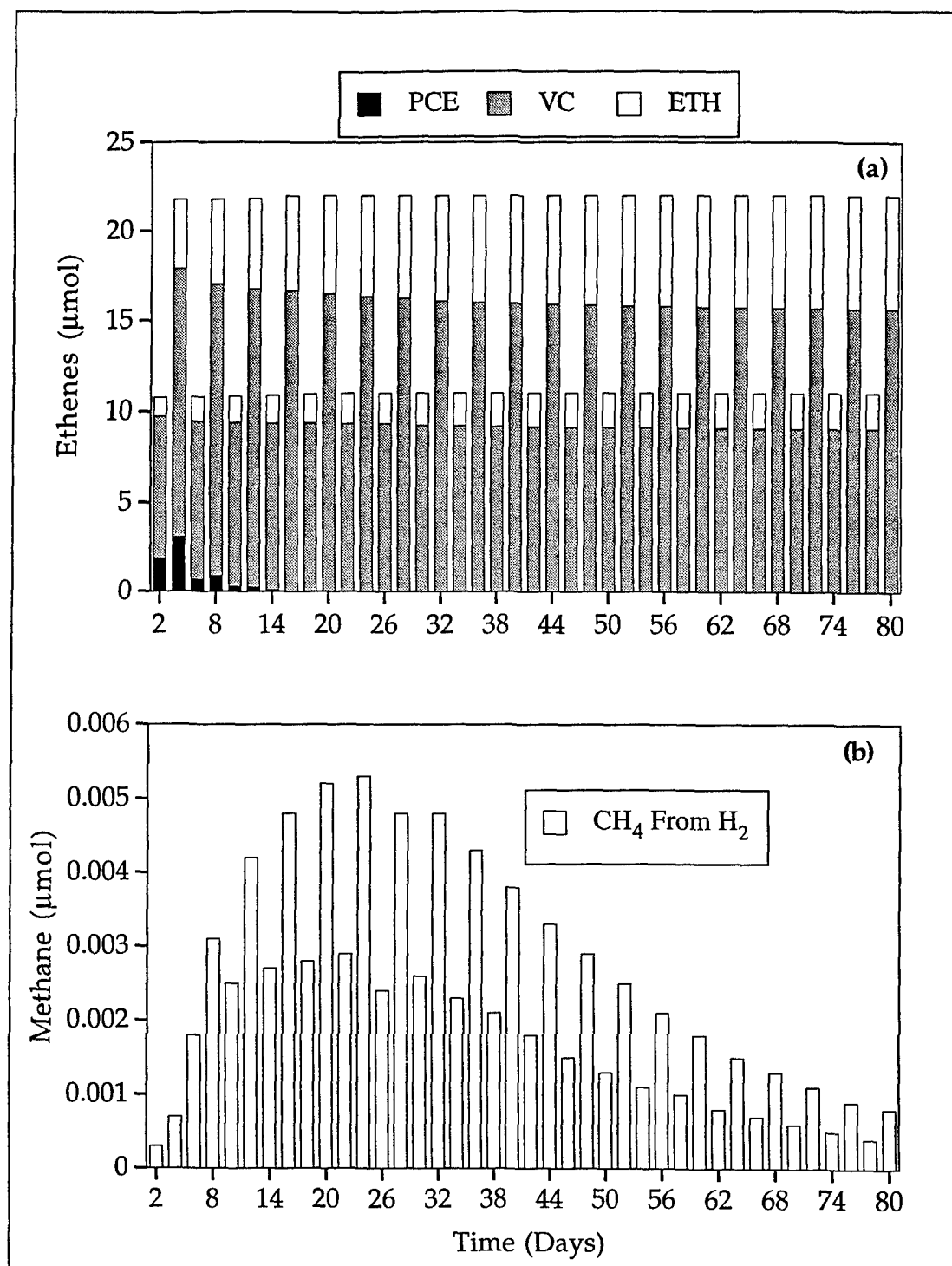
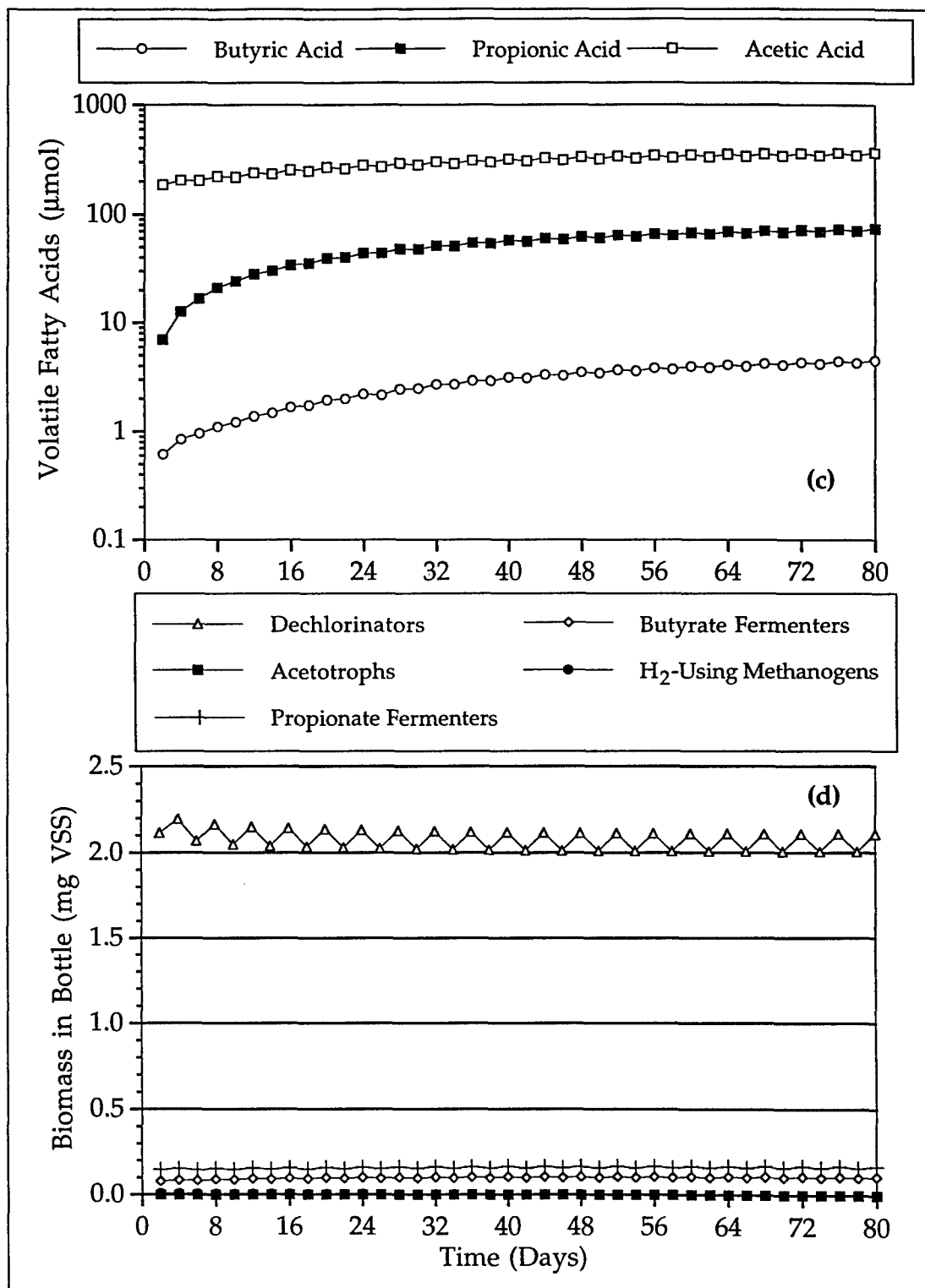


Figure 6.20. Long-term simulation of propionic-acid-amended culture fed 1:1 donor to PCE ratio with FYE amendment: (a) dechlorination; (b) methane from hydrogen; (c) VFAs; and (d) biomass.

Figure 6.20 (Continued)



of a culture operated under these conditions shown in Figure 4.5. In this case, after some initial, incomplete dechlorination, the system did dechlorinate fully and no PCE residual remained (Figure 6.20a).

Interestingly, the addition of small amounts of a somewhat more rapidly degraded donor (butyric acid) appeared to benefit the propionic-acid-fed system over what was observed in the simulation of a system where FYE was withheld. The PCE residual disappeared after 14 days in the system that was amended with FYE (Figure 6.20a), while in the simulation without FYE, the PCE residual persisted until Day 50 (Figure 6.19a).

Biomass levels were stable in this simulation (Figure 6.20d) and the total final biomass was 2.38 mg VSS/100 mL, very similar to that estimated to be in the 1:1 propionic-acid-enrichment from PON analysis, 2.28 mg VSS/L (Table 5.15).

6.E.2. Simulations of Ethanol at a 1:1 Ratio

Figure 6.21 depicts a 1:1 ethanol to PCE ratio TIS simulation with FYE added. This simulation may be compared to Figure 6.3—the same simulation except that no FYE was added. Note that Figure 6.21 also differs in that the length of simulation was 48 hr, while Figure 6.3 depicts a 20 hr run. The overall trend in the simulation is the same, however the presence of the more slowly degraded butyric acid (Figure 6.21c) fuels continued dechlorination of the PCE after ethanol has been depleted.

Figure 6.22 shows the long-term simulation of 1:1 ethanol without FYE addition. This condition was not tested during the experimental study since in actual systems, FYE was thought to be a required nutrient

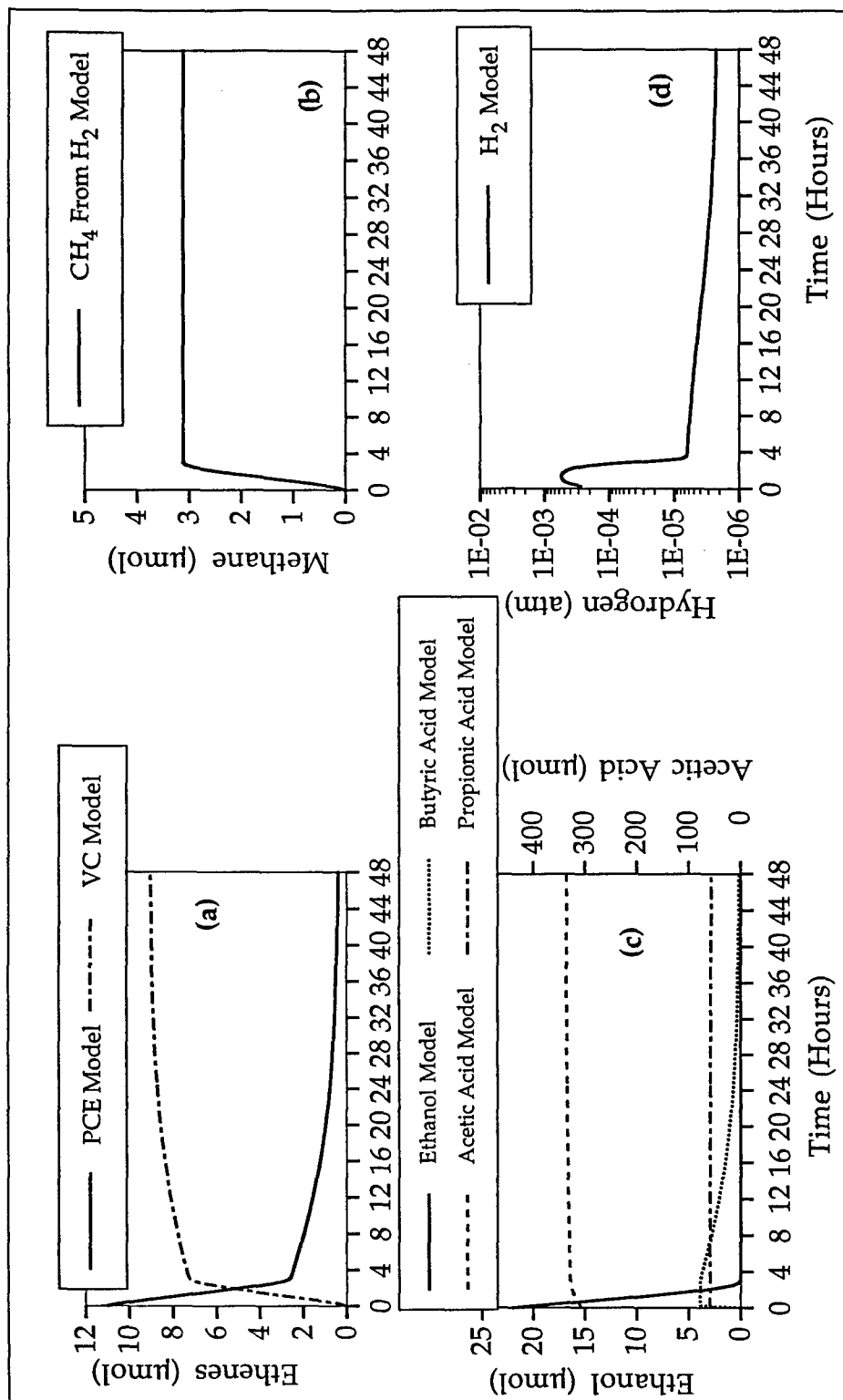


Figure 6.21. Short-term simulation of ethanol-amended culture fed 1:1 donor to PCE ratio and FYE. $\Delta G_{\text{critical}} = -19 \text{ kJ/mol}$. (a) dechlorination; (b) methane; (c) donor and VFAs; and (d) hydrogen.

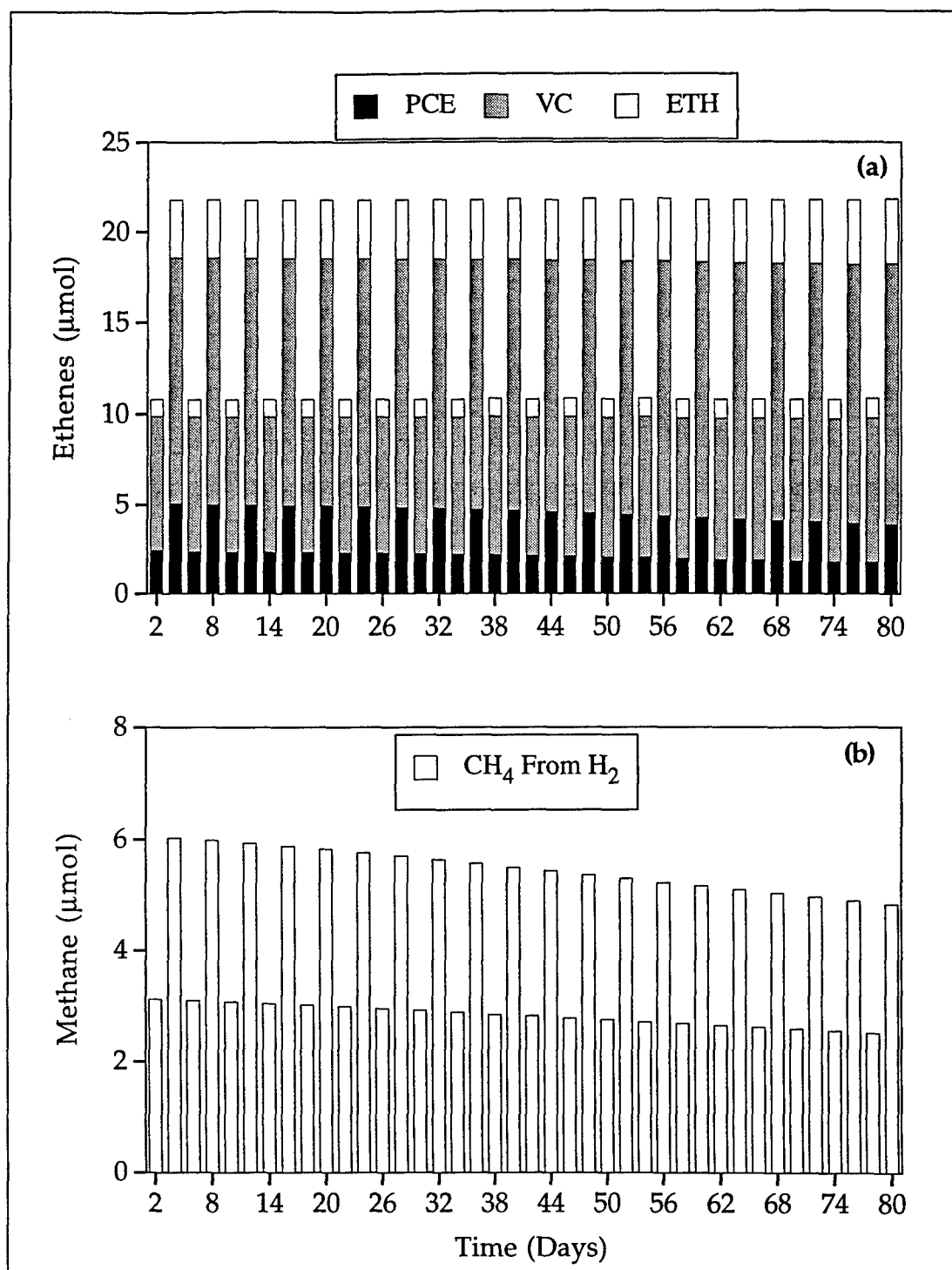
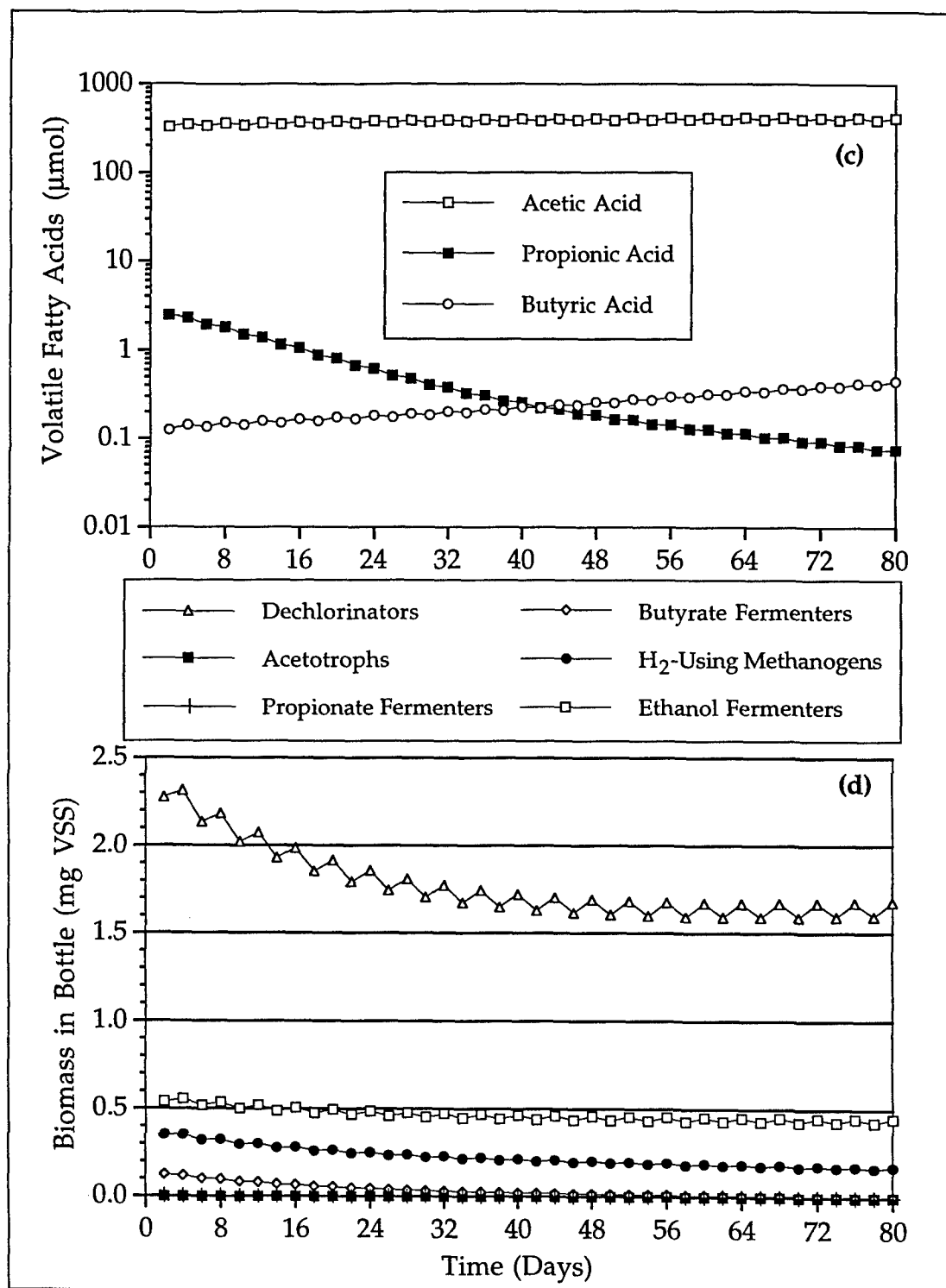


Figure 6.22. Long-term simulation of ethanol-amended culture fed 1:1 donor to PCE ratio without FYE amendment: (a) dechlorination; (b) methane from hydrogen; (c) VFAs; and (d) biomass.

Figure 6.22 (Continued)



amendment. PCE dechlorination was incomplete during this simulation although the amount of PCE being dechlorinated increased over time (Figure 6.22a). CH_4 production decreased during the simulation (Figure 6.22b). A trace amount of propionic acid, entered as a model input to mimic the small amount that was found in actual ethanol-fed cultures was slowly depleted from the system through biodegradation and washout, since there was no further input of propionic acid. Butyric acid accumulated slowly as a result of its generation through biomass decay (Figure 6.22c). Dechlorinator biomass (Figure 6.22d) initially dropped from its estimated steady-state level (assuming FYE addition) of 2.3 mg, to approximately 1.7 mg. The mass of the other organism types, including hydrogenotrophic methanogens, also decreased. The final biomass content of the simulated culture was 2.3 mg/100 mL.

Figure 6.23 shows the same 1:1 ethanol simulation, but with FYE amendment. This can be compared to the long-term operation of an actual culture run under these conditions shown in Figure 4.3. PCE dechlorination—initially incomplete with residual PCE—eventually became complete to VC and ETH and continued to improve (Figure 6.23a). Hydrogenotrophic methanogenesis decreased over time (Figure 6.23b). Traces of both propionic acid and butyric acid accumulated in the culture from the FYE input (Figure 6.23c). Note that dechlorinator biomass did not decrease to the same extent as it did in the simulation where FYE was omitted, but some loss of hydrogenotrophic methanogen biomass was observed (Figure 6.23d). The final biomass content was 2.74 mg/100 mL compared to a biomass content in the 1:1 ethanol-amended culture estimated by PON analysis to be 3.2 mg VSS/100 mL (Table 5.15).

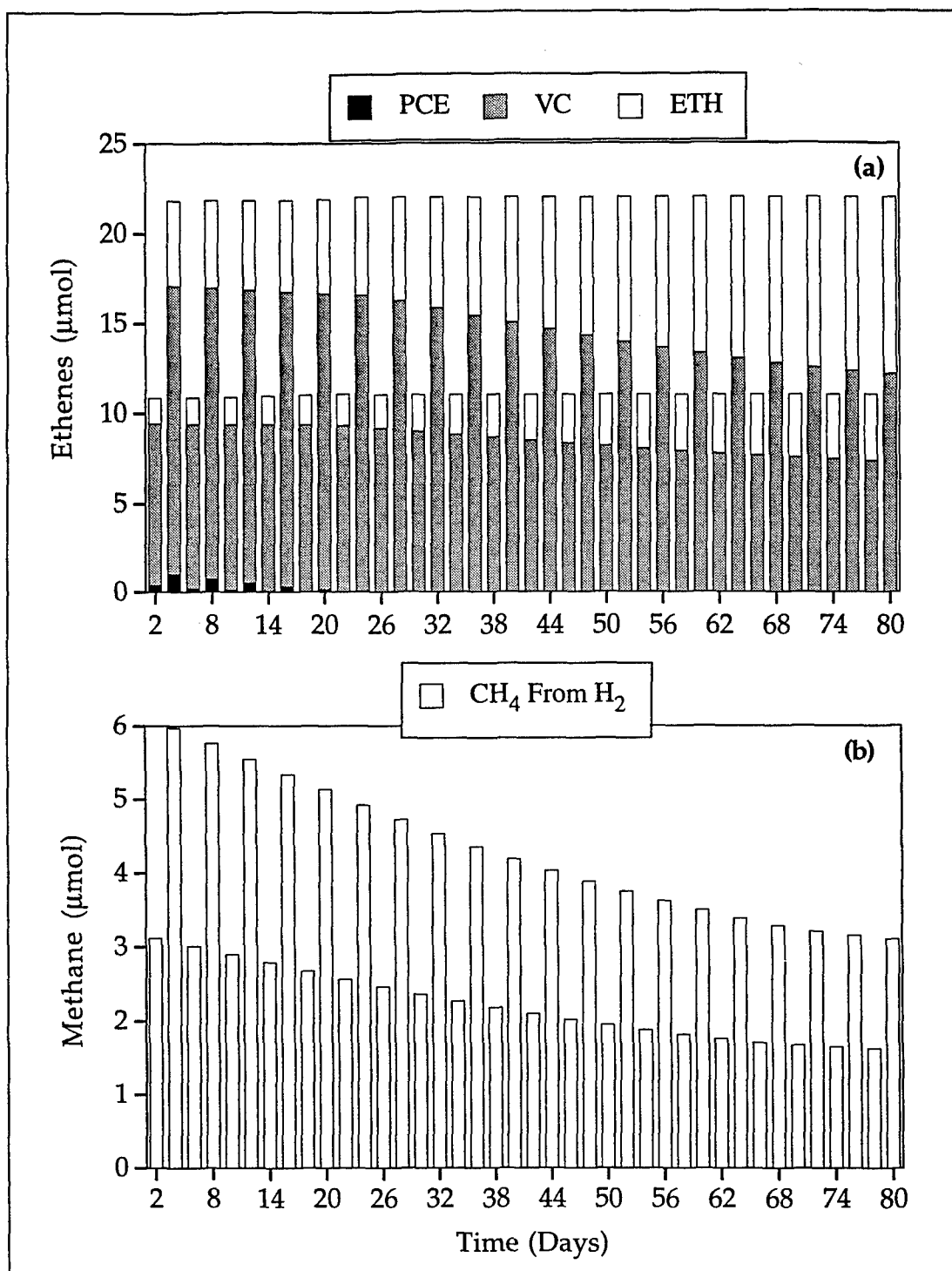
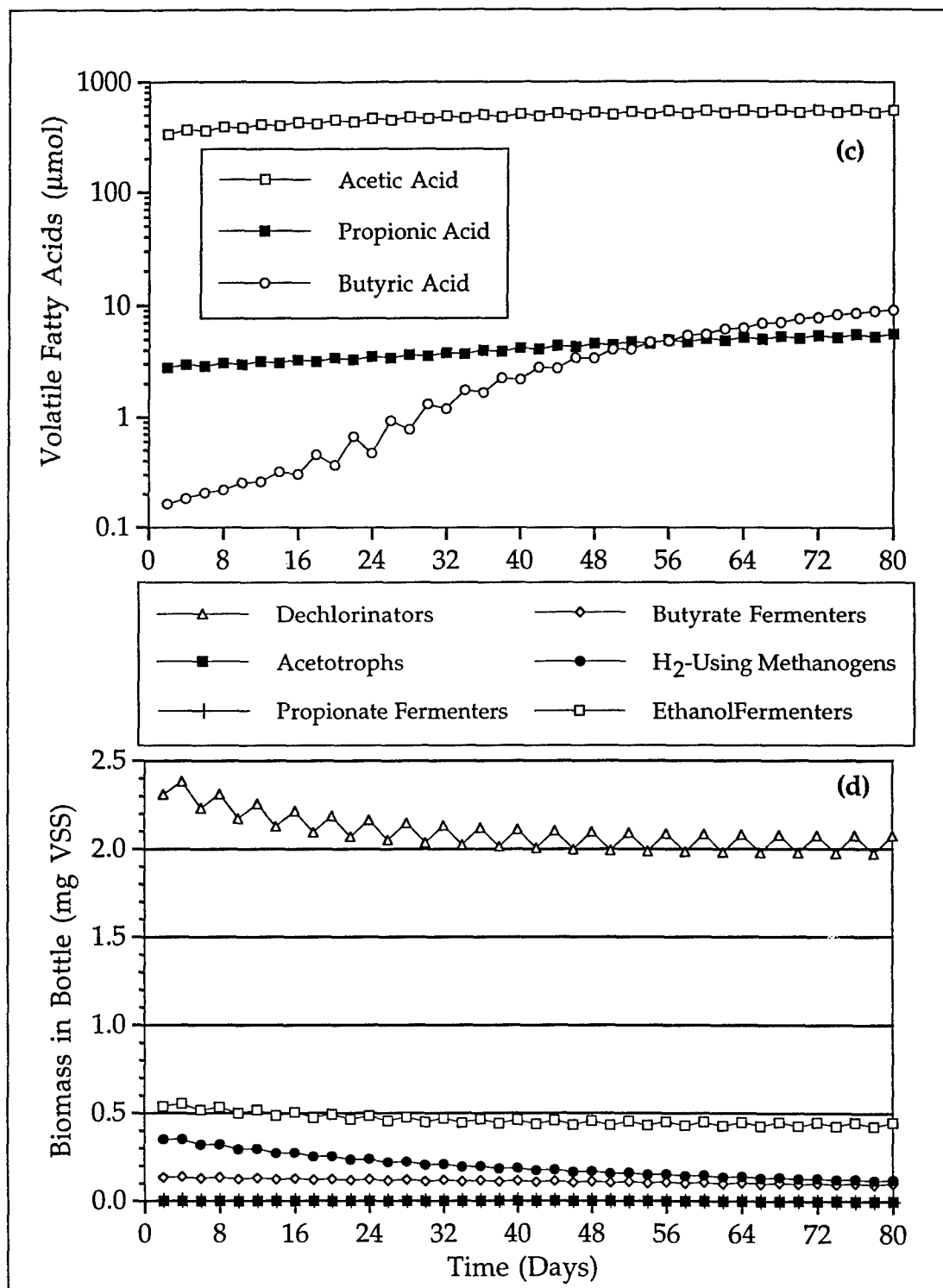


Figure 6.23. Long-term simulation of ethanol-amended culture fed 1:1 donor to PCE ratio with FYE amendment: (a) dechlorination; (b) methane from hydrogen; (c) VFAs; and (d) biomass.

Figure 6.23 (Continued)



6.E.3. Simulation of Ethanol at a 10:1 Ratio

A 10:1 ethanol to PCE ratio simulation was run to determine whether by simply adding a higher donor to PCE ratio, ethanol (without FYE) *could* support complete dechlorination. The repetitive amendments for this simulation were: 220 μmol ethanol; 11 μmol PCE; and no FYE. The initial biomass settings were the same as those used for the 1:1 ethanol to PCE simulation. The results are shown in Figure 6.24. While initially complete, dechlorination eventually failed in the simulated system (Figure 6.24a). Methanogenesis from H_2 predominated as a bioprocess (Figure 6.24b). The hydrogenotrophic methanogenic population increased 5-fold and the dechlorinator biomass decreased during the run (Figure 6.24d). Although butyric acid accumulated (through endogenous decay) (Figure 6.24c), the amount contributed did not restore complete dechlorination. The total biomass accumulated by the end of the run was 12 mg/100 mL.

Since actual cultures were not run under these conditions, and since FYE is thought to supply some unidentified nutrient, it is not possible to confirm these model results at this time.

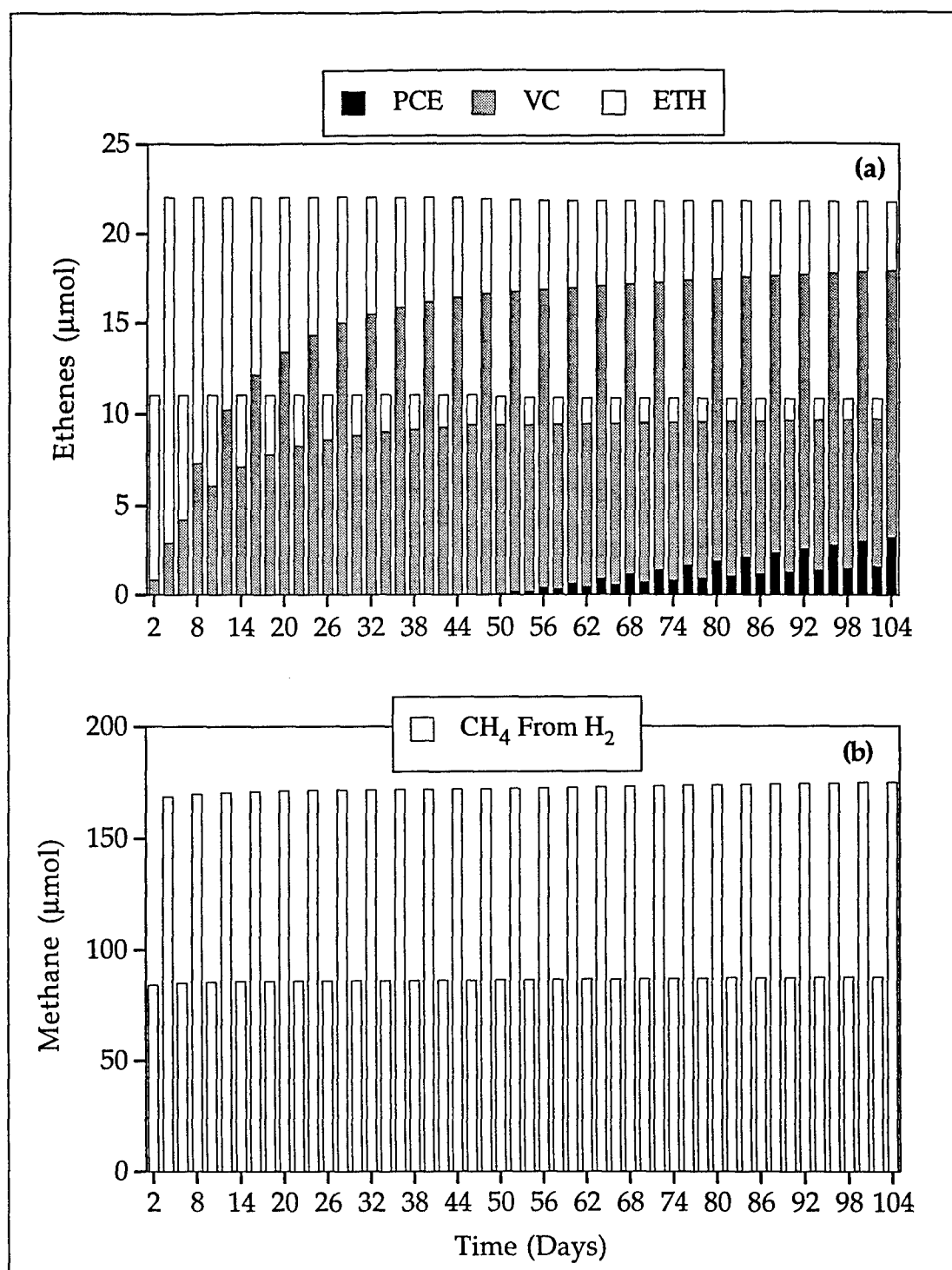
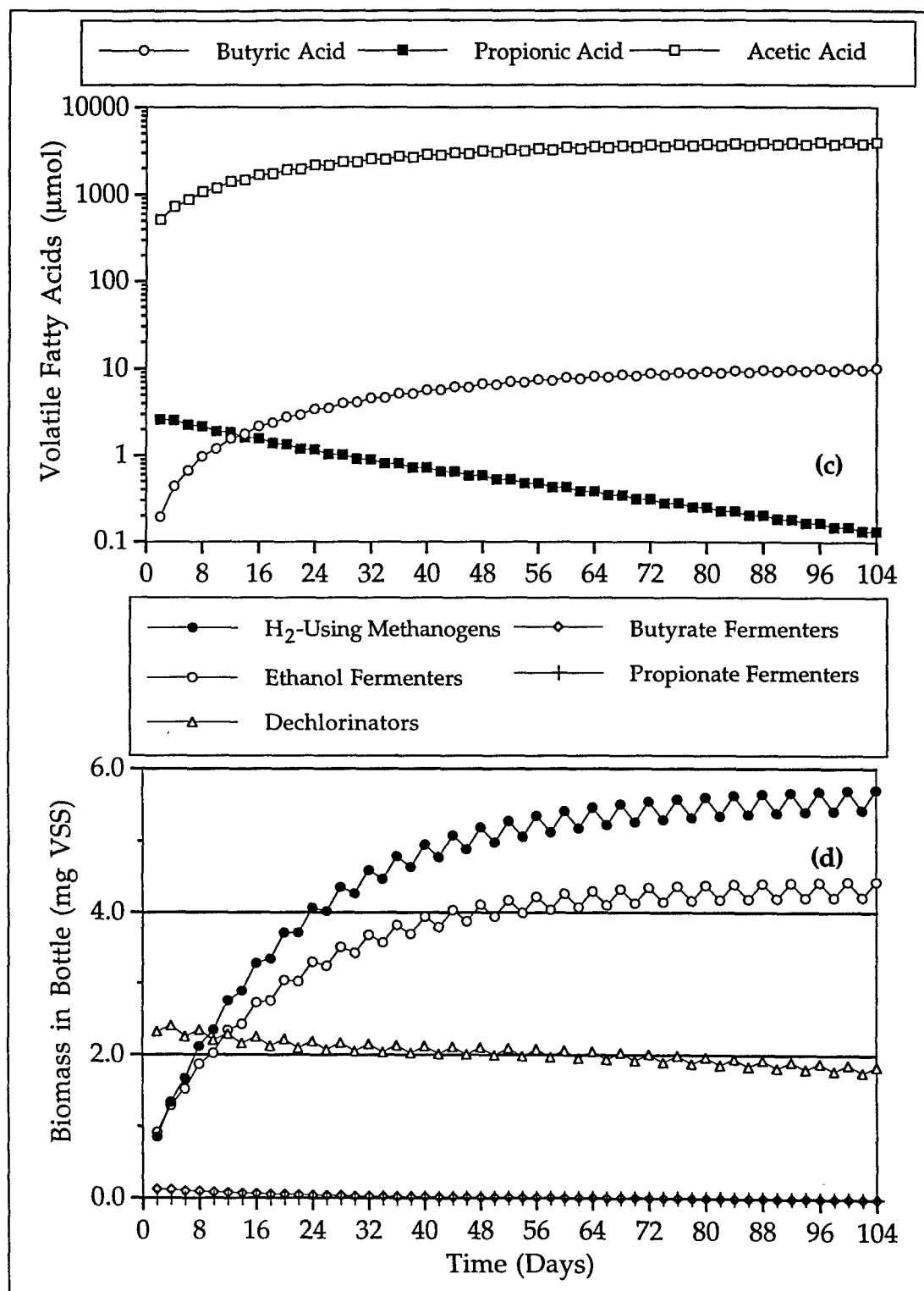


Figure 6.24. Long-term simulation of ethanol-amended culture fed 10:1 donor to PCE ratio without FYE amendment: (a) dechlorination; (b) methane from hydrogen; (c) VFAs; and (d) biomass.

Figure 6.24 (Continued)



CHAPTER SEVEN

DISCUSSION

7.A. Experimental Results

During the long-term electron donor comparison experiments, it was expected that significant differences in the partitioning of reduction equivalents to PCE dechlorination and methanogenesis would be observed when different H₂ donors were used. It was thought that in cultures fed lactic acid or ethanol, which are degraded more rapidly and could, in theory, produce higher levels of H₂ that could be utilized for both dechlorination and competing methanogenesis, dechlorination might eventually fail as methanogens came to predominate the culture and dechlorinators were marginalized. Conversely, it was hypothesized that cultures fed the more slowly fermentable donors, butyric and propionic acids, that are only able to generate low levels of H₂ would result in a predominance of dechlorination since methanogens could not compete for the available H₂. However, during long-term operation, nearly equally good establishment and maintenance of dechlorination was observed, and regardless of the electron donor, PCE was dechlorinated to VC and ETH. The only difference noted among the donors was the significantly slower development of hydrogenotrophic methanogenic activity in the lactic-acid- (at 1:1 donor to PCE ratio) and propionic-acid-fed cultures. While the propionic-acid enrichment did exclude a vigorous methanogenic population from start-up, it did not then concurrently result in significantly better dechlorination than enrichments using other electron donors. Indeed, the relative amounts of VC and ETH formed were similar

for each of the four different H_2 donors. At a 1:1 ratio, a significant portion of the lactate was fermented to propionate—thus, this culture performed very similarly to the propionic-acid-amended cultures, at least in the beginning, with a minimum of methanogenesis. The slow rate of propionic acid fermentation resulted in its accumulation in the cultures, and the full donor amendment was often not fully degraded prior to the subsequent feeding.

The expected differences among electron donors *were* observed during short-term TISs. The slowly-degraded, low- H_2 -producing substrates (butyric and propionic acids) did support complete dechlorination of PCE to VC and ETH while minimizing—and in the case of propionic acid, essentially excluding—methanogenic competition. At a 1:1 donor to PCE ratio, lactic-acid degradation also produced an order of magnitude lower H_2 peak (10^{-4} atm) than ethanol ($10^{-2.7}$ atm) and resulted in less competing methanogenesis. In contrast, the degradation of ethanol, both at a 1:1 and 2:1 ratios and lactic acid at a 2:1 ratio resulted in orders of magnitude higher H_2 that fueled initial rapid dechlorination and methanogenesis. However, as the donor and H_2 were depleted, dechlorination slowed drastically, often leaving significant quantities of PCE which were then only slowly degraded.

If the amount of dechlorination products formed was compared to the total amount of donor fermented, on a H_2 equivalents basis, then during TISs at 1:1 donor to PCE ratio, 100, 99, 87, and 49 percent of the total amount of the donor equivalents released via fermentation were channeled to dechlorination for propionic-acid-, butyric-acid-, lactic-acid-, and ethanol-fed cultures, respectively. When these tests were performed at

a 2:1 ratio, 52, 50.7, 23, and 38.2 percent of the donor equivalents released were channeled to dechlorination for propionic-acid-, butyric-acid-, lactic-acid-, and ethanol-fed cultures, respectively.

The relative peak levels of H_2 produced by the different donors (Figure 4.15) were roughly in accordance with expectations from thermodynamic considerations (Figure 1.1). Peak H_2 levels from propionic acid, butyric acid, ethanol, and lactic acid at a 1:1 ratio were approximately $10^{-5.1}$, 10^{-5} , $10^{-2.9}$, 10^{-4} atm. At a 2:1 ratio the peak H_2 levels from propionic acid, butyric acid, ethanol, and lactic acid were approximately $10^{-5.1}$, $10^{-4.2}$, $10^{-2.7}$, and $10^{-2.9}$ atm, respectively. Thermodynamic upper limits to H_2 —i.e., H_2 levels causing each of the four fermentations to yield zero free energy—were estimated to be $10^{-4.4}$, $10^{-3.5}$, $10^{-1.2}$, and $10^{+0.5}$ atm, respectively. That the experimentally observed H_2 peaks were below thermodynamic limits is not surprising. In the first place, physiological upper H_2 limits must provide some finite free energy to the fermenting organisms. Secondly, the observed peak H_2 levels represent dynamic steady-state conditions where rates of H_2 production and use were balanced; therefore, rates of H_2 production were non-zero, unlike the situation at the physiological limit.

In their studies of anaerobic aquifer sediments from a Traverse City, Michigan site which were amended with fatty-acid mixtures, Gibson *et al.* [87] observed that lactate was quickly depleted and probably did not persist long enough to support dechlorination, whereas butyric acid persisted for a longer period of time and was a better amendment for stimulating dechlorination. Propionic acid degradation was not observed in their microcosms amended with fatty-acid mixtures—perhaps, they suggested,

because of inhibition by high levels of H_2 and/or acetic acid. In microcosms amended with propionic acid alone, dechlorination was supported after a lag period [88]. This study does confirm that donor persistence, in addition to H_2 level generated, is a very important consideration for sustaining dechlorination. The more persistent donors, butyric and propionic acids, were better at stimulating complete dechlorination, although rates were slower.

It was suspected and then confirmed through additional testing that the addition of FYE (an unfortunately required micronutrient supplement) significantly influenced the outcome of the long-term operation. Time-intensive studies of ethanol with added supplements clearly showed the importance of FYE-contributed VFAs—most notably butyric acid—in providing slowly-released H_2 to fuel the continued dechlorination of PCE which remained after ethanol was depleted. While it is known that nutrients contained in yeast extract are required for growth of pure cultures of the dechlorinating organism [152] and for the high-PCE/methanol source culture [61], it has not yet been determined whether this nutritional requirement holds for cultures amended with the electron donors tested in this study. It is possible that the complex mixed community itself would provide the missing nutrients and that the FYE could be omitted. What is perhaps more important is the finding that addition of even relatively small amounts of slowly available donors that produce low levels of H_2 upon fermentation, greatly affected the outcome by persisting and providing reduction products to fuel continued slow dechlorination of remaining chloroethenes.

The stability of the low-PCE/butyric-acid-amended source culture and the high ETH production that it exhibited at non-inhibitory PCE concentrations in the presence of a vigorous methanogenic community are encouraging. This information demonstrates the ability of dechlorinators to maintain their important role in a community containing other hydrogenotrophs that are also competing for reducing equivalents. The long-term operation of the low-PCE/butyric acid source culture also provided an important example concerning the nutritional requirements of "*Dehalococcoides ethenogenes*" strain 195 in mixed culture. While other researchers were delineating the nutritional needs of strain 195 [151, 152], the low-PCE/butyric acid source culture was initiated without knowledge that the purified cultures were routinely amended with a vitamin solution. The high-PCE/methanol source culture had never been amended with the vitamin solution, and dechlorination was supported very well without it [61]. Once switched to butyric acid, however, the dechlorinators eventually failed in the culture. Upon addition of the vitamin solution, dechlorination again flourished. It was concluded that in the high-PCE/methanol culture, corrinoid compounds were most likely supplied to strain 195 by the methanol-using acetogens in the culture [152].

The pathway of fermentation of the donor will greatly determine whether it is suitable or not for stimulating enhanced reductive dechlorination. Propionic acid accumulation was observed in lactic-acid- and perhaps to a lesser extent in ethanol-fed cultures. We have also observed propionic acid accumulation in microcosms from a sulfate-rich aquifer amended with these donors [20, 209]. These pathways are well documented. Lactic acid can be degraded to propionic acid by *Veillonella*

[55], *Propionibacterium*, *Megasphaera*, *Selenomonas*, and various clostridia [94]. Ethanol fermentation to propionic acid was first reported [183] for *Clostridium neopropionicum* [240], and is also carried out by *Desulfobulbus propionicus* [126] and *Pelobacter propionicus* [187]. This pathway has been shown to be important in some natural environments [191]. Other possibilities of alternative donor fermentation pathways exist, for example butyric acid formation from ethanol and acetate has been documented for *Clostridium kluyveri* [94]. The accumulated propionic acid (in addition to FYE) in our cultures contributed to the pool of slowly degradable donor and undoubtedly facilitated continued dechlorination after the primary donors were depleted.

Thus, not all of the fermentation pathways of the donors tested followed the simple model of degradation to acetic acid and H_2 . A more complicated scenario was observed in some cases. In some ecosystems, adding ethanol or lactic acid may be almost the equivalent of adding propionic acid—a slow-release H_2 donor. Cost and ease of handling could then help determine which type of addition is more practical. In many of the described laboratory studies and field investigations, the fate of the added electron donor is not followed or reported. This is unfortunate since the fate of the donor and the efficiency with which it is directed to dechlorination would be expected to have an enormous impact on the success or failure of a system. This study has shown that fate of electron donors and their fermentation products—including not only H_2 , but other intermediates as well—is of critical importance for understanding dechlorinating communities. Site by site determinations, not only of dechlorinating capacity, but also of donor fate, will be useful for evaluating

treatment schemes for bioremediation, and will also be of great value in understanding results at naturally attenuated sites.

7.B. Modeling

In general, a thermodynamically-based model of donor fermentation, coupled with H_2 competition between dechlorinators and methanogens and methanogenesis captured the overall shape and trends of the experimentally-obtained data. This is perhaps best demonstrated by comparing the H_2 level produced by the fermentation of each of the donors during simulation to those observed during actual TISs. Figure 7.1 shows model-generated H_2 levels overlaid on data from selected TISs for both 1:1 (Figure 7.1a) and 2:1 (Figure 7.1b) donor to PCE ratios. While some differences exist, the model does capture the duration and level of H_2 production for each of the donors fairly accurately.

Discrepancies did exist between the model and the data in terms of rates of dechlorination and donor fermentation in some cases, and in total amounts of products formed—especially CH_4 and ETH. Since much of the collected data were obtained at non-steady-state conditions, the modeling certainly suffered from not having precise numbers of microbial biomass present for each group of organisms. (Biomass was estimated from steady-state analysis predictions.) Also, dependence upon literature values for organism yield may have affected the outcomes since populations could have been over- or under- predicted.

There were two significant problems with the model that were not resolved during this study. The first concerned the choice of $\Delta G_{\text{critical}}$ for the model. Analysis of the Gibbs free energy of reaction available at each

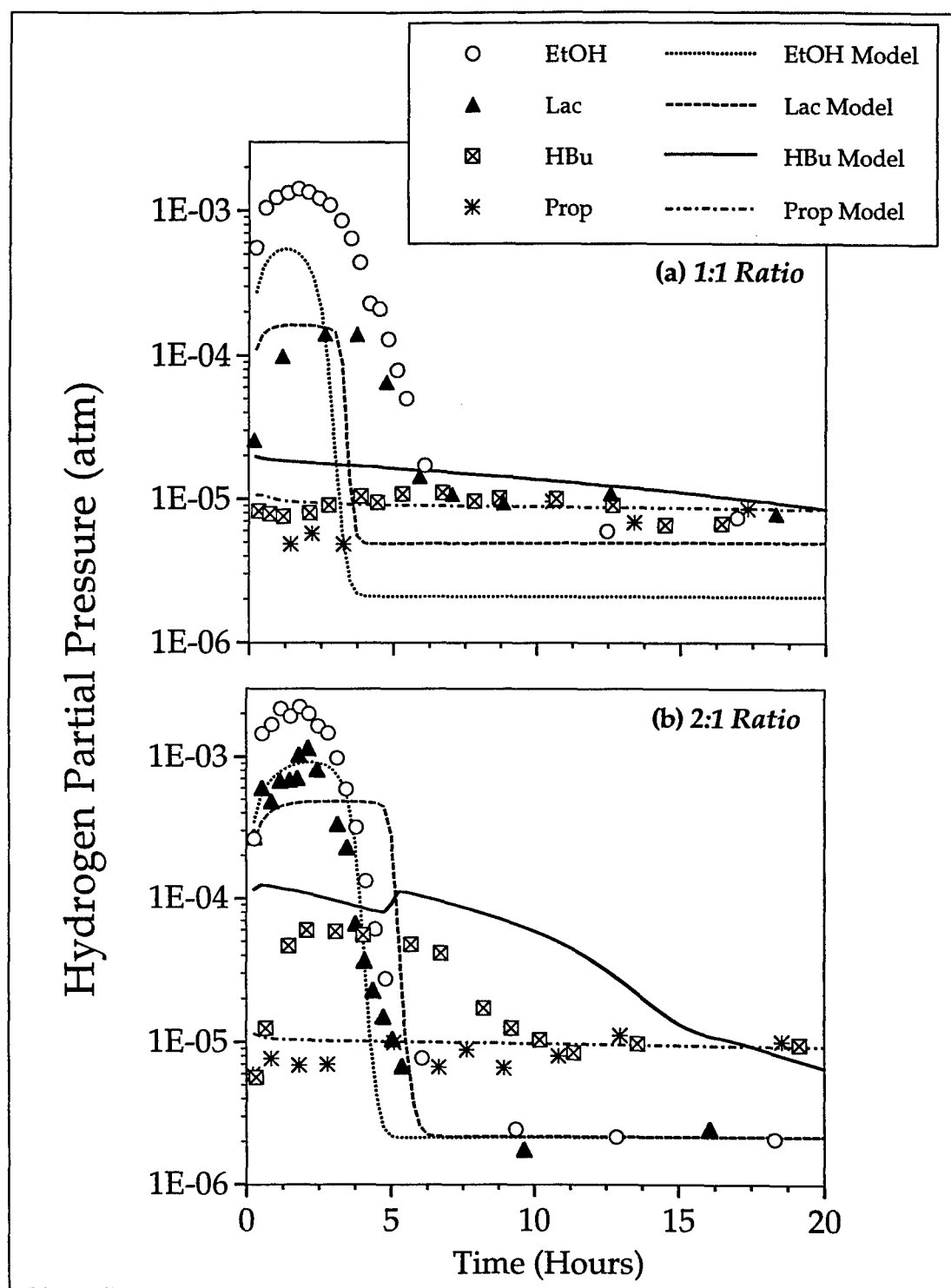
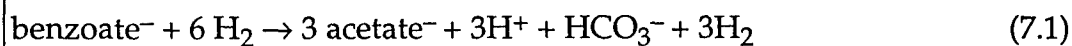


Figure 7.1. Comparison of model predictions with hydrogen production from the degradation of ethanol, lactic acid, butyric acid, and propionic acid at a (a) 1:1 donor to PCE ratio and (b) 2:1 donor to PCE ratio.

step of the TISs generally showed that reactions were proceeding at near -20 kJ/mol donor or at even more negative free energy levels (thus -19 kJ/mol donor was chosen as a model input). However, in cases where a significant background level of acetate was present, propionic acid and butyric acid fermentation continued and the free energy available increased to as high as -5 kJ/mol donor. This is difficult to explain, and difficult to model. Other studies have reported values for $\Delta G_{\text{critical}}$ of -10 kJ/mol ethanol to -20 kJ/mol butyrate degraded [67, 190, 201, 202, 257].

At least one set of studies in the literature supports the idea that thermodynamics—determined not only by H_2 accumulation, but also by acetate accumulation—limits the extent of fermentation of these energetically unfavorable substrates. Benzoate—a substrate that is fermented as per Equation 7.1—with an unfavorable standard free energy of reaction of 74 kJ/mol benzoate [109] was studied in this respect.



It was reported that benzoate fermentation ceased, even in the presence of acetate levels that corresponded to a free energy of reaction of about -54 kJ/mol benzoate [109]. This “threshold” of benzoate below which it was not fermented differed depending upon the background acetate level present. The authors speculated that the “threshold” was the result of thermodynamic limitation and demonstrated that the lack of degradation was not due to inhibition by formate (which was not present); or inhibition by acetate or by nutritional deficiency since re-amendment with benzoate resulted in immediate continuation of the fermentation back down to a similar “threshold” benzoate level. In a follow-up study, the thermodynamic limitation was confirmed and a $\Delta G_{\text{critical}}$ for benzoate

degradation of near -30 kJ/mol benzoate was reported [258]. Thus, while the phenomenon of a thermodynamic ceiling being controlled not only by H_2 , but also by acetate has been reported—and was the basis of the modeling of donor fermentation in this study—the fermentation of propionic acid and butyric acid appeared to continue even though the thermodynamics of the fermentation became less favorable than the presumed $\Delta G_{\text{critical}}$. This is an as yet unresolved issue from this study.

Beaty and McInerney [18] observed inhibition of butyrate fermentation by *S. wolfei* by acetate and other organic acid anions. No free-energy analysis was presented in their study. They speculated that acetate turnover, not thermodynamic inhibition, was the important factor in the inhibition since acetate concentrations that inhibited butyrate fermentation in cocultures containing *S. wolfei* and a hydrogenotrophic methanogen did not inhibit butyrate fermentation in a tri-culture that additionally contained an acetotrophic methanogen (where acetate was actively being turned over).

It has also been reported that despite the apparent thermodynamic unfavorability, the fermentation of propionic and butyric acids occurred in diverse anaerobic environments. It was speculated that the reactions occur in microniches where bulk H_2 concentrations do not control the reactions. [48]. That would, however, be an unlikely explanation for this study. Assuming that flocs existed in the cultures used during this study, and that the flocs were made up of a mixed matrix of organisms—i.e. H_2 producers and H_2 users—then if H_2 is on the rise, the concentration within the floc would be higher than bulk; if H_2 is falling, the concentration would be lower in the floc than in bulk. If H_2 is steady, the concentration must be

identical within the floc and in the bulk liquid—i.e., if the source of H_2 is the floc itself. This would not be true if H_2 was being produced somewhere outside the flocs—an unlikely scenario.

The second discrepancy with the modeling concerns the apparently better dechlorination and greater amount of reducing equivalents available during the long-term operation of the low-PCE/butyric acid source culture than was predicted through modeling. The amount of ETH produced was slightly under-predicted by the model. A possible explanation is that H_2 is available for scavenging during the conversion of acetate to CH_4 . In some cases, this source of reductant could be fueling slow, continued dechlorination in the absence of other primary donors. That would explain why, for example, dechlorination continued in butyric-acid-amended cultures after butyric acid was depleted, but acetate remained and was slowly converted to CH_4 . Perhaps the most likely explanation for the discrepancy was that during normal operation of the 9-L source culture, purging to remove volatile products was performed for only 2 to 5 min every fourth day. It is possible that this amount of purging did not completely remove all of the volatile reduction products in the bottle and these residuals (i.e. VC, ETH, and CH_4) would then have been measured as part of the new total amount produced at the subsequent analysis. That would have made the sum of the reduction products higher than expected and would also explain why more ethenes were detected (12 to 15 μmol per feeding) than was expected (11 μmol per feeding).

Long-term simulations of the donors ethanol and propionic acid with and without FYE amendment supported the obvious fact that FYE amendment, with its contribution of the slowly-available, low-level H_2

producing substrates propionic and butyric acids, vastly improved the amount of dechlorination supported with ethanol amendment. Simulations of FYE-amended, propionic-acid-fed cultures also showed improved performance over simulations where propionate was added without FYE. Addition of butyric acid from FYE allowed more reducing equivalents to be produced under conditions (higher acetate and H_2 levels) where propionic acid fermentation was highly limited. In general, the dechlorinator population was more stable when FYE addition was included, since these organisms can out-compete hydrogenotrophic methanogens for low-level H_2 . Simulation of 1:1 ethanol-amended cultures that did not receive FYE showed failure of dechlorination, while when FYE was added, dechlorination reached completion. Increasing the ethanol amendment to a 10:1 ratio (in the absence of FYE addition) initially allowed for complete dechlorination of the PCE to VC and ETH. After prolonged operation, however, dechlorination eventually became incomplete. This is reminiscent of the "spiral to failure" phenomenon observed with methanol as an electron donor at intermediate PCE levels [224].

CHAPTER EIGHT

CONCLUSIONS

The following conclusions can be drawn from this study of hydrogen donors for stimulating anaerobic reductive dechlorination of PCE:

- (1) Amendment with either the rapidly fermented donors (ethanol at a 1:1 and 2:1 donor to PCE ratios, and lactic acid at a 2:1 ratio) that were capable of producing high-levels of H_2 (10^{-3} atm), or with the slowly available donors (lactic acid at a 1:1 ratio, butyric and propionic acids at 1:1 and 2:1 ratios) that produced only lower levels of H_2 (10^{-5} to 10^{-4} atm), equally stimulated dechlorination of PCE to VC and ETH during long-term operation.
- (2) Significant differences in the completeness of dechlorination, H_2 level formed, amount of H_2 channeled to dechlorination, and donor persistence *were* observed during short-term, time-intensive studies. Fermentation of propionic acid (or lactic acid that was subsequently fermented to propionate) generated slowly-released, low-level H_2 that sharply limited the formation of hydrogenotroph methanogenic populations while supporting dechlorination. Fermentation of ethanol resulted in rapidly-produced high H_2 levels that supported both methanogenesis and dechlorination initially; however, the donor was rapidly depleted and dechlorination was incomplete.
- (3) Addition of a trace nutrient solution—fermented yeast extract (FYE)—greatly influenced the extent of dechlorination during long-term operation by contributing extra reducing equivalents—primarily in

the form of propionate, butyrate, or other apparently slowly-available substrates. This effect could be mimicked by adding a surrogate FYE consisting of butyric, propionic, and other longer-chain fatty acids.

- (4) Dechlorination proceeded at aqueous H_2 concentrations as low as 1.5 nM in cultures amended with no donor or FYE.
- (5) Complete dechlorination of PCE to primarily ETH and lesser quantities of VC was maintained for a period of 2.2 yr in a 2:1 donor to PCE ratio butyric-acid-amended source culture at intermediate, noninhibitory PCE concentrations (110 μ M) in the presence of a vigorous, hydrogenotrophic methanogenic population.
- (6) The successful conversion of a high-PCE/methanol source culture to low-PCE/butyric acid source culture required the addition of vitamin B_{12} . Vitamin B_{12} was apparently not required when methanol was fed, perhaps because the high numbers of acetogens in the high-PCE/methanol culture provided some form of the corrinoid compound to the dechlorinators.
- (7) A comprehensive model incorporating Michaelis-Menten-type kinetics for donor fermentation, dechlorination, and methanogenesis—and featuring thermodynamically limited donor fermentation and H_2 thresholds for dechlorination and methanogenesis—did a very good job of capturing the short-term, dynamic behavior patterns observed with each of the donors.
- (8) The model also produced a very good fit of the long-term, steady-state behavior of the low-PCE/butyric-acid-amended source culture.
- (9) Simulations comparing long-term operation of ethanol- and propionic-acid-amended cultures with and without FYE amendment,

provided evidence that, not only did addition of the FYE to ethanol-amended cultures improve dechlorination; but its addition to propionic-acid-amended cultures also improved dechlorination since the added butyric acid could be more rapidly fermented under circumstances where propionic acid fermentation was very slow.

- (10) During a model simulation, increasing the ethanol amendment to a 10:1 ratio to PCE (in the absence of FYE and its trace addition of the more slowly degraded donors propionate and butyrate) initially stimulated complete dechlorination; but, eventually, the dechlorinator population decreased, the hydrogenotrophic methanogenic population rapidly increased, and dechlorination eventually failed. This reinforces the concept of the greater importance of the "quality" of the donor over the quantity of donor.

CHAPTER NINE

ENGINEERING SIGNIFICANCE

Successful stimulation of biological reductive dechlorination of the chlorinated ethenes requires the presence of an electron donor. In engineered, *in situ* systems, if no co-spilled donor or high-level background organic matter is present, it will be necessary to add donor. Several of the recently described dehalorespiring organisms use H_2 as an electron donor, thus, H_2 is an important donor to consider when planning how best to stimulate bioremediation in aquifers contaminated with chlorinated solvents. A question arises of how to supply H_2 in such systems. Delivery of gaseous H_2 is problematic in terms of engineering considerations since it is an explosive gas with some risk involved in its handling; and, it is difficult to deliver uniform concentrations of this sparingly soluble and very rapidly turned-over substrate to an aquifer. Also, ensuring that the applied H_2 gets channeled to the desired reaction—i.e., dechlorination of the pollutants instead of to competing sinks such as methanogenesis and sulfate reduction is also a problem when applying gaseous H_2 . Studies in this laboratory have also shown that addition of H_2 alone as a donor eventually resulted in failure of dechlorination because of nutritional deficiencies that were overcome only by amendment with complex nutrient sources such as culture extracts.

Recently reported experimental evidence has demonstrated that dechlorinators have a higher affinity for H_2 than do hydrogenotrophic methanogens, as quantified through $K_{S(H_2)dechlor}$ values. Thus, if H_2 could be delivered at low levels, a competitive advantage might be imparted to the dechlorinators.

There is some disagreement about whether competition for donors is actually an important or overriding issue for *in situ* bioremediation. Some argue that as an overall cost consideration, the donor makes up a relatively small fraction of the total cost of the project and therefore simply applying excessive amounts of any donor will provide ample reducing equivalents for chlorinated solvent dechlorination. While the cost issue is likely true, limiting competition for donor is nonetheless important for a variety of reasons. If one considers H_2 amendment as an example, it is not difficult to imagine that injecting H_2 into an aquifer would result in very active biological zones consisting of both desired dechlorinators and undesirable alternate sinks such as sulfate-reducing bacteria and hydrogenotrophic methanogens near the injection point that would then rapidly deplete the added H_2 . Very little of the donor amendment would be transported far from the injection point. Even injection of a substrate like ethanol, which is rapidly degraded to fairly high H_2 levels might have the same result. Injection of such rapidly degraded donors would result in biofouling and inefficient delivery of the donor to distance portions of the aquifer.

An alternative, tested in this study, is amendment with slowly fermented donors such as propionic and butyric acids. Amendment with a substrate such as propionic or butyric acid would accomplish two important goals. First, H_2 would be generated at low concentrations that are still useable at appreciable rates by dechlorinators but less accessible—either below the threshold or simply too low to be used at appreciable rates—by competing hydrogenotrophic methanogens. Second, these slowly available compounds are persistent in even vigorous microbial cultures and they are perhaps more amenable to being delivered more uniformly to all portions of the contaminated aquifer under remediation.

Since the reductive dechlorination process depends upon the presence of a donor, fate and persistence of donors are critical to the success or failure of a bioremediation attempt. Enhanced, *in situ* chlorinated solvent bioremediation systems have incorporated the use of electron donors such as methanol, molasses, lactic acid, and yeast extract. Microcosm studies have reported other donors such as ethanol, butyrate, benzoate and propionate. Unfortunately, in many of these reports, the fate of the donors was not determined. For example, some of the studies simply reported measurements of TOC or COD, but did not determine the specific components making up the organic carbon. If the fate of the donor is not determined, then an important piece of the data base is missing and the opportunity for determining the reason for success or failure may be lost. It is evident from the results generated during this study and from the few studies which have reported donor fate, that donor fate is a crucial factor. For example, microcosm studies performed on microcosms from Fallon Air Station in Nevada reported ethanol and lactate conversion to propionate upon addition [20, 209]. Thus, donors that might be expected to be rapidly degraded were instead converted to a slowly degraded donor, propionate, that produces low levels of H_2 . The fate of the donor from site to site would not be known without specific investigation through site sampling or microcosm studies.

The fate of the all-critical donor, if known, should then be incorporated into pollutant fate and transport models. Currently, few such models include this aspect of chlorinated solvent dechlorination, nor do they include full kinetics for solvent dechlorination. Most of the models available include only first-order type kinetics for contaminant disappearance. Type, availability, and competition for donor is included in only a few of these

models. Although it was beyond the scope of this study, it is hoped that, eventually, the model developed during this study will be incorporated into an existing fate-and-transport model as a plug-in descriptor of the microbiological processes involved. In the case of chlorinated solvent bioremediation, more complexity is certainly needed in site models to bring a better understanding and, thus, more widespread application of the process to field situations.

APPENDIX I

KINETIC PARAMETERS FOR SUBSTRATE USE—LITERATURE VALUES

Appendix I contains tables of literature values of kinetic parameters for fermentation of butyric acid (Table A1.1), ethanol (Table A1.2), lactic acid (Table A1.3), and propionic acid (Table A1.4); H₂ use by methanogens (Table A1.5); and acetate use by methanogens (Table A1.6). These values were used for judging whether parameters used in modeling were reasonable, and/or as sources of model parameters.

Table A1.1. Kinetic parameters for butyric acid fermentation.

Organism	Temp. (°C)	k ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)	K _S (μM)	Yield (mg VSS/ μmol)	Reference
thermophilic butyrate degrader (in triculture)	60	7.17 to 11.44	76.4 65.2	0.00279	[4]
<i>Syntrophomonas wolfei</i>	37			0.01314 (on crotonate)	[17]
<i>Syntrophomonas wolfei</i> (in coculture with <i>M. hungatei</i>)	35		470		[268]
<i>Syntrophomonas wolfei</i> (in coculture with <i>M. hungatei</i>)	35			0.0087	[18]
Defined species granules	35		190		[270]
Digester sludge	35		190		[270]
Laboratory digester	35	7.4	57		[131]

Table A1.2. Kinetic parameters for ethanol fermentation.

Organism	Temp. (°C)	K _S (μM)	Yield (mg VSS/μmol)	Reference
<i>Pelobacter acetylenicus</i> (to acetate)	32		0.00198 0.00153	[202]
<i>Clostridium neopropionicum</i> (to propionate)	30		0.00297	[240]
<i>Pelobacter propionicus</i> strain Ott Bd1 (to propionate)	30		0.00126	[187]
<i>Pelobacter propionicus</i> (to propionate)	28		0.00225	[228]
Anaerobic methanogenic laboratory reactor	35	1000		[211]

Table A1.3. Kinetic parameters for lactic acid fermentation.

Organism	Temp. (°C)	Yield (mg VSS/μmol)	Reference
<i>Syntrophobacter pfennigii</i> strain KoProp 1 (to acetate)	37	0.00351	[256]
<i>Pelobacter propionicus</i> strain Ott Bd1 (to propionate)	30	0.005625	[187]
<i>Desulfobulbus propionicus</i> (to propionate)	30	0.00273	[218]
<i>Veillonella</i> species (to propionate)	33	0.00315	[207]
<i>Veillonella</i> species (to propionate)	30	0.00396	[127]
<i>Veillonella alcalescens</i> (to propionate)	30	0.00774	[252]
<i>Propionibacterium freudenreichii</i> (to propionate)	30	0.00729 0.00918	[253]
<i>Propionibacterium pentosaceum</i>	30	0.00918 to 0.0116	[253]

Table A1.4. Kinetic parameters for propionic acid fermentation.

Organism	Temp. (°C)	k ($\mu\text{mol}/\text{mg}$ VSS-hr)	K _S (μM)	Yield (mg VSS/ μmol)	Reference
<i>Syntrophobacter pfennigii</i> strain KoProp 1 (coculture w/ <i>M. hungatei</i>)	37			0.00144	[256]
<i>Syntrophobacter pfennigii</i> strain KoProp 1 pure culture grown with sulfate as an electron acceptor	37			0.0027	[256]
Strain MPOB pure culture grown with sulfate as an electron acceptor	37			0.00135	[125]
Defined species granules	35		38		[270]
Anaerobic digester	33		94		[115]
Propionate-acclimatized sludge	37	0.09	2000		[83]
Laboratory digester	35	5.4	430		[131]
Anaerobic digester (fast- growing organisms)	35		150		[100]
Anaerobic digester (slow- growing organisms)	35		4500		[100]

Table A1.5. Kinetic parameters for hydrogenotrophic methanogenesis.

Organism	Temp. (°C)	k ($\mu\text{mol}/\text{mg}$ VSS-hr)	K _S (μM)	Threshold for H ₂ Use (μM H ₂)	Yield (mg VSS/ μmol)	Reference
<i>Methanobacterium thermoautotrophicum</i>	65				0.00036 to 0.000675	[197]
<i>Methanobacterium thermoautotrophicum</i>	65				0.000135 to 0.00036	[231]
<i>Methanobacterium bryantii</i> (Strain M.o.H.)	40				0.000495 to 0.000558	[179]
<i>Methanobacterium</i> Strain AZ	33				0.000515 to 0.000603	[276]
<i>Methanosarcina barkeri</i>	37				0.00144	[261]
<i>Methanobacterium formicium</i>	37				0.0007875	[185]
<i>Methanosarcina barkeri</i>	37	10.31	13		0.000256 (ave)	[180]
<i>Methanospirillum hungatei</i> JF-1		6.56	5			
<i>Methanospirillum</i> PM1		8.44	2.5			
<i>Methanospirillum</i> PM2		0.61	4.1			
<i>Methanospirillum hungatei</i>	28-34			0.023		[50]
<i>Methanobrevibacter smithii</i>				0.075		
<i>Methanobrevibacter arboriphilus</i>				0.068		
<i>Methanobacterium formicium</i>				0.021		
<i>Methanococcus vannieli</i>				0.056		[139]
<i>Methanobacterium formicium</i>	39			0.069		
<i>Methanobacterium bryantii</i>				0.050		
<i>Methanospirillum hungatei</i>				0.069		
Mixed Digester	35		0.0076			[90]
Mixed Digester	35		0.39	0.028		[92]
Mixed Culture	35		0.96			[210]

Table A1.6. Kinetic parameters for acetotrophic methanogenesis.

Organism	Temp. (°C)	k (μmol acetate/mg VSS-hr)	K _S (μM)	Yield (mg VSS/ μmol)	Reference
<i>Methanotrix soehngenii</i>	33		460	0.00099 to 0.00126	[274]
<i>Methanosarcina barkeri</i>	37			0.00144 to 0.00171	[260]
<i>Methanosarcina barkeri</i>	37			0.00099	[110]
<i>Methanosarcina barkeri</i> strain 227	35 - 37		5000	0.00162 to 0.00243	[212]
<i>Methanosarcina barkeri</i> strain 227	35			0.001989 to 0.00288	[213]
<i>Methanosarcina barkeri</i>	50 55		4500	0.00162	[281]
<i>Methanotrix soehngenii</i> 5 strains	37	1.08 to 5.65	390 to 1190		[169]
Digestor sludge	33		320		[115]

APPENDIX II

ANALYSIS OF REDUCING EQUIVALENT CONTRIBUTION BY FYE

Although different batches of FYE were used during the study, the amount of reduction products formed per μL FYE added as estimated from results during short-term tests and long-term operation was similar. Two serum bottle tests were performed to specifically determine the reducing equivalent contribution of FYE.

A2.1. FYE Reduction Product Formation in Low-PCE/Butyric Acid Source Culture

Four bottles containing 100-mL aliquots of 100 percent butyric-acid-enriched culture were set up directly from the source culture as described in Section 3.B.1 and operated for four days with the low-PCE/butyric acid culture protocol to ensure a successful transfer. The TIS was initiated as described previously in Section 3.B.4 and was run for two days. Two bottles were amended with PCE and FYE and two bottles were amended only with PCE. The total amount of reduction products formed in the bottles amended only with PCE was subtracted from the total amount formed in the bottle amended with FYE and PCE to determine the amount of reduction products that were formed from FYE alone. Results are shown in Table A2.1.

A2.2. FYE Reduction Product Formation in an Ethanol-Enriched Culture

The second test to determine reduction product formation from FYE was performed using two sets of two serum-bottle-grown cultures that were originally set up from a 20 percent dilution of the high-PCE/methanol source culture as described in Section 3.B.1. Upon set-up, Day 0, the bottles were amended with 88 μmol H_2 , 11 μmol PCE, 40 μL FYE, and vitamins. On Day 2 and thereafter, the bottles were amended with 44 μmol ethanol, 11 μmol PCE, and 40 μL FYE. Vitamins were added every fourth day. On Day 8, TISs were performed during which one bottle of each set was amended with PCE and vitamins only and the other bottle of the set was amended with FYE, vitamins and PCE. The production of reduction products was followed over approximately two days and the difference between the reduction products formed in the bottle amended with PCE and vitamins only was subtracted from the amount formed in the bottle amended with PCE, FYE, and vitamins to determine the amount of reduction products formed from FYE alone. Results are shown in Table A2.1.

A2.3 Summary of FYE Results

It was determined from the average of the two studies that the degradation of an FYE addition of 40 μL resulted in the formation of approximately 31 μeq of reduction products in two days. Assuming that 10 percent of the contributed reduction equivalents were channeled to biomass synthesis, then the FYE contribution was approximately 34 μeq per 40 μL added per two days.

Table A2.1. Reduction product formation (μeq) from endogenous decay and FYE in two culture types.

Low-PCE/Butyric Acid Source Culture	Bottle Set 1	Bottle Set 2	Ave
FYE Plus Endogenous Decay	56.2	48.2	52.2
Endogenous Decay	26.2	27.5	26.9
FYE			25.4
Ethanol-Amended Cultures (20% High-PCE/Methanol Culture)	Bottle Set 1	Bottle Set 2	Ave
FYE Plus Endogenous Decay	46.7	42.1	44.4
Endogenous Decay	4.4	9.8	7.1
FYE			37.3

APPENDIX III

H₂ComPCE VERSION 4.4.1

Appendix III contains a printout of the entire STELLA Research[®] model that was constructed for this study. The model was named H₂ComPCE (hydrogen competition and PCE dechlorination). The model consists of the following: the upper-level mapping layer that contains scrollable instructions, sectors for aid in navigating to the model level, sliders with which to adjust input values for many of the model parameters, and table and graph pads for viewing simulation results; the model construction layer containing the icon-based model constructed from stocks, flows, and converters; and, finally, the list of equations generated by STELLA from the relationships represented by the model.

For all simulations, the integration method used was Runge-Kutta 4 with a time step (dt) of 0.03125 hr. To prevent division errors, initial stock values were set to 1×10^{-20} in lieu of zero.

A3.1. STELLA[®] Mapping Layer

This section contains a printout of the mapping layer of the STELLA[®] model.

H2ComPCE Version 4.4.1

H2ComPCE Version 4.4.1 models anaerobic reductive dechlorination of PCE by a hydrogenotrophic direct dechlorinator in a mixed community of microorganisms. Parameter input is via sliders and output is published to editions that are subscribed to by Excel.

GENERAL INFORMATION

Simulations may be carried out with one or combinations of more than one of the hydrogen donors: butyric acid, ethanol, lactic acid, and propionic acid. The nutritional supplement fermented yeast extract can also be added. The model produces output showing the fate of the hydrogen donor, the use of evolved hydrogen by methanogenesis or dechlorination, acetate production and its subsequent conversion to methane, and the status of the biomass of the various organism

TO RUN A SIMULATION

To run a simulation with H2ComPCE Version 4.4.1, enter parameters by using the sliders below. Sliders are included for the following four processes:

- (1) Donor fermentations;
- (2) Fermented yeast extract addition;
- (3) Hydrogenotrophic processes—dechlorination and

HYDROGEN DONORS

The rate of disappearance of an added hydrogen donor depends upon kinetics, the size of the biomass pool of the donor fermenter, and on the thermodynamics and favorability of the particular fermentation. Fermentation of all donors produces hydrogen and acetate. Ethanol and lactic acid may be fermented to propionate if

▽ BUTYRIC ACID
BUTYRIC ACID

▽ PROPIONIC ACID
PROPIONIC ACID

▽ ETHANOL
ETHANOL

▽ LACTIC ACID
LACTIC ACID

▽ THERMODYNAMICS
THERMODYNAMICS

▽ DONOR FERMENTER BIOMASS
DONOR FERMENTER BIOMASS

GLOBAL INPUTS

To adjust global values such as pH, temperature, Henry's constants, event times, and FYE content, click on the down arrow in the upper right-hand corner of the global inputs sector below. The

▽ GLOBAL INPUTS
GLOBAL INPUTS

COMPETITION FOR EVOLVED HYDROGEN

The rate of hydrogen evolution from donor fermentation, the ultimate aqueous phase concentration that it attains, the affinity that each set of organisms has for hydrogen, and the populations of hydrogen users are the parameters that determine the rate and extent of dechlorination or hydrogenotrophic methanogenesis that occurs.

Scroll over.....

HYDROGEN

HYDROGEN

DECHLORINATION

DECHLORINATION

DECHLORINATOR BIOMASS

DECHLORINATOR BIOMASS

METHANE FROM HYDROGEN

METHANE FROM HYDROGEN

HYDROGENOTROPHIC METHANOGEN

HYDROGENOTROPHIC METHANOGEN BIOMASS

ACETATE

The hydrogen donors also produce acetate upon fermentation. Acetate may accumulate or be converted to methane by acetoclastic methanogens. The acetate concentration effects the thermodynamics and favorability of donor fermentation.

ACETATE

ACETATE

METHANE FROM ACETATE

METHANE FROM ACETATE

ACETOTROPHIC METHAN

ACETOTROPHIC METHANOGEN BIOMASS

FERMENTED YEAST EXTRACT

Fermented yeast extract (FYE) is a nutritional supplement added to dechlorinating cultures to supply trace nutrients. FYE also contains acetate, propionate, butyrate, and longer-chained VFAs, and it contributes a significant amount of extra reducing equivalents over

FERMENTED YEAST EXTRACT

FERMENTED YEAST EXTRACT

SIMULATION RESULTS

At the end of a simulation, data are stored in the six tables below: (1) Donor HAc (2) Ethenes (3) Biomass-Donors (4) Biomass-Others (5) CH₄ / H₂, and (6) delta G rxn. At the end of the simulation, the tables publish output from H2CompCE 4.4.1 to editions which have the same names plus 4.4 on the end. The editions are subscribed to by an Excel file 'H2CompCE Output File 4.4'. The ending values can be examined and used in subsequent runs if desired. Note that currently, the tables are set up to collect data every 0.25 hours for 192 hours (8 days). If you wish to retain the data from each simulation, it must be copied to a new Excel worksheet or to a Cricket Graph or other type of application

Tables



Donors HAc



Ethenes



delta G rxn

CH₄ H₂

Biomass Donors



Biomass Others

Graphs



Donors and HAc



Ethenes



Thermo Factors



Hydrogen



Biomass



Biomass 2

DONOR FERMENTATIONS

adjust input using sliders....

	Pulse Input (μmol)		Initial Value (μmol)		k ($\mu\text{mol}/\text{mg VSS-hr}$)	
BUTYRIC ACID	Pulse Value Butyric Acid	<div><div>0.00</div><div>88.00</div><div>?</div></div>	Initial Butyrate	<div><div>0.00</div><div>100.00</div><div>0.00</div></div>	k Butyrate	<div><div>0.00</div><div>20.00</div><div>4.59</div></div>
					k Ethanol to Acetate	<div><div>0.00</div><div>21.92</div><div>21.92</div></div>
ETHANOL	Pulse Value Ethanol	<div><div>0.00</div><div>440.00</div><div>0.00</div></div>	Initial Ethanol	<div><div>0.00</div><div>100.00</div><div>0.00</div></div>	k Ethanol to Propionate	<div><div>0.00</div><div>21.92</div><div>21.92</div></div>
					k Lactate to Acetate	<div><div>0.00</div><div>20.00</div><div>8.57</div></div>
LACTIC ACID	Pulse Value Lactic Acid	<div><div>0.00</div><div>88.00</div><div>0.00</div></div>	Initial Lactate	<div><div>0.00</div><div>100.00</div><div>0.00</div></div>	k Lactate to Propionate	<div><div>0.00</div><div>20.00</div><div>8.57</div></div>
					k Propionate	<div><div>0.00</div><div>20.00</div><div>2.21</div></div>
PROPIONIC ACID	Pulse Value Propionic Acid	<div><div>0.00</div><div>88.00</div><div>0.00</div></div>	Initial Propionate	<div><div>0.00</div><div>100.00</div><div>0.00</div></div>		

DONOR FERMENTATIONS

adjust input using sliders....

Ks ($\mu\text{mol/L}$)		Initial Biomass (mg VSS)		Yield (mg VSS/ μmol)		Decay (/hr)	
Ks Butyrate	<div><div></div><div>0.00</div><div>50.00</div></div>	Initial X Butyrate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Y Butyrate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Decay Butyrate Fermenters	<div><div></div><div>0.000</div><div>1.000</div></div>
	<div><div>34.25</div><div>0.00</div><div>50.00</div></div>		<div><div>0.000</div><div>0.000</div><div>5.000</div></div>		<div><div>2.790</div><div>0.000</div><div>5.000</div></div>		<div><div>1.000</div><div>0.000</div><div>1.000</div></div>
Ks Ethanol to Acetate	<div><div></div><div>0.00</div><div>50.00</div></div>	Initial X Ethanol to Acetate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Y Ethanol to Acetate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Decay Ethanol to Acetate Fermenters	<div><div></div><div>0.000</div><div>1.000</div></div>
	<div><div>16.95</div><div>0.00</div><div>50.00</div></div>		<div><div>0.000</div><div>0.000</div><div>5.000</div></div>		<div><div>1.980</div><div>0.000</div><div>5.000</div></div>		<div><div>1.000</div><div>0.000</div><div>1.000</div></div>
Ks Ethanol to Propionate	<div><div></div><div>0.00</div><div>50.00</div></div>	Initial X Ethanol to Propionate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Y Ethanol to Propionate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Decay Ethanol to Propionate Fermenters	<div><div></div><div>0.000</div><div>1.000</div></div>
	<div><div>16.95</div><div>0.00</div><div>50.00</div></div>		<div><div>0.000</div><div>0.000</div><div>5.000</div></div>		<div><div>2.970</div><div>0.000</div><div>5.000</div></div>		<div><div>1.000</div><div>0.000</div><div>1.000</div></div>
Ks Lactate to Acetate	<div><div></div><div>0.00</div><div>50.00</div></div>	Initial X Lactate to Acetate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Y Lactate to Acetate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Decay Lactate to Acetate Fermenters	<div><div></div><div>0.000</div><div>1.000</div></div>
	<div><div>2.52</div><div>0.00</div><div>50.00</div></div>		<div><div>0.000</div><div>0.000</div><div>5.000</div></div>		<div><div>3.510</div><div>0.000</div><div>5.000</div></div>		<div><div>1.000</div><div>0.000</div><div>1.000</div></div>
Ks Lactate to Propionate	<div><div></div><div>0.00</div><div>50.00</div></div>	Initial X Lactate to Propionate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Y Lactate to Propionate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Decay Lactate to Propionate Fermenters	<div><div></div><div>0.000</div><div>1.000</div></div>
	<div><div>2.52</div><div>0.00</div><div>50.00</div></div>		<div><div>0.000</div><div>0.000</div><div>5.000</div></div>		<div><div>0.000</div><div>0.000</div><div>5.000</div></div>		<div><div>1.000</div><div>0.000</div><div>1.000</div></div>
Ks Propionate	<div><div></div><div>0.00</div><div>50.00</div></div>	Initial X Propionate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Y Propionate Fermenters	<div><div></div><div>0.000</div><div>5.000</div></div>	Decay Propionate Fermenters	<div><div></div><div>0.000</div><div>1.000</div></div>
	<div><div>11.30</div><div>0.00</div><div>50.00</div></div>		<div><div>0.000</div><div>0.000</div><div>5.000</div></div>		<div><div>1.440</div><div>0.000</div><div>5.000</div></div>		<div><div>1.000</div><div>0.000</div><div>1.000</div></div>

HYDROGENOTROPIC PROCESSES

adjust input using sliders....

DECHLORINATION

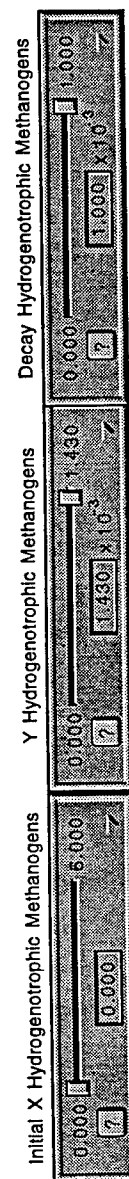
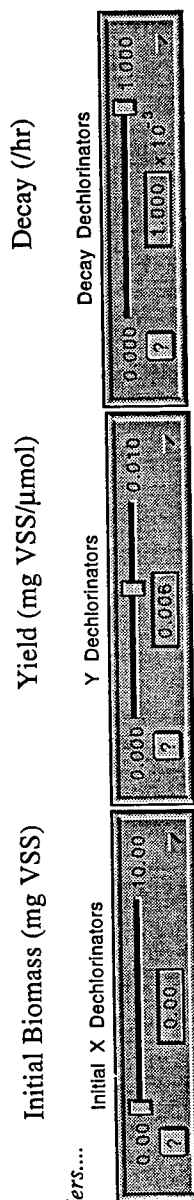
Pulse Input (μmol)	k ($\mu\text{mol}/\text{mg VSS}\cdot\text{hr}$)	Ks ($\mu\text{mol}/\text{L}$)
<div style="border: 1px solid black; padding: 5px;"> Pulse Value PCE 0.00 ————— 44.00 <input type="text" value="11.00"/> </div>	<div style="border: 1px solid black; padding: 5px;"> k PCE 0.000 ————— 5.000 <input type="text" value="1.815"/> </div>	<div style="border: 1px solid black; padding: 5px;"> Ks PCE 0.000 ————— 5.000 <input type="text" value="0.540"/> </div>
DECHLORINATION		
	<div style="border: 1px solid black; padding: 5px;"> k TCE 0.000 ————— 5.000 <input type="text" value="3.000"/> </div>	<div style="border: 1px solid black; padding: 5px;"> Ks TCE 0.000 ————— 5.000 <input type="text" value="0.540"/> </div>
	<div style="border: 1px solid black; padding: 5px;"> k DCE 0.000 ————— 5.000 <input type="text" value="3.000"/> </div>	<div style="border: 1px solid black; padding: 5px;"> Ks DCE 0.000 ————— 5.000 <input type="text" value="0.540"/> </div>
	<div style="border: 1px solid black; padding: 5px;"> k VC 0.000 ————— 5.000 <input type="text" value="3.000"/> </div>	<div style="border: 1px solid black; padding: 5px;"> Ks VC 0.0 ————— 500.0 <input type="text" value="290.0"/> </div>
		<div style="border: 1px solid black; padding: 5px;"> Ks H2 Dechlor 0.000 ————— 5.000 <input type="text" value="0.100"/> </div>

HYDROGENOTROPIC METHANOGENESIS

Pulse Value Hydrogen	k H2 methane	Ks H2 methane
<div style="border: 1px solid black; padding: 5px;"> Pulse Value Hydrogen 0.00 ————— 88.00 <input type="text" value="0.08"/> </div>	<div style="border: 1px solid black; padding: 5px;"> k H2 methane 0.0 ————— 100.0 <input type="text" value="40.0"/> </div>	<div style="border: 1px solid black; padding: 5px;"> Ks H2 methane 0.000 ————— 5.000 <input type="text" value="0.500"/> </div>

HYDROGENOTROPIC PROCESSES

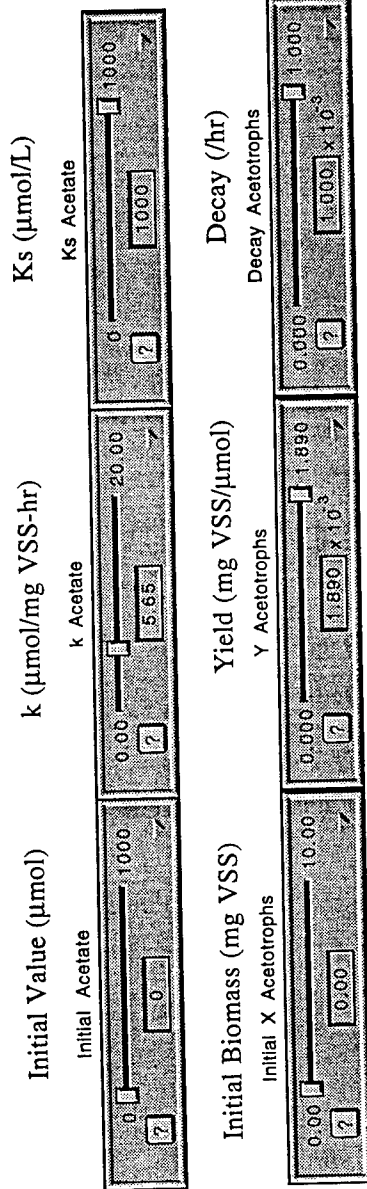
adjust input using sliders....



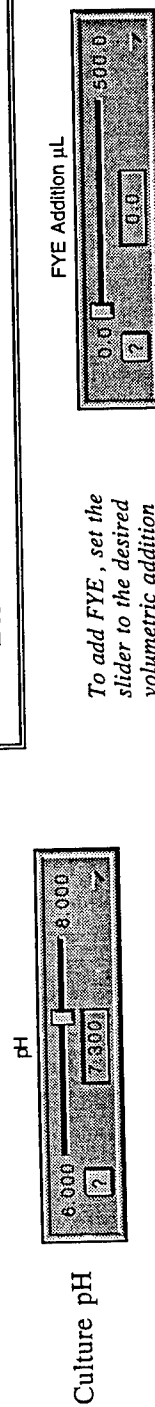
ACETOTROPIC PROCESSES

adjust input using sliders....

ACETOTROPIC METHANOGENESIS



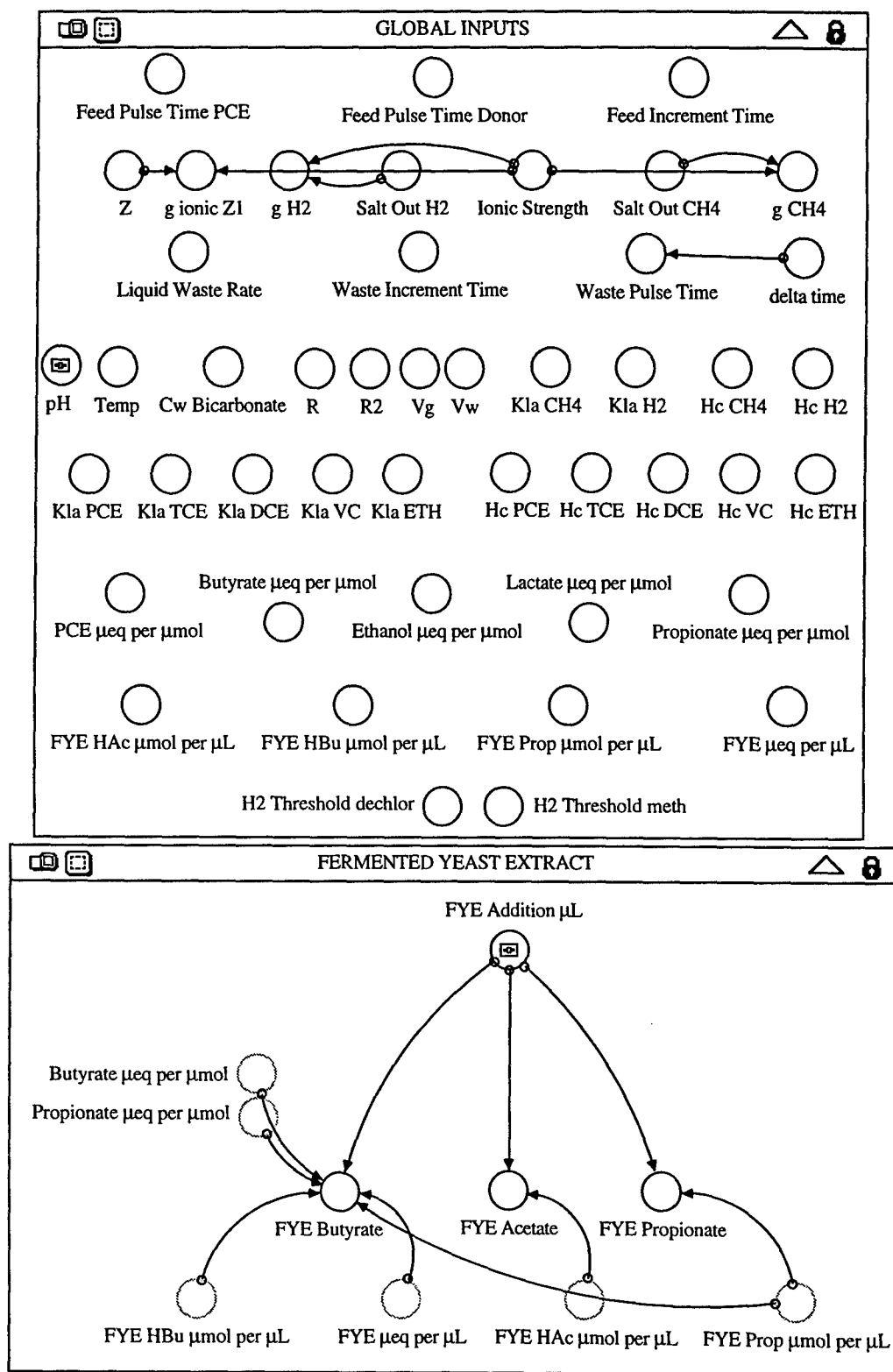
FERMENTED YEAST EXTRACT

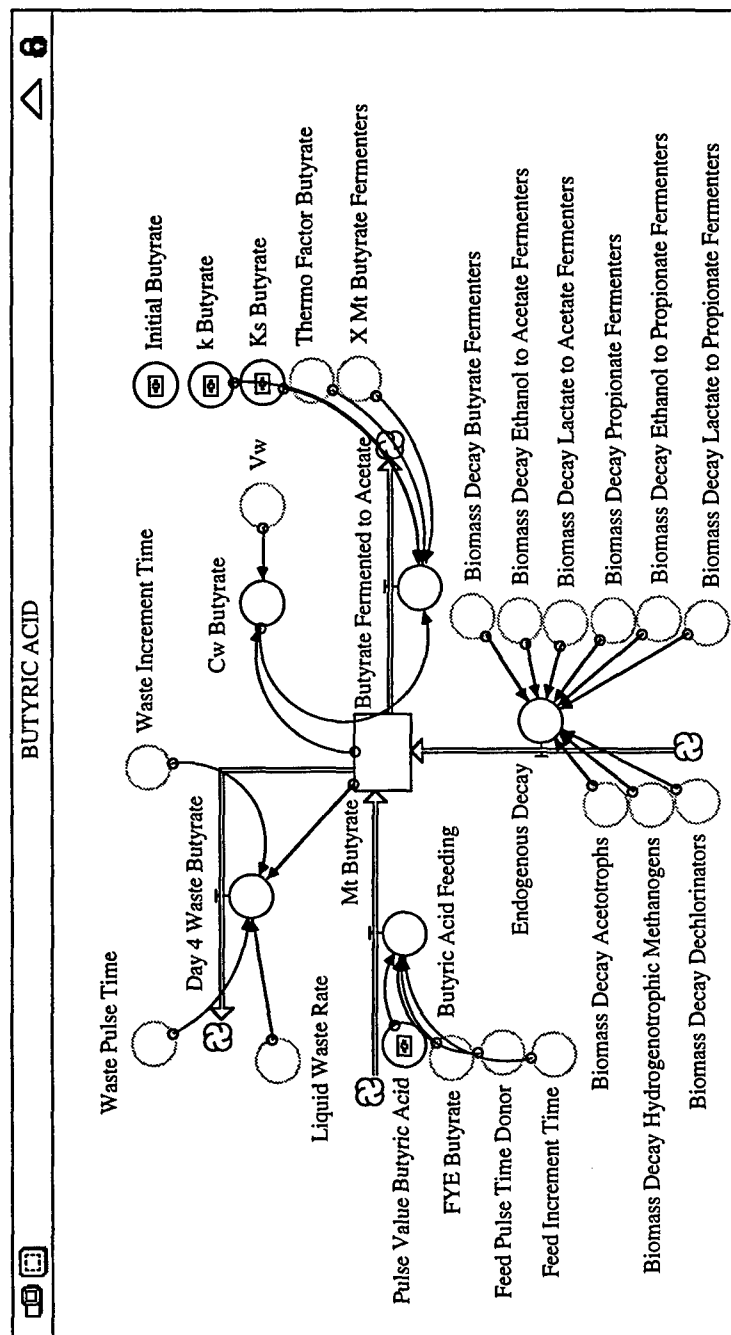


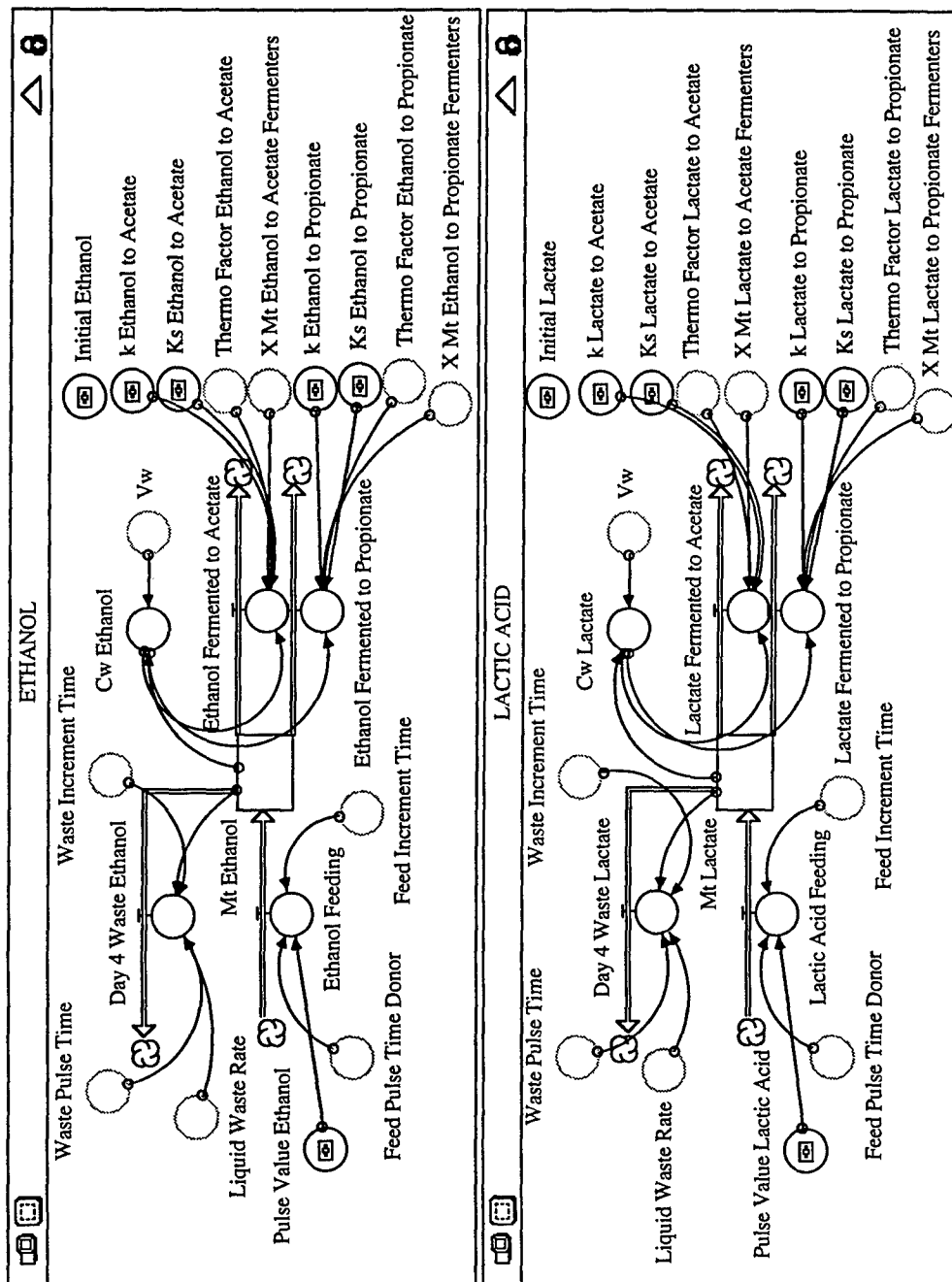
A3.2. STELLA® Model Construction Layer

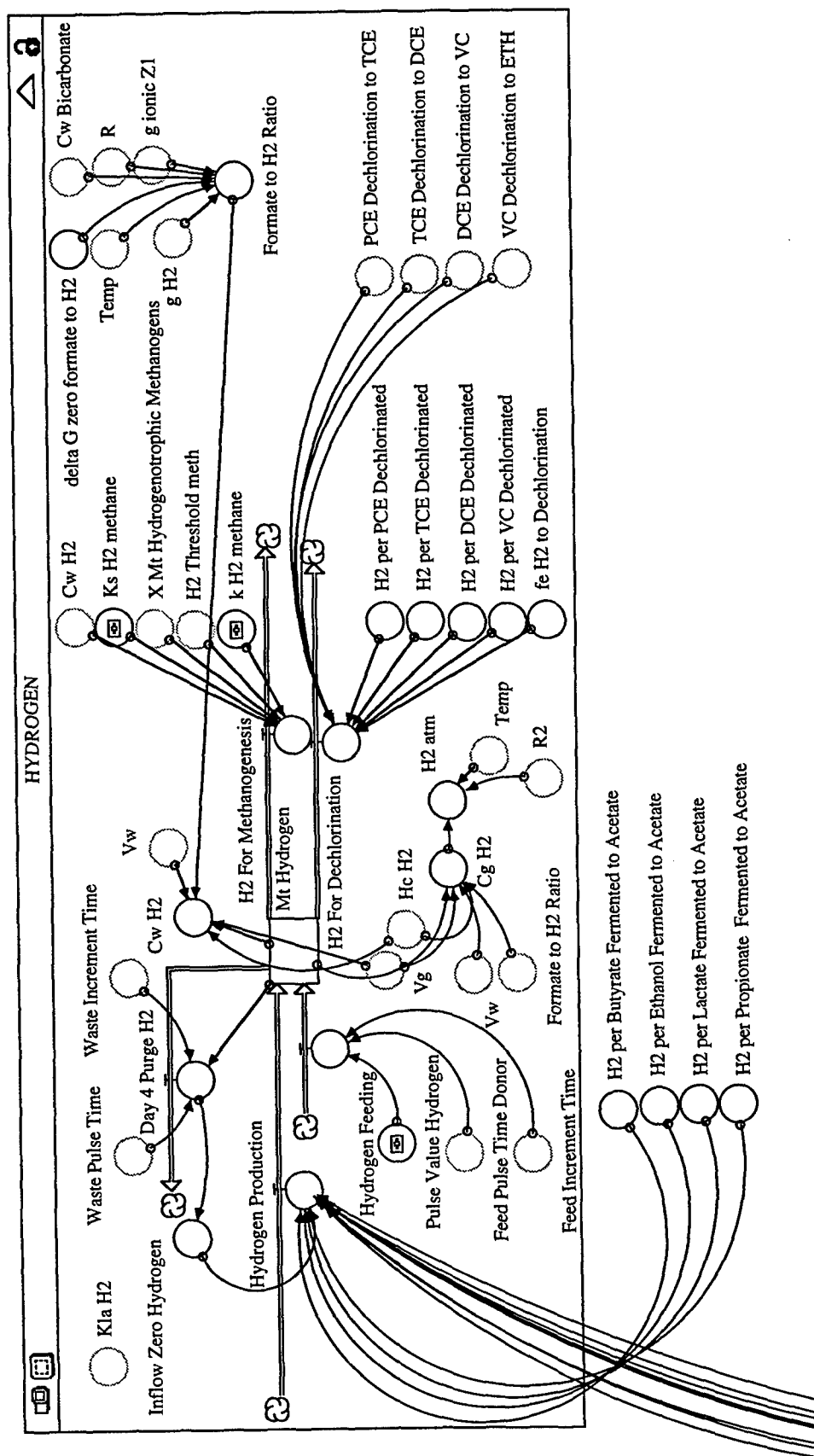
This section contains a printout of the model construction layer of the STELLA® model.

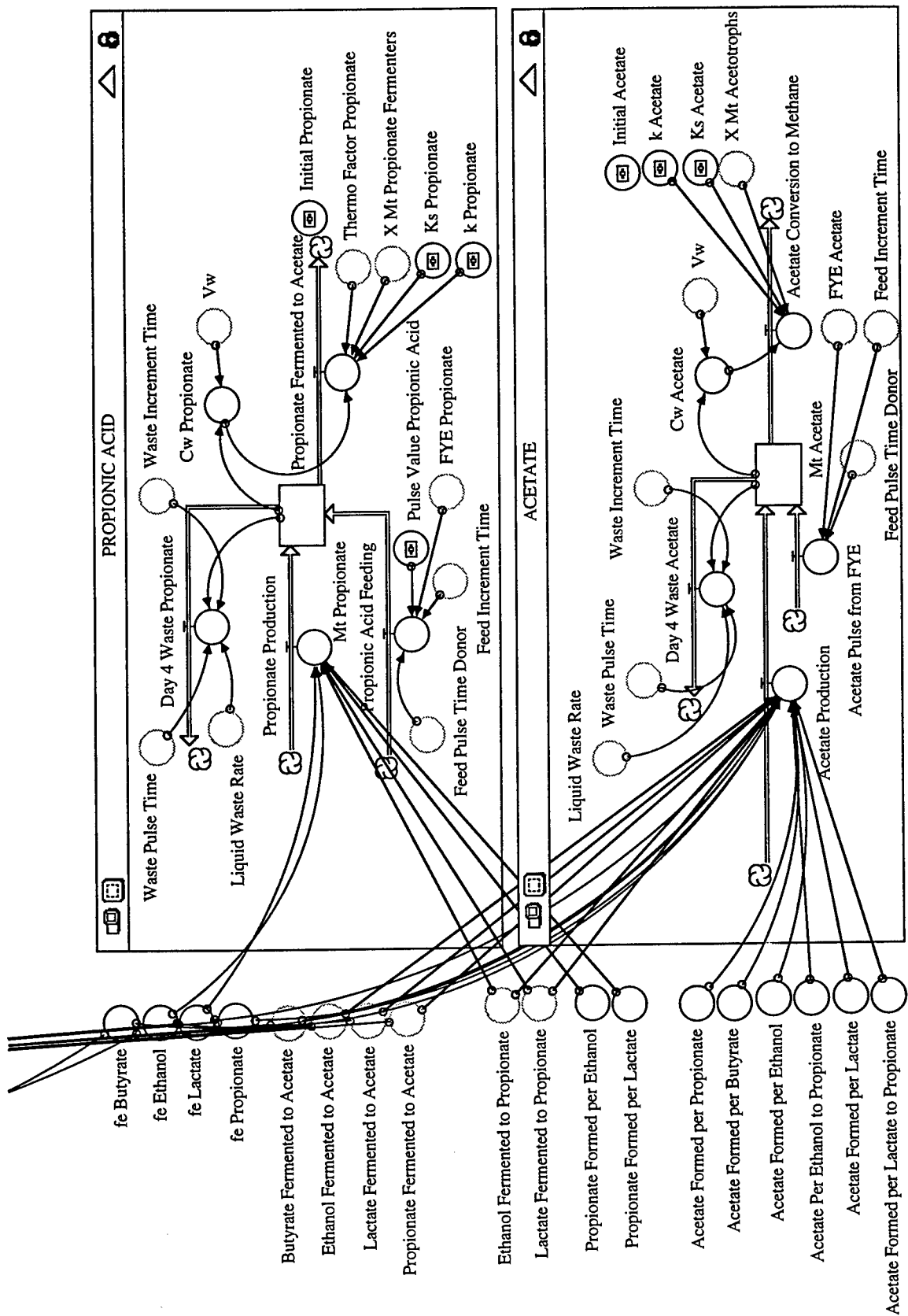
H2ComPCE Model Version 4.4.1

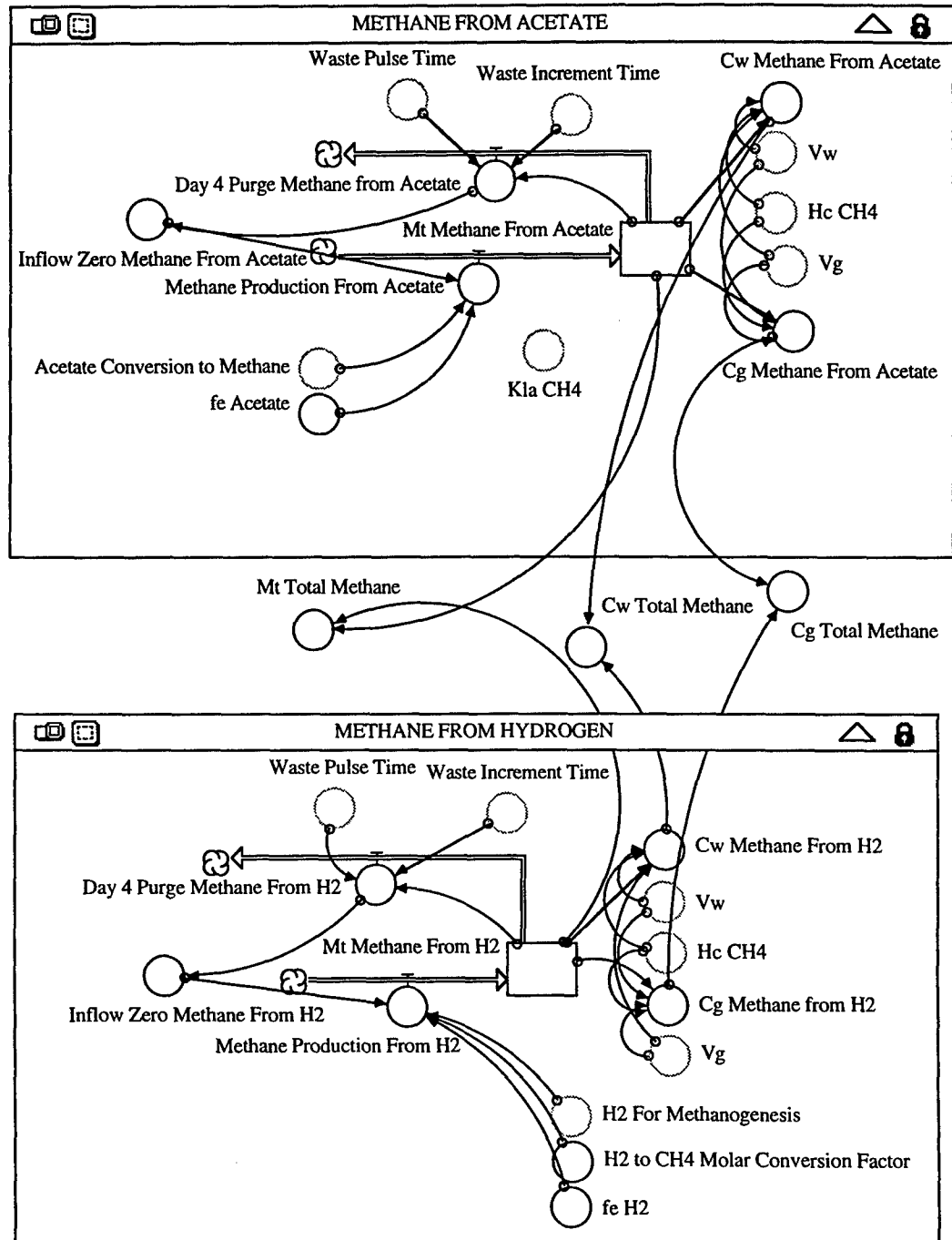


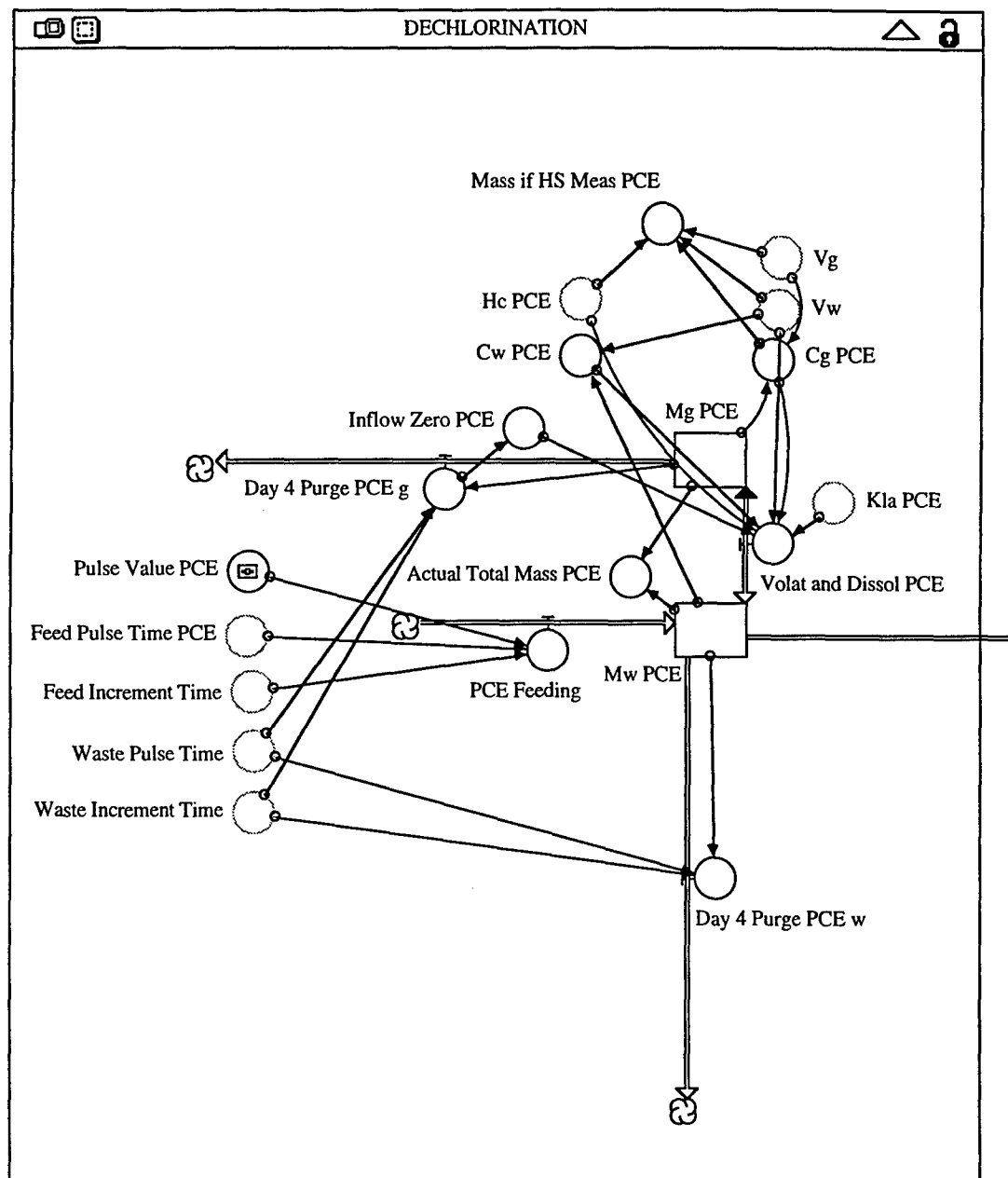


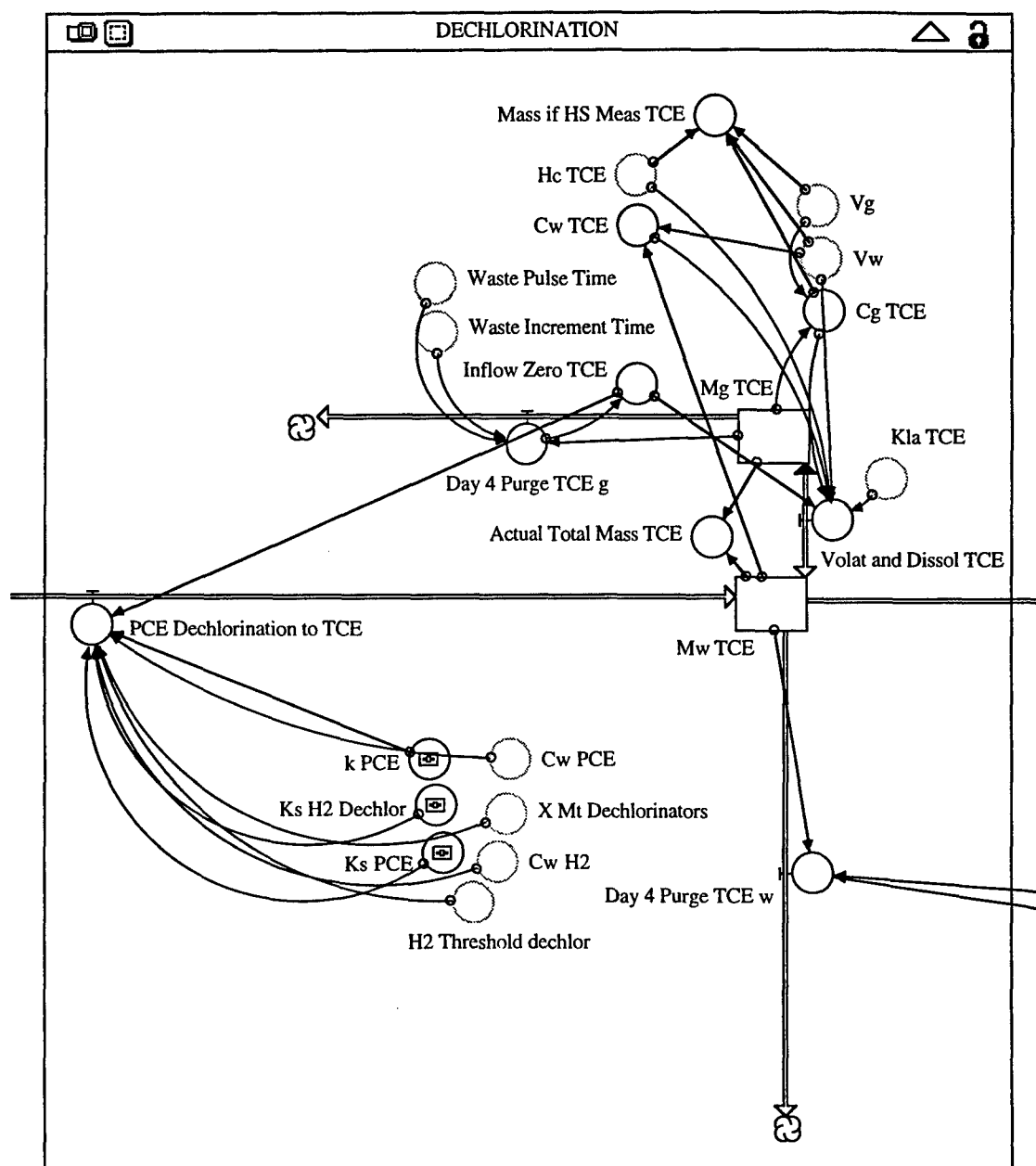


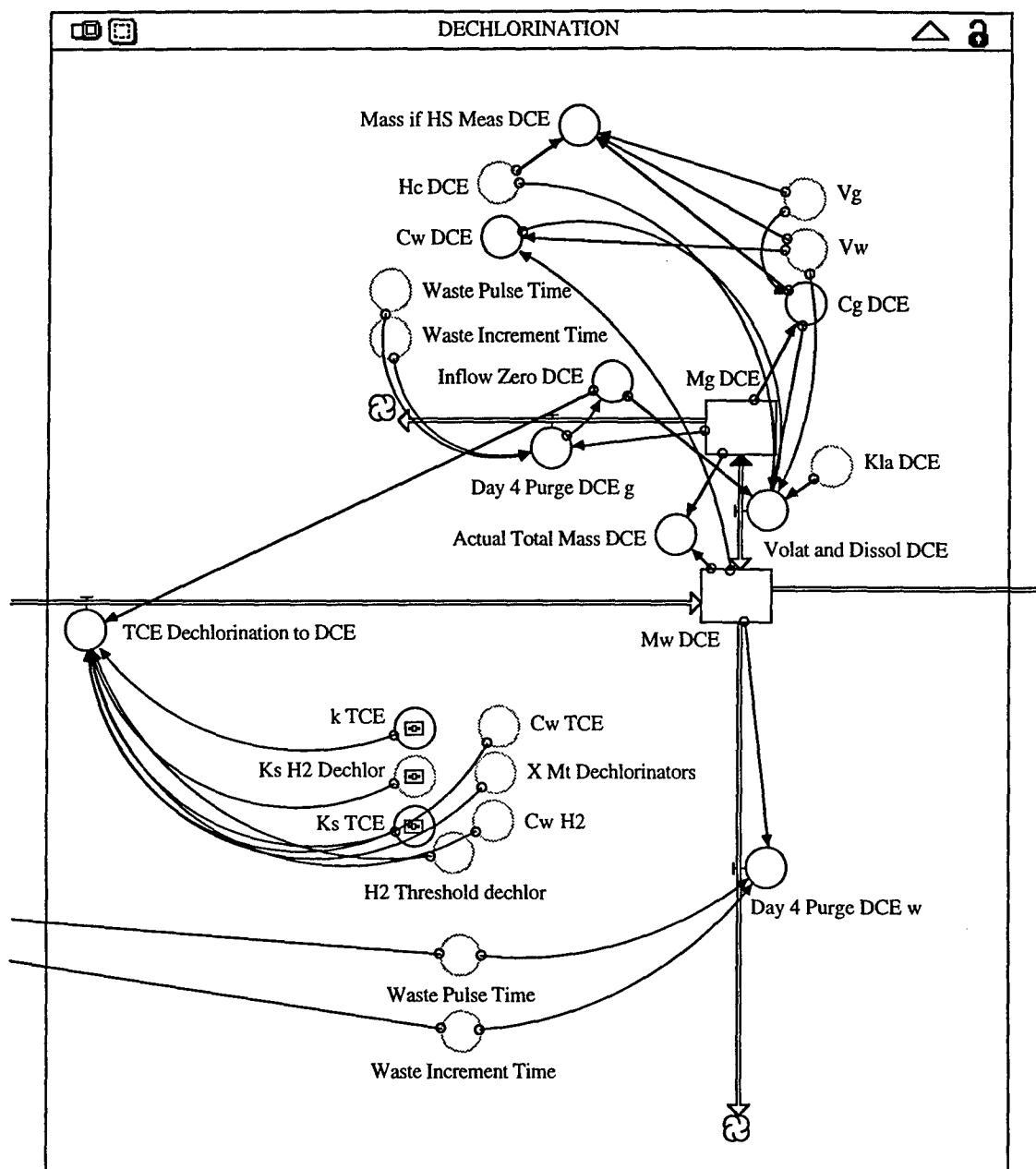


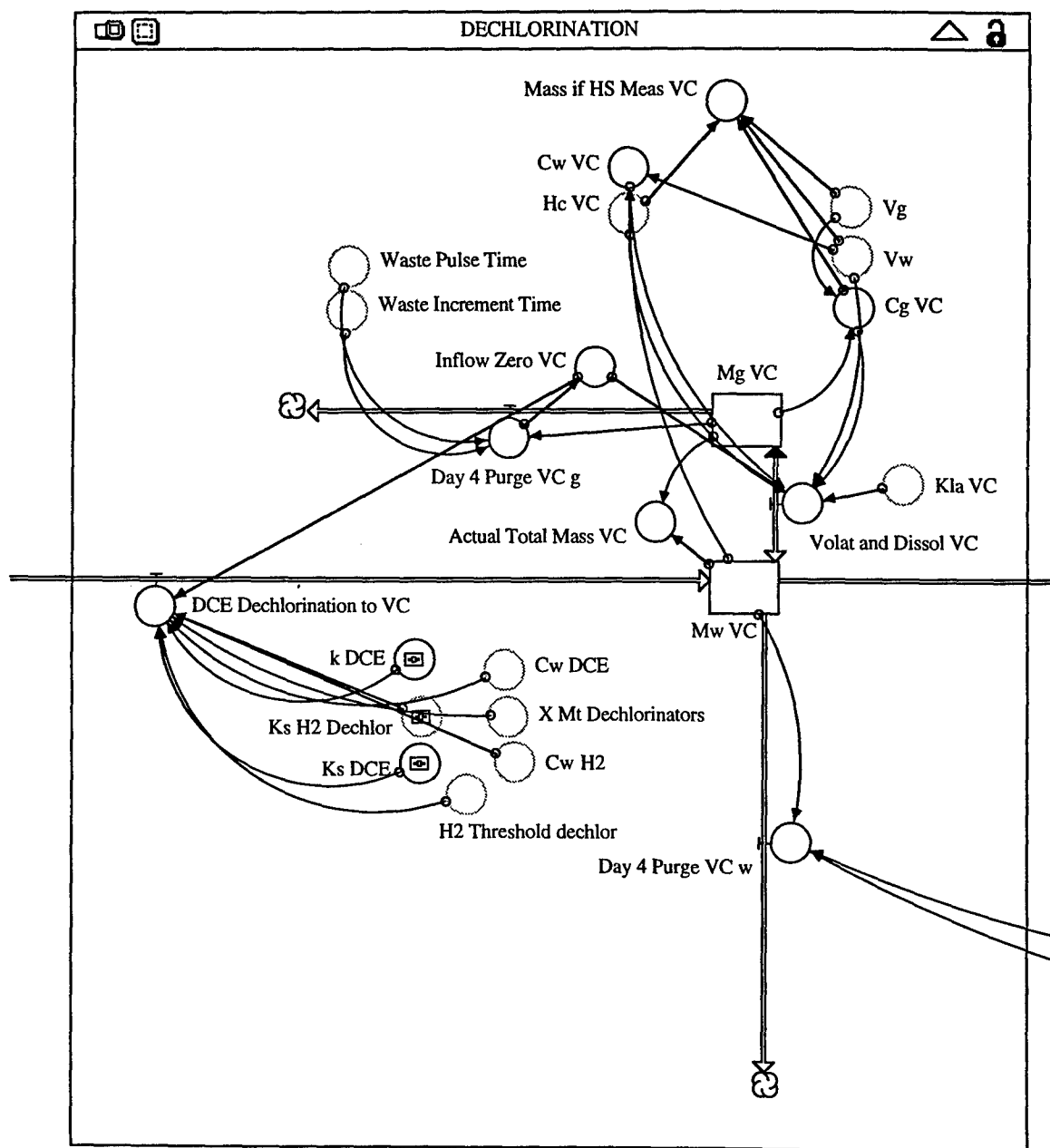


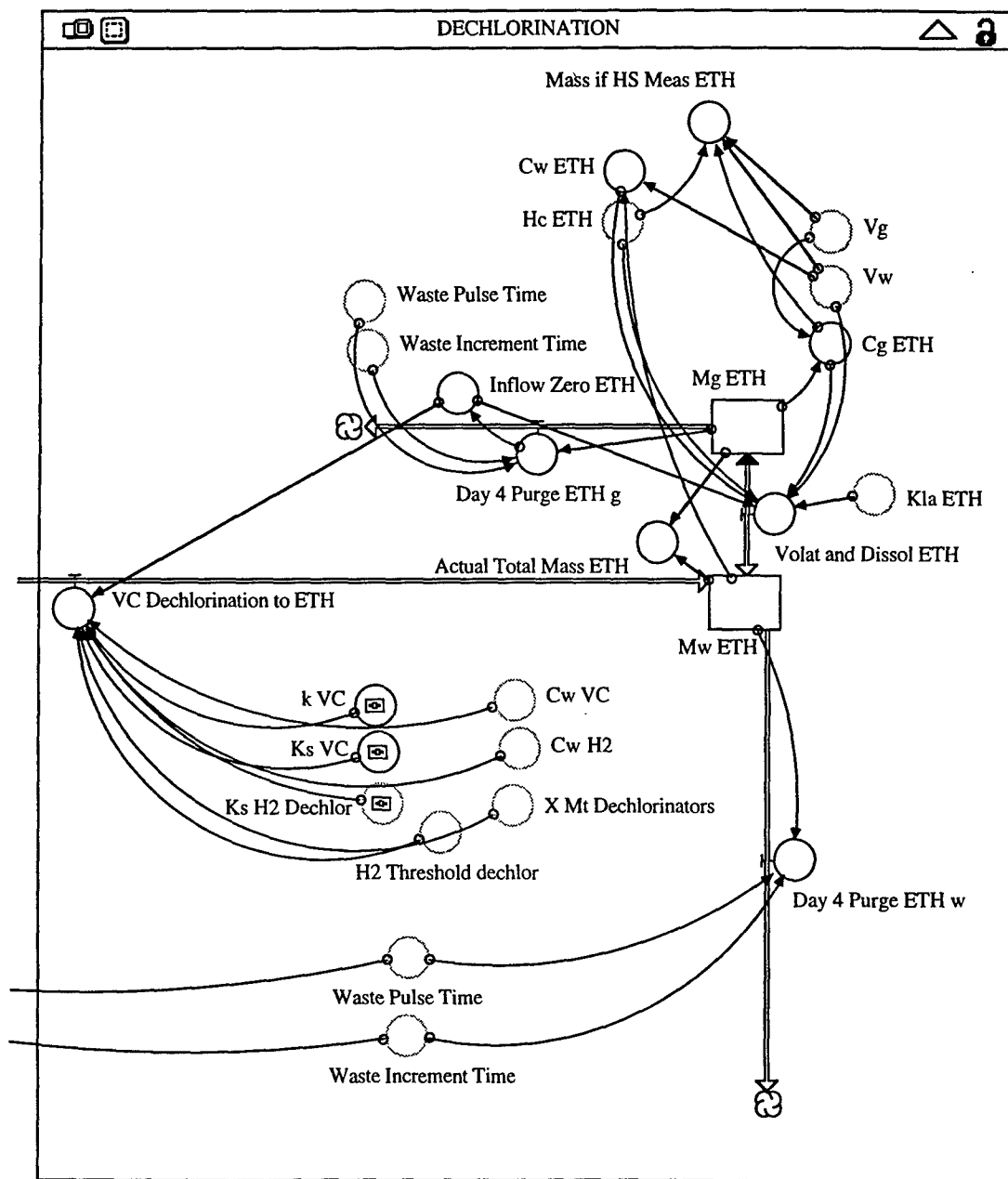


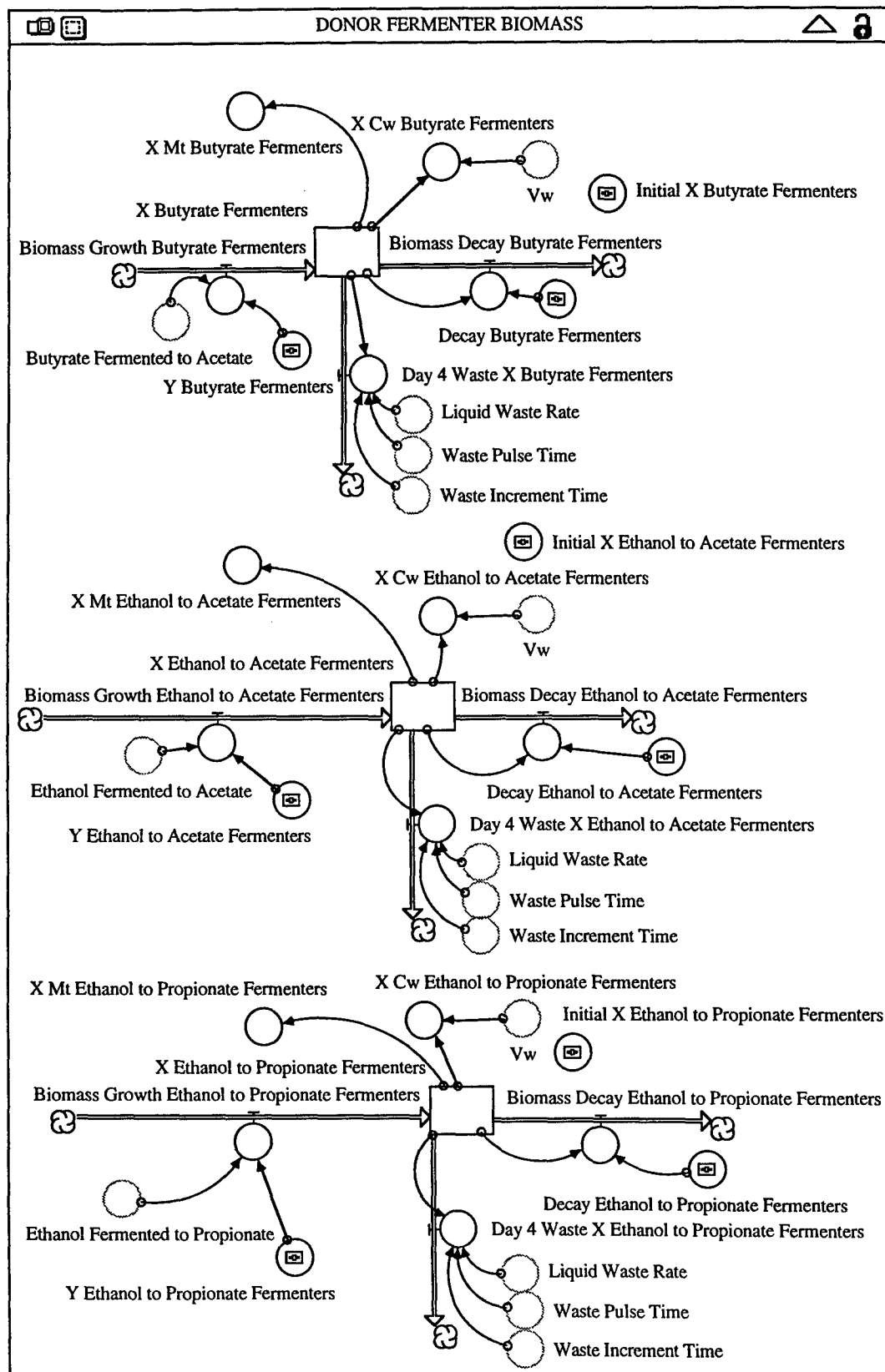


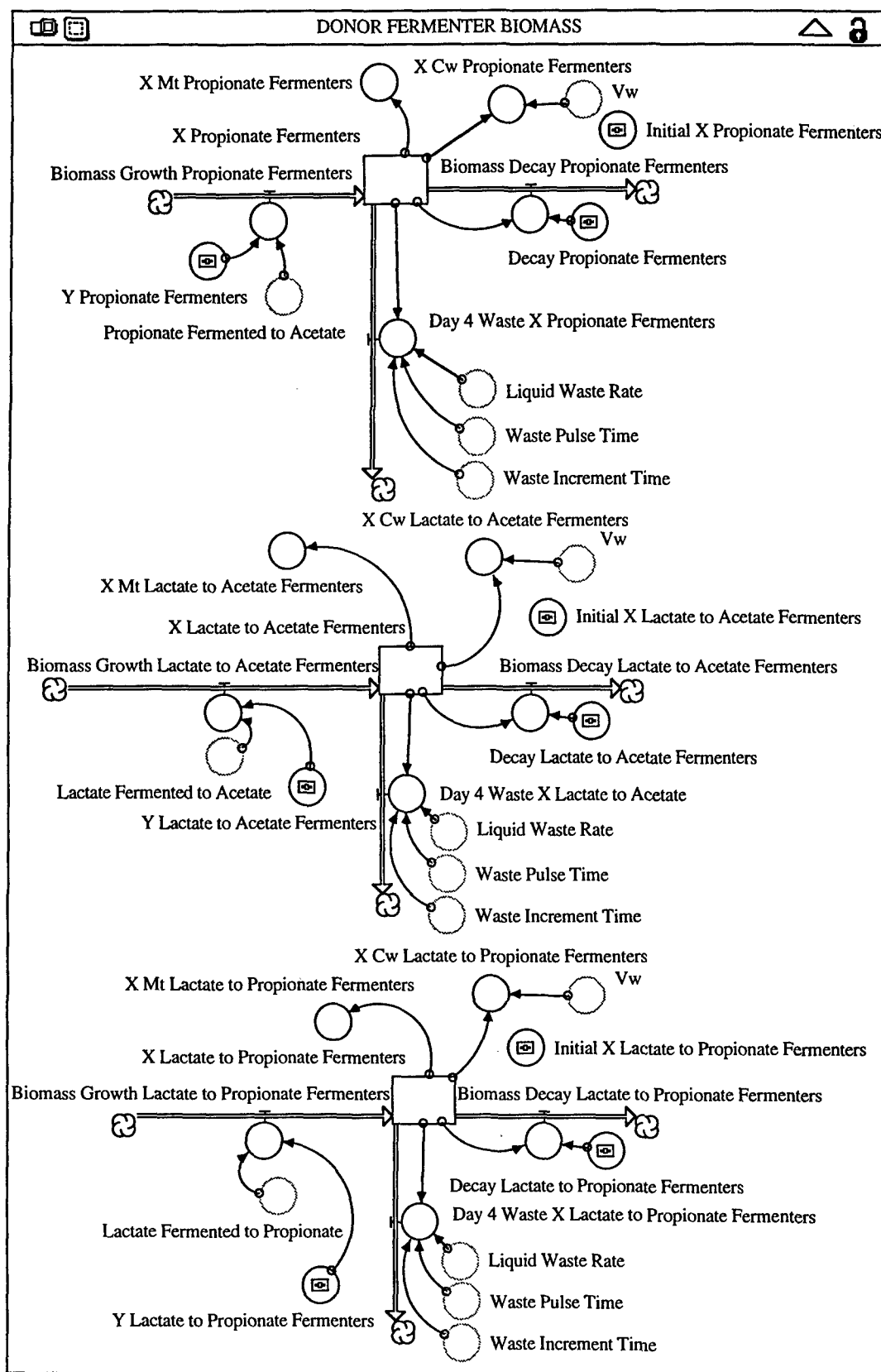


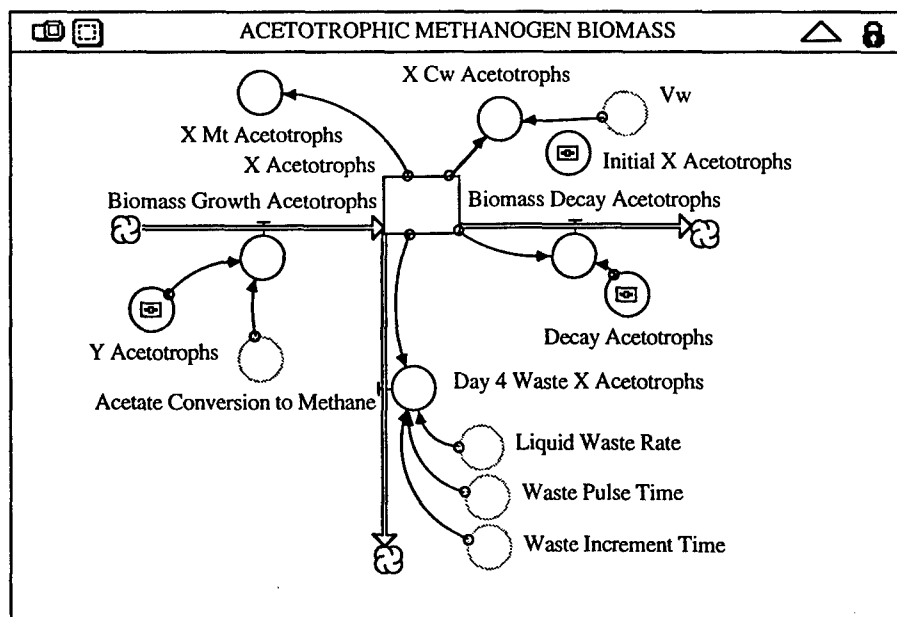
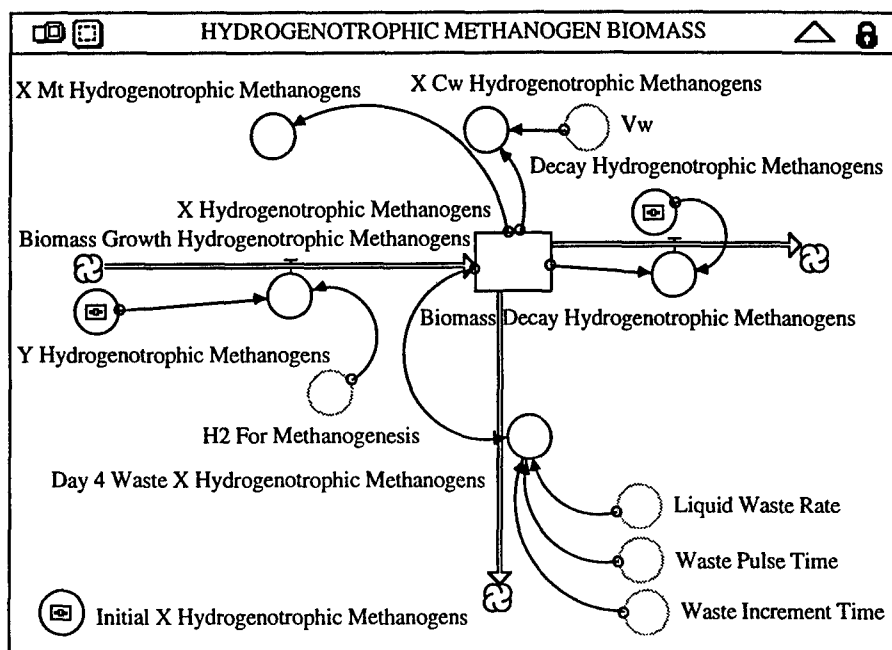


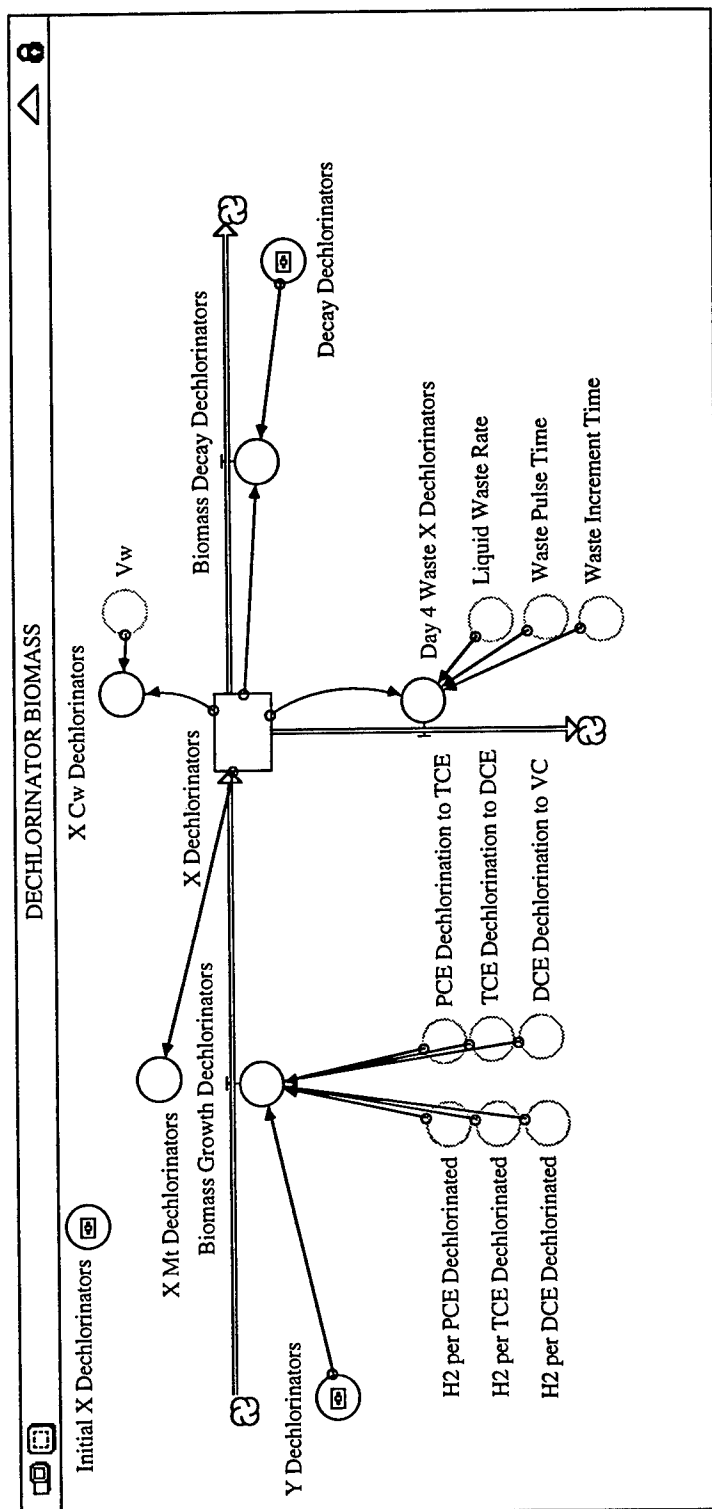


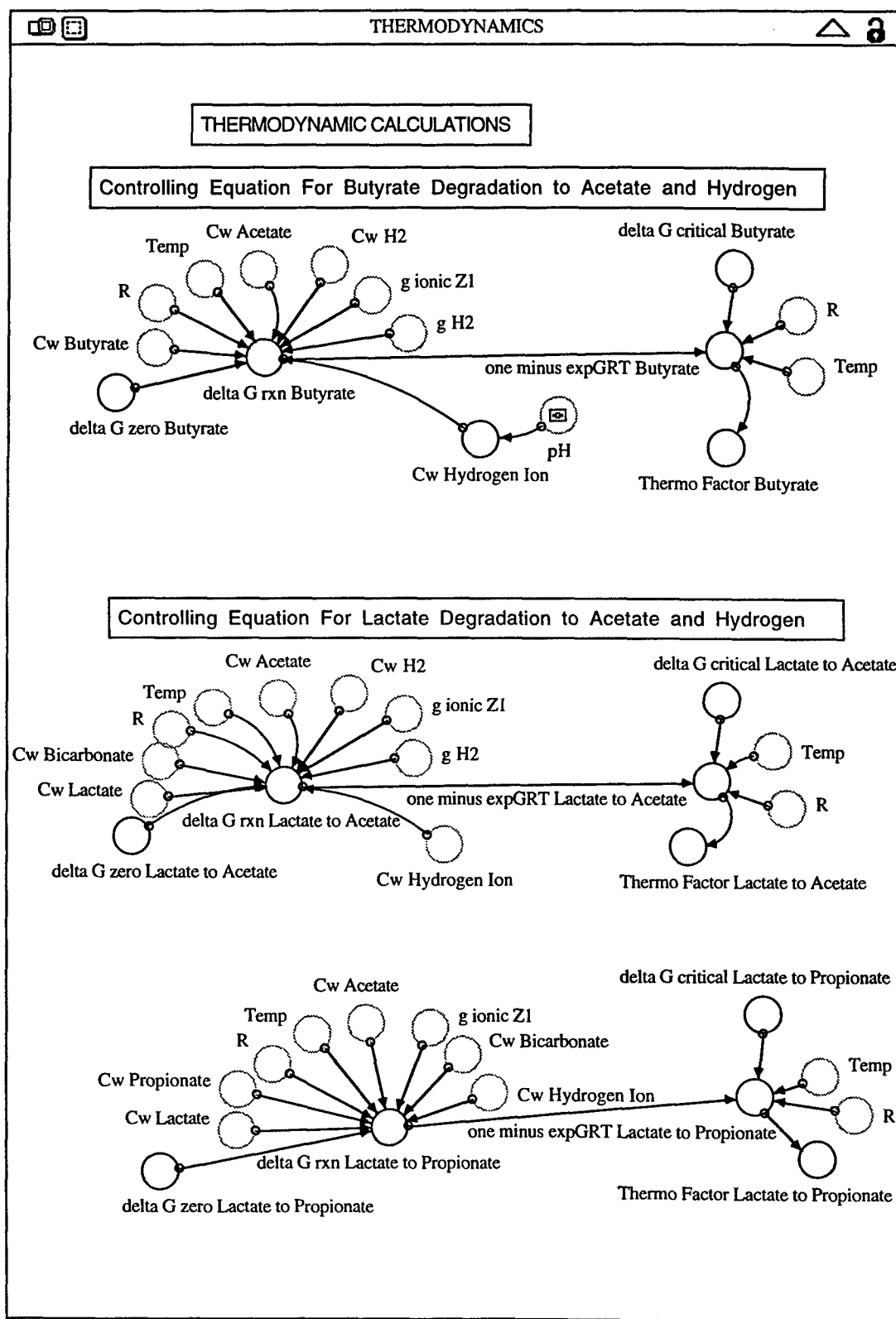


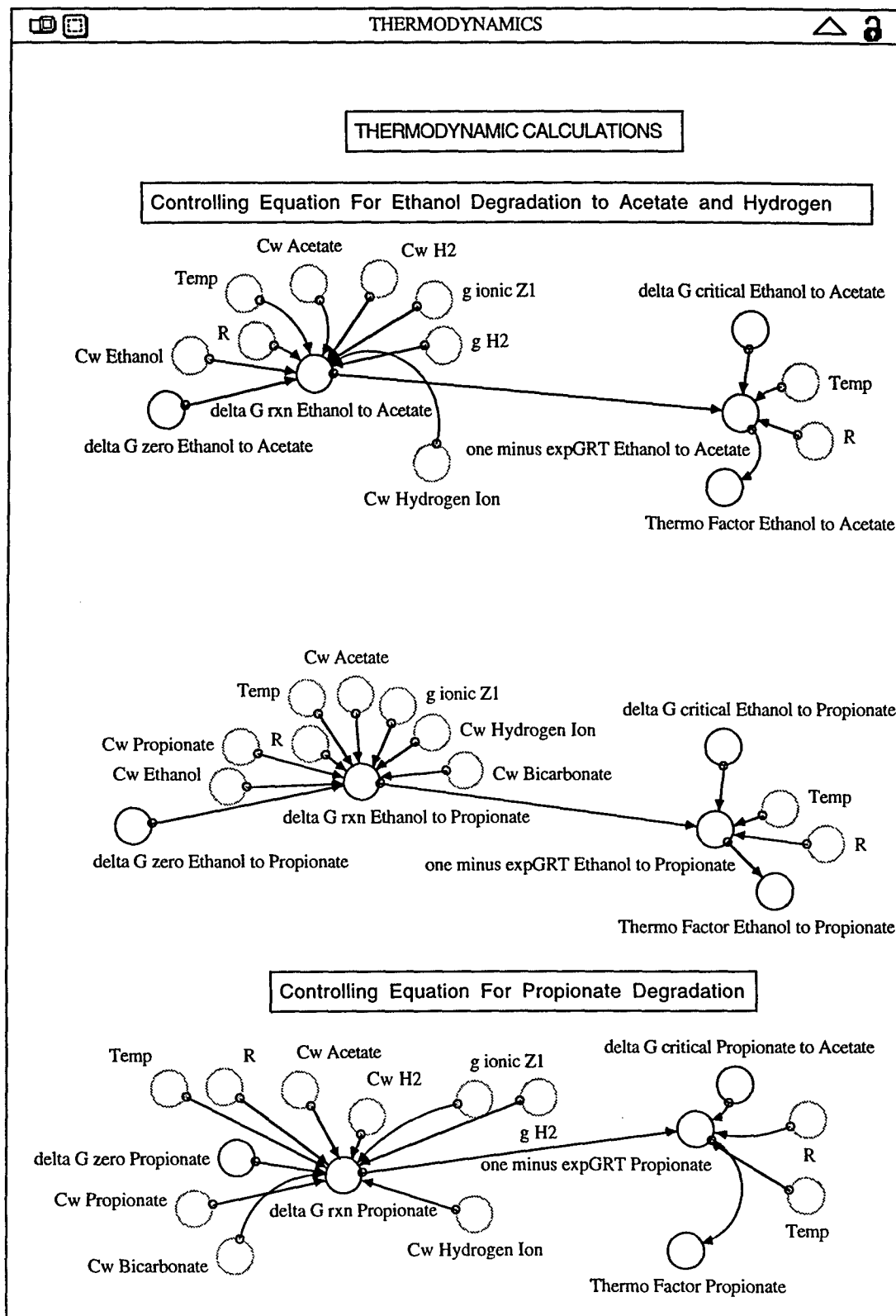












A3.3. STELLA® Equations

This section contains a listing of the STELLA® equations.

ACETIC ACID

$Mt_Acetate(t) = Mt_Acetate(t - dt) + (Acetate_Production +$
 $Acetate_Pulse_from_FYE - Day_4_Waste_Acetate -$
 $Acetate_Conversion_to_Methane) * dt$

INIT $Mt_Acetate = Initial_Acetate$

DOCUMENT: Reservoir representing acetic acid (μmol).

INFLOWS:

$Acetate_Production = fe_Butyrate * Butyrate_Fermented_to_Acetate *$

$Acetate_Formed_per_Butyrate + fe_Ethanol *$

$Ethanol_Fermented_to_Propionate *$

$Acetate_Per_Ethanol_to_Propionate + fe_Ethanol *$

$Ethanol_Fermented_to_Acetate * Acetate_Formed_per_Ethanol +$

$fe_Lactate * Lactate_Fermented_to_Acetate *$

$Acetate_Formed_per_Lactate + fe_Lactate *$

$Lactate_Fermented_to_Propionate *$

$Acetate_Formed_per_Lactate_to_Propionate + fe_Propionate *$

$Propionate_Fermented_to_Acetate * Acetate_Formed_per_Propionate$

DOCUMENT: Acetate production from all donors ($\mu\text{mol/hr}$). Acetate Produced
 $= \text{Sum of \{donor fermentation flow * stoichiometric conversion (HAc/Donor) *}$
 $fe\}$. Where fe is the fraction of the donor that is used for energy.

$Acetate_Pulse_from_FYE = PULSE(FYE_Acetate,$

$Feed_Pulse_Time_Donor, Feed_Increment_Time)$

DOCUMENT: This is a pulse input of acetate that is contributed by FYE at each feeding (μmol).

OUTFLOWS:

$Day_4_Waste_Acetate = PULSE(Mt_Acetate * Liquid_Waste_Rate,$
 $Waste_Pulse_Time, Waste_Increment_Time)$

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This decreases the amount of soluble constituents by 10 percent. Volatile constituents

are not affected since they are purged out after each 96 hr period (μmol every 96 hr).

$$\text{Acetate_Conversion_to_Methane} = (k_Acetate * X_Mt_Acetotrophs * Cw_Acetate) / (Ks_Acetate + Cw_Acetate)$$

DOCUMENT: Acetate conversion to methane ($\mu\text{mol/hr}$).

$$Cw_Acetate = Mt_Acetate / Vw \text{ } (\mu\text{mol/L})$$

DOCUMENT: Converter to allow display of acetic acid in concentration units ($\mu\text{mol/L}$).

$$\text{Initial_Acetate} = 1E-20$$

DOCUMENT: Initial amount of acetate present (μmol).

$$Ks_Acetate = 1000$$

DOCUMENT: Half-velocity coefficient for acetate degradation. $1000 \mu\text{mol/L}$. Ohtsubo et al., 1992; Zehnder et al., 1980.

$$k_Acetate = 5.65$$

DOCUMENT: Rate of acetate degradation. $5.65 \mu\text{mol/mg VSS-hr}$. Ohtsubo et al., 1992.

ACETOTROPHIC METHANOGENS

$$X_Acetotrophs(t) = X_Acetotrophs(t - dt) + (\text{Biomass_Growth_Acetotrophs} - \text{Biomass_Decay_Acetotrophs} - \text{Day_4_Waste_X_Acetotrophs}) * dt$$

$$\text{INIT } X_Acetotrophs = \text{Initial_X_Acetotrophs} \text{ } (\text{mg VSS})$$

DOCUMENT: Reservoir representing acetotrophic methanogen biomass (mg VSS).

INFLOWS:

$$\text{Biomass_Growth_Acetotrophs} = Y_Acetotrophs *$$

$$\text{Acetate_Conversion_to_Methane} \text{ } (\text{mg VSS/hr})$$

OUTFLOWS:

$$\text{Biomass_Decay_Acetotrophs} = \text{Decay_Acetotrophs} * X_Acetotrophs \text{ } (\text{mg VSS/hr})$$

$$\text{Day_4_Waste_X_Acetotrophs} = \text{PULSE}(X_Acetotrophs *$$

$$\text{Liquid_Waste_Rate, Waste_Pulse_Time, Waste_Increment_Time}) \text{ } (\text{mg VSS wasted every 96 hr})$$

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

$$\text{Decay_Acetotrophs} = 0.001\{\text{/hr}\}$$

DOCUMENT: 0.001/hr. Generic number.

$$\text{Initial_X_Acetotrophs} = 0$$

DOCUMENT: Initial amount of acetotrophic methanogen biomass present {mg VSS}.

$$\text{X_Cw_Acetotrophs} = \text{X_Acetotrophs}/\text{Vw} \{\text{mg VSS/L}\}$$

DOCUMENT: Converter to allow reporting of acetotrophic methanogen biomass as a concentration {mg VSS/L}.

$$\text{X_Mt_Acetotrophs} = \text{X_Acetotrophs} \{\text{mg VSS}\}$$

$$\text{Y_Acetotrophs} = 0.00189$$

DOCUMENT: Yield for acetotrophic methanogens (0.00189 mg VSS/ μmol acetic acid) Smith and Mah, 1978.

BUTYRIC ACID

$$\begin{aligned} \text{Mt_Butyrate}(t) = & \text{Mt_Butyrate}(t - dt) + (\text{Butyric_Acid_Feeding} + \\ & \text{Endogenous_Decay} - \text{Day_4_Waste_Butyrate} - \\ & \text{Butyrate_Fermented_to_Acetate}) * dt \end{aligned}$$

$$\text{INIT Mt_Butyrate} = \text{Initial_Butyrate} \{\mu\text{mol}\}$$

DOCUMENT: Reservoir representing butyric acid { μmol }.

INFLOWS:

$$\text{Butyric_Acid_Feeding} = \text{PULSE}((\text{Pulse_Value_Butyric_Acid} + \text{FYE_Butyrate}), \text{Feed_Pulse_Time_Donor}, \text{Feed_Increment_Time})$$

DOCUMENT: Pulse input of butyric acid { μmol } beginning at time = 0 hr and occurring every 48 hr thereafter.

$$\begin{aligned} \text{Endogenous_Decay} = & \text{Biomass_Decay_Acetotrophs} + \\ & \text{Biomass_Decay_Butyrate_Fermenters} + \text{Biomass_Decay_Dechlorinators} \\ & + \text{Biomass_Decay_Ethanol_to_Acetate_Fermenters} + \\ & \text{Biomass_Decay_Ethanol_to_Propionate_Fermenters} + \\ & \text{Biomass_Decay_Hydrogenotrophic_Methanogens} + \end{aligned}$$

Biomass_Decay_Lactate_to_Acetate_Fermenters+
 Biomass_Decay_Lactate_to_Propionate_Fermenters+
 Biomass_Decay_Propionate_Fermenters

DOCUMENT: Endogenous decay is modeled as an input to the butyric acid pool. Decaying cells contribute to the pool of electron donor and probably to the pool of *slowly* degraded electron donor. To model decay products as being degraded under a thermodynamic ceiling, it is arbitrarily modeled as butyrate. Note that 1 mol biomass, C₅H₇O₂N, goes to 1 mol butyrate regardless of the organism pool from which the biomass comes {μmol/hr}.



OUTFLOWS:

Day_4_Waste_Butyrate = PULSE(Mt_Butyrate*Liquid_Waste_Rate,
 Waste_Pulse_Time,Waste_Increment_Time) {μmol every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

Butyrate_Fermented_to_Acetate =
 (k_Butyrate*X_Mt_Butyrate_Fermenters*Cw_Butyrate*
 Thermo_Factor_Butyrate)/(Ks_Butyrate+Cw_Butyrate)

DOCUMENT: Butyrate fermentation to acetate and hydrogen {μmol/hr}.

Cw_Butyrate = Mt_Butyrate/Vw {μmol/L}

DOCUMENT: Converter to allow reporting of butyrate as a concentration {μmol/L}.

Initial_Butyrate = 1E-20

DOCUMENT: The initial amount of butyrate present {μmol}.

Ks_Butyrate = 34.25

DOCUMENT: Half-velocity coefficient for butyrate fermentation. 34.25 μmol/L, Fennell est., 1996.

k_Butyrate = 4.86

DOCUMENT: Apparent rate of butyrate degradation was 0.567 {μmol butyrate/mg VSS-hr, est., Fennell}.

This occurred under a thermodynamic ceiling (ave) of -20 kJ/mol butyrate fermented (on average) for the 1:1 TISs and -34 kJ/mol for the 2:1 TISs. Delta G critical was set at -19 kJ/mol and the thermo factor was calculated for each condition. There is less butyrate-degrader biomass than the total VSS measured. From my biomass estimates the butyrate degraders make up 20.9 % for 1:1 TISs and 21.6 % for 2:1 TISs, of the total biomass in the bottle.

The rate that would be observed in the absence of a thermodynamic limit and accounting for the fraction of relevant biomass is:

rate = apparent rate / (thermo factor * fraction of relevant VSS).

AVE k = 4.86 $\mu\text{mol}/\text{mg}$ butyrate VSS-hr

Pulse_Value_Butyric_Acid = 0

DOCUMENT: This is the amount of butyric acid fed at each pulse beginning at 0 hr and occurring every 48 hr (μmol).

DECHLORINATION

PCE

$\text{Mg_PCE}(t) = \text{Mg_PCE}(t - dt) + (-\text{Volat_and_Dissol_PCE} - \text{Day_4_Purge_PCE_g}) * dt$

INIT Mg_PCE = 0 (μmol)

DOCUMENT: Reservoir representing PCE in the gaseous phase (μmol).

OUTFLOWS:

$\text{Volat_and_Dissol_PCE} =$

$\text{Inflow_Zero_PCE} * \text{Vw} * \text{Kla_PCE} * ((\text{Cg_PCE} / \text{Hc_PCE}) - \text{Cw_PCE})$

DOCUMENT: This biflow simulates the exchange of PCE between the gaseous and liquid phases of the bottle.

$\text{Day_4_Purge_PCE_g} =$

$\text{PULSE}((\text{Mg_PCE}), \text{Waste_Pulse_Time}, \text{Waste_Increment_Time})$

DOCUMENT: The pulse output simulates the purge of PCE that remained undechlorinated from the bottle every 4 days (96 hr).

$\text{Mw_PCE}(t) = \text{Mw_PCE}(t - dt) + (\text{PCE_Feeding} + \text{Volat_and_Dissol_PCE} - \text{Day_4_Purge_PCE_w} - \text{PCE_Dechlorination_to_TCE}) * dt$

INIT Mw_PCE = 0 (μmol)

DOCUMENT: Reservoir representing PCE in the liquid phase (μmol).

INFLOWS:

PCE_Feeding = PULSE(Pulse_Value_PCE,
Feed_Pulse_Time_PCE,Feed_Increment_Time)

DOCUMENT: 11 μmol PCE is pulse fed every 48 hr beginning at time = 0 hr.

Volat_and_Dissol_PCE =
Inflow_Zero_PCE*Vw*Kla_PCE*((Cg_PCE/Hc_PCE)-Cw_PCE)

DOCUMENT: This biflow simulates the exchange of PCE between the gaseous and liquid phases of the bottle.

OUTFLOWS:

Day_4_Purge_PCE_w =
PULSE((Mw_PCE),Waste_Pulse_Time,Waste_Increment_Time)

DOCUMENT: The pulse output simulates the purge of PCE that remained undechlorinated from the bottle every 4 days (96 hr).

PCE_Dechlorination_to_TCE = Inflow_Zero_TCE*
((k_PCE*X_Mt_Dechlorinators*Cw_PCE)/(Ks_PCE+Cw_PCE))*
((Cw_H2-H2_Threshold_dechlor)/(Ks_H2_Dechlor+
(Cw_H2-H2_Threshold_dechlor))) { μmol PCE converted to TCE/hr}

TCE

Mg_TCE(t) = Mg_TCE(t - dt) + (- Volat_and_Dissol_TCE -
Day_4_Purge_TCE_g) * dt
INIT Mg_TCE = 0 { μmol }

DOCUMENT: Reservoir representing TCE in the gaseous phase { μmol }.

OUTFLOWS:

Volat_and_Dissol_TCE = Inflow_Zero_TCE*Vw*Kla_TCE*
((Cg_TCE/Hc_TCE)-Cw_TCE)

DOCUMENT: This biflow simulates the exchange of TCE between the gaseous and liquid phases of the bottle.

Day_4_Purge_TCE_g =
PULSE((Mg_TCE),Waste_Pulse_Time,Waste_Increment_Time)

DOCUMENT: The pulse output simulates the purge of accumulated TCE from the bottle every 4 days (96 hr).

$Mw_TCE(t) = Mw_TCE(t - dt) + (Volat_and_Dissol_TCE +$
 $PCE_Dechlorination_to_TCE - Day_4_Purge_TCE_w -$
 $TCE_Dechlorination_to_DCE) * dt$

INIT Mw_TCE = 0 {μmol}

DOCUMENT: Reservoir representing TCE in the liquid phase {μmol}.

INFLOWS:

Volat_and_Dissol_TCE =

Inflow_Zero_TCE*Vw*Kla_TCE*((Cg_TCE/Hc_TCE)-Cw_TCE)

DOCUMENT: This biflow simulates the exchange of TCE between the gaseous and liquid phases of the bottle.

$PCE_Dechlorination_to_TCE = Inflow_Zero_TCE*$
 $((k_PCE*X_Mt_Dechlorinators*Cw_PCE)/(Ks_PCE+Cw_PCE))*$
 $((Cw_H2-H2_Threshold_dechlor)/(Ks_H2_Dechlor+$
 $(Cw_H2-H2_Threshold_dechlor)))$ {μmol PCE converted to TCE/hr}

OUTFLOWS:

Day_4_Purge_TCE_w =

PULSE((Mw_TCE),Waste_Pulse_Time,Waste_Increment_Time)

DOCUMENT: The pulse output simulates the purge of accumulated TCE from the bottle every 4 days (96 hr).

$TCE_Dechlorination_to_DCE = Inflow_Zero_DCE*$
 $((k_TCE*X_Mt_Dechlorinators*Cw_TCE)/(Ks_TCE+Cw_TCE))*$
 $((Cw_H2-H2_Threshold_dechlor)/(Ks_H2_Dechlor+$
 $(Cw_H2-H2_Threshold_dechlor)))$ {μmol TCE converted to DCE/hr}

DCE

$Mg_DCE(t) = Mg_DCE(t - dt) + (- Volat_and_Dissol_DCE -$
 $Day_4_Purge_DCE_g) * dt$

INIT Mg_DCE = 0 {μmol}

DOCUMENT: Reservoir representing DCE in the gaseous phase {μmol}.

OUTFLOWS:

Volat_and_Dissol_DCE =

Inflow_Zero_DCE*Vw*Kla_DCE*((Cg_DCE/Hc_DCE)-Cw_DCE)

DOCUMENT: This biflow simulates the exchange of DCE between the gaseous and liquid phases of the bottle.

Day_4_Purge_DCE_g =
PULSE((Mg_DCE),Waste_Pulse_Time,Waste_Increment_Time)

Mw_DCE(t) = Mw_DCE(t - dt) + (Volat_and_Dissol_DCE +
TCE_Dechlorination_to_DCE - Day_4_Purge_DCE_w -
DCE_Dechlorination_to_VC) * dt
INIT Mw_DCE = 0 {μmol}

DOCUMENT: Reservoir representing DCE in the liquid phase {μmol}.

INFLOWS:

Volat_and_Dissol_DCE =
Inflow_Zero_DCE*Vw*Kla_DCE*((Cg_DCE/Hc_DCE)-Cw_DCE)
DOCUMENT: This biflow simulates the exchange of DCE between the gaseous
and liquid phases of the bottle.

TCE_Dechlorination_to_DCE = Inflow_Zero_DCE*
((k_TCE*X_Mt_Dechlorinators*Cw_TCE)/(Ks_TCE+Cw_TCE))*
((Cw_H2-H2_Threshold_dechlor)/(Ks_H2_Dechlor+
(Cw_H2-H2_Threshold_dechlor))) {μmol TCE converted to DCE/hr}

OUTFLOWS:

Day_4_Purge_DCE_w =
PULSE((Mw_DCE),Waste_Pulse_Time,Waste_Increment_Time)
DOCUMENT: The pulse output simulates the purge of accumulated DCE from
the bottle every 4 days (96 hr).

DCE_Dechlorination_to_VC = Inflow_Zero_VC*
((k_DCE*X_Mt_Dechlorinators*Cw_DCE)/(Ks_DCE+Cw_DCE))*
((Cw_H2-H2_Threshold_dechlor)/(Ks_H2_Dechlor+
(Cw_H2-H2_Threshold_dechlor))) {μmol DCE converted to VC/hr}

VC

Mg_VC(t) = Mg_VC(t - dt) + (- Volat_and_Dissol_VC -
Day_4_Purge_VC_g) * dt
INIT Mg_VC = 0 {μmol}
DOCUMENT: Reservoir representing VC in the gaseous phase {μmol}.

OUTFLOWS:

Volat_and_Dissol_VC =

Inflow_Zero_VC*Vw*Kla_VC*((Cg_VC/Hc_VC)-Cw_VC) {μmol/hr}

DOCUMENT: This biflow simulates the exchange of VC between the gaseous and liquid phases of the bottle.

Day_4_Purge_VC_g =

PULSE((Mg_VC),Waste_Pulse_Time,Waste_Increment_Time)

DOCUMENT: The pulse output simulates the purge of accumulated VC from the bottle every 4 days (96 hr).

Mw_VC(t) = Mw_VC(t - dt) + (Volat_and_Dissol_VC +
DCE_Dechlorination_to_VC - VC_Dechlorination_to_ETH -
Day_4_Purge_VC_w) * dt

INIT Mw_VC = 0 {μmol}

DOCUMENT: Reservoir representing VC in the liquid phase {μmol}.

INFLOWS:

Volat_and_Dissol_VC =

Inflow_Zero_VC*Vw*Kla_VC*((Cg_VC/Hc_VC)-Cw_VC) {μmol/hr}

DOCUMENT: This biflow simulates the exchange of VC between the gaseous and liquid phases of the bottle.

DCE_Dechlorination_to_VC = Inflow_Zero_VC*
((k_DCE*X_Mt_Dechlorinators*Cw_DCE)/(Ks_DCE+Cw_DCE))*
((Cw_H2-H2_Threshold_dechlor)/(Ks_H2_Dechlor+
(Cw_H2-H2_Threshold_dechlor))) {μmol DCE converted to VC/hr}

OUTFLOWS:

VC_Dechlorination_to_ETH = Inflow_Zero_ETH*
((k_VC*X_Mt_Dechlorinators*Cw_VC)/(Ks_VC+Cw_VC))*
((Cw_H2-H2_Threshold_dechlor)/(Ks_H2_Dechlor+
(Cw_H2-H2_Threshold_dechlor))) {μmol VC converted to ETH/hr}

Day_4_Purge_VC_w =

PULSE((Mw_VC),Waste_Pulse_Time,Waste_Increment_Time)

DOCUMENT: The pulse output simulates the purge of accumulated VC from the bottle every 4 days (96 hr).

ETH

$Mg_ETH(t) = Mg_ETH(t - dt) + (- Volat_and_Dissol_ETH -$
 $Day_4_Purge_ETH_g) * dt$

INIT $Mg_ETH = 0 \text{ } \{\mu\text{mol}\}$

DOCUMENT: Reservoir representing ETH in the gaseous phase $\{\mu\text{mol}\}$.

OUTFLOWS:

$Volat_and_Dissol_ETH = Inflow_Zero_ETH * Vw * K_{la_ETH} * ((Cg_ETH/Hc_ETH) - Cw_ETH) \text{ } \{\mu\text{mol/hr}\}$

DOCUMENT: This biflow simulates the exchange of ETH between the gaseous and liquid phases of the bottle.

$Day_4_Purge_ETH_g =$

$PULSE((Mg_ETH), Waste_Pulse_Time, Waste_Increment_Time)$

DOCUMENT: The pulse output simulates the purge of accumulated ETH from the bottle every 4 days (96 hr).

$Mw_ETH(t) = Mw_ETH(t - dt) + (VC_Dechlorination_to_ETH +$
 $Volat_and_Dissol_ETH - Day_4_Purge_ETH_w) * dt$

INIT $Mw_ETH = 0 \text{ } \{\mu\text{mol}\}$

DOCUMENT: Reservoir representing ETH in the liquid phase $\{\mu\text{mol}\}$.

INFLOWS:

$VC_Dechlorination_to_ETH = Inflow_Zero_ETH * ((k_VC * X_Mt_Dechlorinators * Cw_VC) / (Ks_VC + Cw_VC)) * ((Cw_H2 - H2_Threshold_dechlor) / (Ks_H2_Dechlor + (Cw_H2 - H2_Threshold_dechlor))) \text{ } \{\mu\text{mol VC converted to ETH/hr}\}$

$Volat_and_Dissol_ETH = Inflow_Zero_ETH * Vw * K_{la_ETH} * ((Cg_ETH/Hc_ETH) - Cw_ETH) \text{ } \{\mu\text{mol/hr}\}$

DOCUMENT: This biflow simulates the exchange of ETH between the gaseous and liquid phases of the bottle.

OUTFLOWS:

$Day_4_Purge_ETH_w =$

$PULSE((Mw_ETH), Waste_Pulse_Time, Waste_Increment_Time)$

DOCUMENT: The pulse output simulates the purge of accumulated ETH from the bottle every 4 days (96 hr).

Actual_Total_Mass_DCE = Mg_DCE+Mw_DCE { μmol }

DOCUMENT: Mt DCE

Actual_Total_Mass_ETH = Mg_ETH+Mw_ETH { μmol }

DOCUMENT: Mt ETH

Actual_Total_Mass_PCE = Mg_PCE+Mw_PCE { μmol }

DOCUMENT: Mt PCE

Actual_Total_Mass_TCE = Mg_TCE+Mw_TCE { μmol }

DOCUMENT: Mt TCE

Actual_Total_Mass_VC = Mg_VC+Mw_VC { μmol }

DOCUMENT: Mt VC

Cg_DCE = Mg_DCE/Vg { $\mu\text{mol/L}$ }

Cg_ETH = Mg_ETH/Vg { $\mu\text{mol/L}$ }

Cg_PCE = Mg_PCE/Vg { $\mu\text{mol/L}$ }

Cg_TCE = Mg_TCE/Vg { $\mu\text{mol/L}$ }

Cg_VC = Mg_VC/Vg { $\mu\text{mol/L}$ }

Cw_DCE = Mw_DCE/Vw { $\mu\text{mol/L}$ }

Cw_ETH = Mw_ETH/Vw { $\mu\text{mol/L}$ }

Cw_PCE = Mw_PCE/Vw { $\mu\text{mol/L}$ }

Cw_TCE = Mw_TCE/Vw { $\mu\text{mol/L}$ }

Cw_VC = Mw_VC/Vw { $\mu\text{mol/L}$ }

Inflow_Zero_DCE = IF(Day_4_Purge_DCE_g>0)THEN(0)ELSE(1)

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 0 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

Inflow_Zero_ETH = IF(Day_4_Purge_ETH_g>0)THEN(0)ELSE(1)

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 0 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

Inflow_Zero_PCE = IF(Day_4_Purge_PCE_g>0)THEN(0)ELSE(1)

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 0 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

Inflow_Zero_TCE = IF(Day_4_Purge_TCE_g>0)THEN(0)ELSE(1)

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 0 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

Inflow_Zero_VC = IF(Day_4_Purge_VC_g>0)THEN(0)ELSE(1)

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 0 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

Ks_DCE = 0.54

DOCUMENT: Use the same as for PCE.

Ks_H2_Dechlor = 0.1

DOCUMENT: Half-velocity coefficient for H2 for dechlorination, 0.1 $\mu\text{mol/L}$, Smatlak, 1995.

Ks_PCE = 0.54

DOCUMENT: Half-velocity coefficient for PCE dechlorination, 0.54 $\mu\text{mol/L}$, Smatlak, 1995; 0.6 $\mu\text{mol/L}$, Tandoi, 1994.

Ks_TCE = 0.54

DOCUMENT: Use the same as for PCE.

Ks_VC = 290

DOCUMENT: Half-velocity coefficient for VC, 290 $\mu\text{mol/L}$, Smatlak, 1995.

k_DCE = 3

DOCUMENT: Estimated from relative v_{max}/K_s in Tandoi et al., 1994 and the k/K_s for the pure culture for PCE.

k_PCE = 1.815

DOCUMENT: Rate of PCE dechlorination 1.815 $\mu\text{mol PCE to VC/mg VSS-hr}$ (pure culture, Zinder, 1997).

k_TCE = 3

DOCUMENT: Estimated from relative v_{max}/K_s in Tandoi et al., 1994 and the k/K_s for the pure culture for PCE.

k_VC = 3

DOCUMENT: Estimated from relative v_{max}/K_s in Tandoi et al., 1994 and the k/K_s for the pure culture for PCE.

Mass_if_HS_Meas_DCE = $C_{\text{g_DCE}} \cdot (V_{\text{g}} + (V_{\text{w}}/H_{\text{c_DCE}}))$

DOCUMENT: Mt for DCE if determined from a direct measurement of C_{g} .

Mass_if_HS_Meas_ETH = $C_{\text{g_ETH}} \cdot (V_{\text{g}} + (V_{\text{w}}/H_{\text{c_ETH}})) \{\mu\text{mol}\}$

DOCUMENT: Mt for ETH if determined from a direct measurement of C_{g} .

Mass_if_HS_Meas_PCE = $C_{\text{g_PCE}} \cdot (V_{\text{g}} + (V_{\text{w}}/H_{\text{c_PCE}}))$

DOCUMENT: Mt for PCE if determined from a direct measurement of C_{g} .

Mass_if_HS_Meas_TCE = $C_{\text{g_TCE}} \cdot (V_{\text{g}} + (V_{\text{w}}/H_{\text{c_TCE}}))$

DOCUMENT: Mt for TCE if determined from a direct measurement of C_{g} .

Mass_if_HS_Meas_VC = $Cg_VC \cdot (Vg + (Vw/Hc_VC))$ { μ mol}

DOCUMENT: Mt for VC if determined from a direct measurement of Cg.

Pulse_Value_PCE = 11

DOCUMENT: The amount of PCE pulse fed at a feeding event (μ mol) beginning at 0 hr and occurring every 48 hr thereafter.

DECHLORINATOR BIOMASS

$X_Dechlorinators(t) = X_Dechlorinators(t - dt) +$
 $(Biomass_Growth_Dechlorinators - Biomass_Decay_Dechlorinators -$
 $Day_4_Waste_X_Dechlorinators) \cdot dt$

INIT $X_Dechlorinators = Initial_X_Dechlorinators$ {mg VSS}

DOCUMENT: Reservoir representing dechlorinator biomass {mg VSS}.

INFLOWS:

$Biomass_Growth_Dechlorinators = Y_Dechlorinators \cdot$
 $(PCE_Dechlorination_to_TCE \cdot H2_per_PCE_Dechlorinated +$
 $TCE_Dechlorination_to_DCE \cdot H2_per_TCE_Dechlorinated +$
 $DCE_Dechlorination_to_VC \cdot H2_per_DCE_Dechlorinated)$ {mg
VSS/hr}

OUTFLOWS:

$Biomass_Decay_Dechlorinators =$
 $Decay_Dechlorinators \cdot X_Dechlorinators$ {mg VSS/hr}

$Day_4_Waste_X_Dechlorinators = PULSE(X_Dechlorinators \cdot$
 $Liquid_Waste_Rate, Waste_Pulse_Time, Waste_Increment_Time)$
{mg VSS wasted every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

$Decay_Dechlorinators = 0.001$ {/hr}

DOCUMENT: 0.001/hr. Generic number.

$Initial_X_Dechlorinators = 0$ {mg VSS}

DOCUMENT: Initial amount of dechlorinator biomass present (mg VSS).

$X_Cw_Dechlorinators = X_Dechlorinators/Vw$ {mg VSS/L}

DOCUMENT: Converter to allow reporting of dechlorinator biomass as a concentration {mg VSS/L}.

$$X_{Mt_Dechlorinators} = X_{Dechlorinators} \text{ {mg VSS}}$$

$$Y_{Dechlorinators} = 0.00612$$

DOCUMENT: Yield for dechlorinators. Zinder, 1997.

DONOR FERMENTER BIOMASS

BUTYRATE FERMENTERS

$$X_{Butyrate_Fermenters}(t) = X_{Butyrate_Fermenters}(t - dt) + \\ (\text{Biomass_Growth_Butyrate_Fermenters} - \\ \text{Biomass_Decay_Butyrate_Fermenters} - \\ \text{Day_4_Waste_X_Butyrate_Fermenters}) * dt$$

$$\text{INIT } X_{Butyrate_Fermenters} = \text{Initial_X_Butyrate_Fermenters} \text{ {mg VSS}}$$

DOCUMENT: Reservoir representing butyrate fermenter biomass {mg VSS}.

INFLOWS:

$$\text{Biomass_Growth_Butyrate_Fermenters} =$$

$$Y_{Butyrate_Fermenters} * \text{Butyrate_Fermented_to_Acetate} \text{ {mg VSS/hr}}$$

OUTFLOWS:

$$\text{Biomass_Decay_Butyrate_Fermenters} =$$

$$X_{Butyrate_Fermenters} * \text{Decay_Butyrate_Fermenters} \text{ {mg VSS/hr}}$$

$$\text{Day_4_Waste_X_Butyrate_Fermenters} = \text{PULSE}$$

$$(X_{Butyrate_Fermenters} * \text{Liquid_Waste_Rate}, \text{Waste_Pulse_Time}, \\ \text{Waste_Increment_Time}) \text{ {mg VSS wasted every 96 hr}}$$

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

ETHANOL FERMENTERS (to acetate)

$$X_{Ethanol_to_Acetate_Fermenters}(t) =$$

$$X_{Ethanol_to_Acetate_Fermenters}(t - dt) +$$

$$(\text{Biomass_Growth_Ethanol_to_Acetate_Fermenters} -$$

$$\text{Biomass_Decay_Ethanol_to_Acetate_Fermenters} -$$

$$\text{Day_4_Waste_X_Ethanol_to_Acetate_Fermenters}) * dt$$

INIT X_Ethanol_to_Acetate_Fermenters =

Initial_X_Ethanol_to_Acetate_Fermenters {mg VSS}

DOCUMENT: Reservoir representing ethanol to acetate fermenter biomass {mg VSS}.

INFLOWS:

Biomass_Growth_Ethanol_to_Acetate_Fermenters =

Y_Ethanol_to_Acetate_Fermenters*Ethanol_Fermented_to_Acetate
{mg VSS/hr}

OUTFLOWS:

Biomass_Decay_Ethanol_to_Acetate_Fermenters =

X_Ethanol_to_Acetate_Fermenters*

Decay_Ethanol_to_Acetate_Fermenters {mg VSS/hr}

Day_4_Waste_X_Ethanol_to_Acetate_Fermenters =

PULSE(X_Ethanol_to_Acetate_Fermenters*Liquid_Waste_Rate,
Waste_Pulse_Time,Waste_Increment_Time)
{mg VSS wasted every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

ETHANOL FERMENTERS (to propionate)

X_Ethanol_to_Propionate_Fermenters(t) =

X_Ethanol_to_Propionate_Fermenters(t - dt) +

(Biomass_Growth_Ethanol_to_Propionate_Fermenters -

Biomass_Decay_Ethanol_to_Propionate_Fermenters -

Day_4_Waste_X_Ethanol_to_Propionate_Fermenters) * dt

INIT X_Ethanol_to_Propionate_Fermenters =

Initial_X_Ethanol_to_Propionate_Fermenters {mg VSS}

DOCUMENT: Reservoir representing ethanol to propionate fermenter biomass {mg VSS}.

INFLOWS:

Biomass_Growth_Ethanol_to_Propionate_Fermenters =

Y_Ethanol_to_Propionate_Fermenters*

Ethanol_Fermented_to_Propionate {mg VSS/hr}

OUTFLOWS:

Biomass_Decay_Ethanol_to_Propionate_Fermenters =
 X_Ethanol_to_Propionate_Fermenters*
 Decay_Ethanol_to_Propionate_Fermenters {mg VSS/hr}

Day_4_Waste_X_Ethanol_to_Propionate_Fermenters =
 PULSE(X_Ethanol_to_Propionate_Fermenters*Liquid_Waste_Rate,
 Waste_Pulse_Time,Waste_Increment_Time) {mg VSS wasted per 96 hr}
 DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and
 replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This
 decreases the amount of soluble constituents by 10 percent. Volatile constituents
 are not affected since they are purged out after each 96 hr period.

LACTATE FERMENTERS (to acetate)

X_Lactate_to_Acetate_Fermenters(t) =
 X_Lactate_to_Acetate_Fermenters(t - dt) +
 (Biomass_Growth_Lactate_to_Acetate_Fermenters -
 Biomass_Decay_Lactate_to_Acetate_Fermenters -
 Day_4_Waste_X_Lactate_to_Acetate) * dt
 INIT X_Lactate_to_Acetate_Fermenters =
 Initial_X_Lactate_to_Acetate_Fermenters
 DOCUMENT: Reservoir representing lactate to acetate fermenter biomass {mg VSS}.

INFLOWS:

Biomass_Growth_Lactate_to_Acetate_Fermenters =
 Y_Lactate_to_Acetate_Fermenters*Lactate_Fermented_to_Acetate
 {mg VSS/hr}

OUTFLOWS:

Biomass_Decay_Lactate_to_Acetate_Fermenters =
 X_Lactate_to_Acetate_Fermenters*
 Decay_Lactate_to_Acetate_Fermenters {mg VSS/hr}

Day_4_Waste_X_Lactate_to_Acetate =
 PULSE(X_Lactate_to_Acetate_Fermenters*Liquid_Waste_Rate,
 Waste_Pulse_Time,Waste_Increment_Time)
 {mg VSS wasted every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and
 replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This

decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

LACTATE FERMENTERS (to acetate)

$X_{\text{Lactate_to_Propionate_Fermenters}}(t) =$
 $X_{\text{Lactate_to_Propionate_Fermenters}}(t - dt) +$
 $(\text{Biomass_Growth_Lactate_to_Propionate_Fermenters} -$
 $\text{Biomass_Decay_Lactate_to_Propionate_Fermenters} -$
 $\text{Day_4_Waste_X_Lactate_to_Propionate_Fermenters}) * dt$
 $\text{INIT } X_{\text{Lactate_to_Propionate_Fermenters}} =$
 $\text{Initial_X_Lactate_to_Propionate_Fermenters} \text{ \{mg VSS\}}$
 DOCUMENT: Reservoir representing lactate to propionate fermenter biomass {mg VSS}.

INFLOWS:

$\text{Biomass_Growth_Lactate_to_Propionate_Fermenters} =$
 $Y_{\text{Lactate_to_Propionate_Fermenters}} * \text{Lactate_Fermented_to_Propionate}$ {mg VSS/hr}

OUTFLOWS:

$\text{Biomass_Decay_Lactate_to_Propionate_Fermenters} =$
 $X_{\text{Lactate_to_Propionate_Fermenters}} * \text{Decay_Lactate_to_Propionate_Fermenters}$ {mg VSS/hr}

$\text{Day_4_Waste_X_Lactate_to_Propionate_Fermenters} =$
 $\text{PULSE}(X_{\text{Lactate_to_Propionate_Fermenters}} * \text{Liquid_Waste_Rate}, \text{Waste_Pulse_Time}, \text{Waste_Increment_Time})$ {mg VSS wasted every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

PROPIONATE FERMENTERS

$X_{\text{Propionate_Fermenters}}(t) = X_{\text{Propionate_Fermenters}}(t - dt) +$
 $(\text{Biomass_Growth_Propionate_Fermenters} -$
 $\text{Biomass_Decay_Propionate_Fermenters} -$
 $\text{Day_4_Waste_X_Propionate_Fermenters}) * dt$
 $\text{INIT } X_{\text{Propionate_Fermenters}} = \text{Initial_X_Propionate_Fermenters}$
 {mg VSS}

DOCUMENT: Reservoir representing propionate fermenter biomass {mg VSS}.

INFLOWS:

Biomass_Growth_Propionate_Fermenters =
 $Y_{\text{Propionate_Fermenters}} \times \text{Propionate_Fermented_to_Acetate}$
 {mg VSS/hr}

OUTFLOWS:

Biomass_Decay_Propionate_Fermenters =
 $\text{Decay_Propionate_Fermenters} \times X_{\text{Propionate_Fermenters}}$
 {mg VSS/hr}

Day_4_Waste_X_Propionate_Fermenters =
 $\text{PULSE}(X_{\text{Propionate_Fermenters}} \times \text{Liquid_Waste_Rate}, \text{Waste_Pulse_Time}, \text{Waste_Increment_Time})$ {mg VSS wasted every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

Decay_Butyrate_Fermenters = 0.001

DOCUMENT: 0.001/hr. Generic number.

Decay_Ethanol_to_Acetate_Fermenters = 0.001

DOCUMENT: 0.001/hr. Generic number.

Decay_Ethanol_to_Propionate_Fermenters = 0.001

DOCUMENT: 0.001/hr. Generic number.

Decay_Lactate_to_Acetate_Fermenters = 0.001

DOCUMENT: 0.001/hr. Generic number.

Decay_Lactate_to_Propionate_Fermenters = 0.001

DOCUMENT: 0.001/hr. Generic number.

Decay_Propionate_Fermenters = 0.001

DOCUMENT: 0.001/hr. Generic number.

Initial_X_Butyrate_Fermenters = 0

DOCUMENT: Initial amount of butyrate fermenter biomass present (mg VSS).

Initial_X_Ethanol_to_Acetate_Fermenters = 0

DOCUMENT: Initial amount of ethanol to acetate fermenter biomass present (mg VSS).

Initial_X_Ethanol_to_Propionate_Fermenters = 0

DOCUMENT: Initial amount of ethanol to propionate fermenter biomass present (mg VSS).

$\text{Initial_X_Lactate_to_Acetate_Fermenters} = 0$

DOCUMENT: Initial amount of lactate to acetate fermenter biomass present (mg VSS).

$\text{Initial_X_Lactate_to_Propionate_Fermenters} = 0$

DOCUMENT: Initial amount of lactate to propionate fermenter biomass present (mg VSS).

$\text{Initial_X_Propionate_Fermenters} = 0$

DOCUMENT: Initial amount of propionate fermenter biomass present (mg VSS).

$\text{X_Cw_Butyrate_Fermenters} = \text{X_Butyrate_Fermenters}/V_w \quad \{\text{mg VSS/L}\}$

DOCUMENT: Converter to allow reporting of butyrate fermenter biomass as a concentration {mg VSS/L}.

$\text{X_Cw_Ethanol_to_Acetate_Fermenters} =$

$\text{X_Ethanol_to_Acetate_Fermenters}/V_w \quad \{\text{mg VSS/L}\}$

DOCUMENT: Converter to allow reporting of ethanol to acetate fermenter biomass as a concentration {mg VSS/L}.

$\text{X_Cw_Ethanol_to_Propionate_Fermenters} =$

$\text{X_Ethanol_to_Propionate_Fermenters}/V_w \quad \{\text{mg VSS/L}\}$

DOCUMENT: Converter to allow reporting of ethanol to propionate fermenter biomass as a concentration {mg VSS/L}.

$\text{X_Cw_Lactate_to_Acetate_Fermenters} =$

$\text{X_Lactate_to_Acetate_Fermenters}/V_w \quad \{\text{mg VSS/L}\}$

DOCUMENT: Converter to allow reporting of lactate to acetate fermenter biomass as a concentration {mg VSS/L}.

$\text{X_Cw_Lactate_to_Propionate_Fermenters} =$

$\text{X_Lactate_to_Propionate_Fermenters}/V_w \quad \{\text{mg VSS/L}\}$

DOCUMENT: Converter to allow reporting of lactate to propionate fermenter biomass as a concentration {mg VSS/L}.

$\text{X_Cw_Propionate_Fermenters} = \text{X_Propionate_Fermenters}/V_w \quad \{\text{mg VSS/L}\}$

DOCUMENT: Converter to allow reporting of propionate fermenter biomass as a concentration {mg VSS/L}.

$\text{X_Mt_Butyrate_Fermenters} = \text{X_Butyrate_Fermenters} \quad \{\text{mg VSS}\}$

$\text{X_Mt_Ethanol_to_Acetate_Fermenters} =$

$\text{X_Ethanol_to_Acetate_Fermenters} \quad \{\text{mg VSS}\}$

$X_{Mt_Ethanol_to_Propionate_Fermenters} =$
 $X_{Ethanol_to_Propionate_Fermenters} \{mg \text{ VSS}\}$
 $X_{Mt_Lactate_to_Acetate_Fermenters} =$
 $X_{Lactate_to_Acetate_Fermenters} \{mg \text{ VSS}\}$
 $X_{Mt_Lactate_to_Propionate_Fermenters} =$
 $X_{Lactate_to_Propionate_Fermenters} \{mg \text{ VSS}\}$
 $X_{Mt_Propionate_Fermenters} = X_{Propionate_Fermenters} \{mg \text{ VSS}\}$

$Y_{Butyrate_Fermenters} = 0.00279$

DOCUMENT: Yield for butyrate fermenters (0.00279 mg VSS/ μ mol butyrate) Ahring and Westermann, 1987.

$Y_{Ethanol_to_Acetate_Fermenters} = 0.00198$

DOCUMENT: Yield for ethanol fermenters (0.00198 mg VSS/ μ mol ethanol) Seitz et al., 1990.

$Y_{Ethanol_to_Propionate_Fermenters} = 0.00297$

DOCUMENT: Yield for ethanol fermenters that take ethanol to propionate (0.00297 mg VSS/ μ mol EtOH to Prop) Tholozan, 1992.

$Y_{Lactate_to_Acetate_Fermenters} = 0.00351$

DOCUMENT: Yield for lactate fermentation (0.00351 mg VSS/ μ mol lactate) Wallrabenstein, 1995.

$Y_{Lactate_to_Propionate_Fermenters} = 0.00563$

DOCUMENT: Yield for lactate fermentation to propionate (0.00563 mg VSS/ μ mol Lac to Prop) Schink, 1984.

$Y_{Propionate_Fermenters} = 0.00144$

DOCUMENT: Yield for propionate fermentation (0.00144 mg VSS/ μ mol propionate) Wallrabenstein et al., 1995.

ETHANOL

$Mt_Ethanol(t) = Mt_Ethanol(t - dt) + (Ethanol_Feeding -$
 $Day_4_Waste_Ethanol - Ethanol_Fermented_to_Acetate -$
 $Ethanol_Fermented_to_Propionate) * dt$

INIT $Mt_Ethanol = Initial_Ethanol \{ \mu mol \}$

DOCUMENT: Reservoir representing ethanol $\{ \mu mol \}$.

INFLOWS:

$Ethanol_Feeding =$

$PULSE(Pulse_Value_Ethanol, Feed_Pulse_Time_Donor,$
 $Feed_Increment_Time)$

DOCUMENT: Pulse input of ethanol beginning at time = 0 hr and occurring every 48 hr thereafter.

OUTFLOWS:

Day_4_Waste_Ethanol =

PULSE(Mt_Ethanol*Liquid_Waste_Rate,Waste_Pulse_Time,
Waste_Increment_Time) { μmol wasted every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged. Units are μmol every 96 hr.

Ethanol_Fermented_to_Acetate =

$(k_{\text{Ethanol_to_Acetate}} \times X_{\text{Mt_Ethanol_to_Acetate_Fermenters}} \times C_{\text{w_Ethanol}} \times \text{Thermo_Factor_Ethanol_to_Acetate}) / (K_{\text{s_Ethanol_to_Acetate}} + C_{\text{w_Ethanol}})$

DOCUMENT: This flow simulates the fermentation of ethanol to acetate and hydrogen ($\mu\text{mol/hr}$).

Ethanol_Fermented_to_Propionate = $(k_{\text{Ethanol_to_Propionate}} \times X_{\text{Mt_Ethanol_to_Propionate_Fermenters}} \times C_{\text{w_Ethanol}} \times \text{Thermo_Factor_Ethanol_to_Propionate}) / (K_{\text{s_Ethanol_to_Propionate}} + C_{\text{w_Ethanol}})$

DOCUMENT: This flow simulates the fermentation of ethanol to propionate ($\mu\text{mol/hr}$).

$C_{\text{w_Ethanol}} = \text{Mt_Ethanol} / V_{\text{w}}$ { $\mu\text{mol/L}$ }

DOCUMENT: Converter to allow reporting of ethanol as a concentration ($\mu\text{mol/L}$).

Initial_Ethanol = 1E-20

DOCUMENT: The initial amount of ethanol present (μmol).

$K_{\text{s_Ethanol_to_Acetate}} = 16.95$

DOCUMENT: Half-velocity coefficient for ethanol fermentation. 16.95 $\mu\text{mol/L}$, Fennell, est., 1996.

$K_{\text{s_Ethanol_to_Propionate}} = 16.95$

DOCUMENT: Same as for ethanol to acetate.

$$k_{\text{Ethanol_to_Acetate}} = 21.92$$

DOCUMENT: Apparent rate of ethanol degradation was 4.044 { $\mu\text{mol ethanol/mg VSS-hr}$, est., Fennell}.

This occurred under a thermodynamic ceiling (ave) of -23 kJ/mol ethanol fermented (on average) for the 1:1 TISs and -35 kJ/mol for the 2:1 TISs. Delta G critical was set at -19 kJ/mol and the thermo factor was calculated for each condition. There is less ethanol-degrader biomass than the total VSS measured. From my biomass estimates the ethanol degraders make up 16 % for 1:1 TISs and 20 % for 2:1 TISs, of the total biomass in the bottle.

The rate that would be observed in the absence of a thermodynamic limit and accounting for the fraction of relevant biomass is:

$$\text{rate} = \text{apparent rate} / (\text{thermo factor} * \text{fraction of relevant VSS}).$$

$$\text{AVE } k = 21.92 \mu\text{mol/mg ethanol VSS-hr}$$

$$k_{\text{Ethanol_to_Propionate}} = 21.92$$

DOCUMENT: Same as for ethanol to acetate.

$$\text{Pulse_Value_Ethanol} = 0$$

DOCUMENT: This is the amount of ethanol fed { μmol } at each pulse beginning at 0 hr and occurring every 48 hr.

FERMENTED YEAST EXTRACT

$$\text{FYE_Acetate} = \text{FYE_Addition_}\mu\text{L} * \text{FYE_HAc_}\mu\text{mol_per_}\mu\text{L} \\ \{\mu\text{mol Acetate added by FYE}\}$$

$$\text{FYE_Addition_}\mu\text{L} = 0 \{\mu\text{L}\}$$

DOCUMENT: Designates the volumetric FYE solution addition (μL). Typically, the following volumetric additions were added:

donor:PCE ratio ($\mu\text{eq}/\mu\text{eq}$)	FYE Addition
1:1	20 μL
2:1	40 μL

Typical FYE acetate, propionate, butyrate, and reducing equivalents concentrations are entered in the model. These can be changed if other measurements become available.

FYE_Butyrate =

FYE_Addition_μL*(FYE_HBu_μmol_per_μL+(FYE_μeq_per_μL-
Butyrate_μeq_per_μmol*FYE_HBu_μmol_per_μL-
Propionate_μeq_per_μmol*FYE_Prop_μmol_per_μL)*
1/Butyrate_μeq_per_μmol) {μmol butyric acid added from FYE}

DOCUMENT: A measurable amount butyric acid is added by FYE. In addition, this model represents all unaccounted for reducing equivalents added by FYE (excluding the reducing equivalents that are added as measurable butyric acid or propionic acid) as butyric acid. In this way, butyric acid represents a pool of slowly released reducing equivalents donated by FYE that we are unable to completely quantify. Some of this is higher fatty acids, some is probably carbohydrate and protein.

FYE_Propionate = FYE_Addition_μL*FYE_Prop_μmol_per_μL
{μmol Propionate added by FYE}

GLOBAL INPUTS

Butyrate_μeq_per_μmol = 4 {μeq/μmol HBu}

Cw_Bicarbonate = 0.0714 {mol/L}

DOCUMENT: Bicarbonate concentration in the basal salts medium {mol/L}.

delta_time = DT

DOCUMENT: Time Step {hr}.

Ethanol_μeq_per_μmol = 4 {μeq/μmol EtOH}

Feed_Increment_Time = 48 {hr}

DOCUMENT: This is the increment of time {hr} between feedings.

Feed_Pulse_Time_Donor = 0 {hr}

DOCUMENT: The pulse feed time is the time {hr} at which the first feed pulse occurs.

Feed_Pulse_Time_PCE = 0 {hr}

DOCUMENT: The time {hr} when the first pulse input of PCE occurs.

FYE_HAc_μmol_per_μL = 0.0685 {μmol Acetic acid/μL FYE added}

DOCUMENT: Concentration of acetic acid in the batch of FYE in use.

FYE_HBu_μmol_per_μL = 0.0665 {μmol Butyric acid/μL FYE added}

DOCUMENT: Concentration of butyric acid in the batch of FYE in use.

FYE_Prop_μmol_per_μL = 0.016 {μmol Propionic acid/μL FYE solution}

DOCUMENT: Concentration of propionic acid in the batch of FYE in use.

FYE_μeq_per_μL = 0.87 {μeq contributed/μL FYE solution}

DOCUMENT: The amount of reducing equivalents added by the batch of FYE in use.

$g_{CH4} = 10^{(Salt_Out_CH4 * Ionic_Strength)}$

DOCUMENT: Activity coefficient for the nonionic compound, CH₄.

$g_{H2} = 10^{(Salt_Out_H2 * Ionic_Strength)}$

DOCUMENT: Activity coefficient for the nonionic compound, H₂.

$g_{ionic_Z1} = 10^{(-(0.5 * Z^2 * Ionic_Strength^{0.5}) / (1 + Ionic_Strength^{0.5}))}$

DOCUMENT: Activity coefficient for ionic compounds with a charge of 1.

Calculated using the Guntelburg approximation.

H2_Threshold_dechlor = 0.0015 {μmol/L}

DOCUMENT: Estimate, Fennell, 1997 from FYE- or non-fed cultures.

H2_Threshold_meth = 0.008 {μmol/L}

DOCUMENT: Estimate, Fennell, 1997.

Hc_CH4 = 33.1

DOCUMENT: pseudo-dimensionless, DiStefano, 1992

Hc_DCE = 0.216

DOCUMENT: pseudo-dimensionless, for cis-1,2-DCE, Gossett, 1987

Hc_ETH = 9

DOCUMENT: pseudo-dimensionless, DiStefano, 1992

Hc_H2 = 52.7

DOCUMENT: Young, 1981

Hc_PCE = 1.116

DOCUMENT: pseudo-dimensionless, Gossett, 1987

Hc_TCE = 0.591

DOCUMENT: pseudo-dimensionless, Gossett, 1987

Hc_VC = 1.42

DOCUMENT: pseudo-dimensionless, Gossett, 1987

Ionic_Strength = 0.0856

DOCUMENT: Estimated for the basal salts medium {eq/L}.

$K_{la_CH4} = 50 \text{ \{ /hr \}}$

DOCUMENT: Smatlak, 1995

$K_{la_DCE} = 38.2 \text{ \{ /hr \}}$

DOCUMENT: Estimated from the molar volume and Equation 9-26 of Schwarzenbach et al., 1993, and the relationship developed by Smatlak, 1995.

$K_{la_ETH} = 60 \text{ \{ /hr \}}$

DOCUMENT: Smatlak, 1995

$K_{la_H2} = 69.3 \text{ \{ /hr \}}$

DOCUMENT: Smatlak, 1995

$K_{la_PCE} = 25 \text{ \{ /hr \}}$

DOCUMENT: Smatlak, 1995

$K_{la_TCE} = 36 \text{ \{ /hr \}}$

DOCUMENT: Estimated from the molar volume and Equation 9-26 of Schwarzenbach et al., 1993, and the relationship developed by Smatlak, 1995.

$K_{la_VC} = 40 \text{ \{ /hr \}}$

DOCUMENT: Smatlak, 1995

$Lactate_μeq_per_μmol = 4 \text{ \{ μeq/μmol Lac \}}$

$Liquid_Waste_Rate = 0.1$

DOCUMENT: One tenth of the liquid is wasted and replaced with fresh basal medium every fourth day.

$PCE_μeq_per_μmol = 8 \text{ \{ μeq/μmol PCE \}}$

$pH = 7.3$

DOCUMENT: Typical pH of the system.

$Propionate_μeq_per_μmol = 6 \text{ \{ μeq/μmol Prop \}}$

$R = 0.00831441 \text{ \{ kJ/mol-K \}}$

DOCUMENT: For thermodynamic calculations.

$R2 = 0.082054 \text{ \{ L-atm/mol-K \}}$

DOCUMENT: To convert C_g ($μmol/L$) to partial pressure (atm).

$Salt_Out_CH4 = 0.135 \text{ \{ L/mol \}}$

DOCUMENT: Salt effect parameter for CH_4 in aqueous NaCl solution from a review of various studies. In Solubility Data Series, Vol 27/28, Methane, C.L. Young, editor, 1987, Pergamon Press, page 70.

Salt_Out_H2 = 0.102 {L/mol}

DOCUMENT: Salt effect parameter for H2 in aqueous NaCl solution from a review of various studies. In Solubility Data Series, Vol 5/6, Hydrogen and Deuterium, C.L. Young, editor, 1981, Pergamon Press, page 32.

Temp = 308.15 {K}

DOCUMENT: Temperature, K

Vg = 0.06 {L}

DOCUMENT: Volume {L} of the gaseous headspace of the serum bottle

Vw = 0.1 {L}

DOCUMENT: Volume {L} of the aqueous contents of the serum bottle.

Waste_Increment_Time = 96 {hr}

DOCUMENT: This is the time {hr} that elapses between wasting events.

Waste_Pulse_Time = 96-(delta_time) {hr}

DOCUMENT: This is the initial time {hr} at which all waste pulses occur. The waste event occurs just prior to feeding, therefore, the event occurs at 96 hr-dt. Feeding occurs every 48 hr.

Z = 1

DOCUMENT: Charge on ionic species

HYDROGEN

$Mt_Hydrogen(t) = Mt_Hydrogen(t - dt) + (Hydrogen_Production + Hydrogen_Feeding - H2_For_Dechlorination - H2_For_Methanogenesis - Day_4_Purge_H2) * dt$

INIT Mt_Hydrogen = 1E-20 {μmol}

DOCUMENT: This reservoir represents all gaseous and aqueous hydrogen plus the aqueous formate that is in equilibrium with the aqueous hydrogen.

INFLOWS:

Hydrogen_Production = Inflow_Zero_Hydrogen*

(fe_Propionate*Propionate_Fermented_to_Acetate*

H2_per_Propionate__Fermented_to_Acetate+fe_Butyrate*

Butyrate_Fermented_to_Acetate*

H2_per_Butyrate_Fermented_to_Acetate+fe_Ethanol*

Ethanol_Fermented_to_Acetate*

H2_per_Ethanol_Fermented_to_Acetate+fe_Lactate*

Lactate_Fermented_to_Acetate*

H2_per_Lactate_Fermented_to_Acetate) { $\mu\text{mol/hr}$ }

DOCUMENT: Hydrogen production from all donors.

Hydrogen Produced = Sum of {donor fermentation flow * stoichiometric conversion (H2/Donor) * fe}

Where fe is the fraction of the donor that is used for energy.

Hydrogen_Feeding =

PULSE(Pulse_Value_Hydrogen,Feed_Pulse_Time_Donor,
Feed_Increment_Time)

DOCUMENT: Pulse input of hydrogen beginning at time = 0 hr and occurring every 48 hr thereafter.

OUTFLOWS:

H2_For_Dechlorination = (PCE_Dechlorination_to_TCE*
H2_per_PCE_Dechlorinated+TCE_Dechlorination_to_DCE*
H2_per_TCE_Dechlorinated+DCE_Dechlorination_to_VC*
H2_per_DCE_Dechlorinated+VC_Dechlorination_to_ETH*
H2_per_VC_Dechlorinated)/fe_H2_to_Dechlorination { $\mu\text{mol/hr}$ }

H2_For_Methanogenesis = (k_H2_methane*
X_Mt_Hydrogenotrophic_Methanogens*
(Cw_H2-H2_Threshold_meth))/(Ks_H2_methane+
(Cw_H2-H2_Threshold_meth)) { $\mu\text{mol/hr}$ }

Day_4_Purge_H2 = PULSE((Mt_Hydrogen),Waste_Pulse_Time,
Waste_Increment_Time) { μmol wasted every 96 hr}

DOCUMENT: The pulse output simulates the purge of hydrogen from the bottle every 4 days (96 hr-dt).

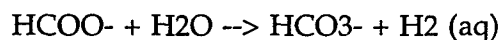
Cg_H2 =

Mt_Hydrogen/((Vw/Hc_H2)+Vg+(Formate_to_H2_Ratio*Vw/Hc_H2))
{ $\mu\text{mol/L}$ }

Cw_H2 = Mt_Hydrogen/(Vw+(Hc_H2*Vg)+(Formate_to_H2_Ratio*Vw))
{ $\mu\text{mol/L}$ }

delta_G_zero_formate_to_H2 = 19.03 {kJ/mol rxn}

DOCUMENT: delta G rxn at 35 C for



$$\text{fe_H}_2\text{_to_Dechlorination} = 0.9023$$

DOCUMENT: fe -- fraction of hydrogen used for energy

$$\text{Formate_to_H}_2\text{_Ratio} = \frac{\text{g_H}_2 \cdot \text{g_ionic_Z1} \cdot \text{Cw_Bicarbonate}}{(\text{g_ionic_Z1} \cdot \text{EXP}(-\Delta G_{\text{zero_formate_to_H}_2} / (R \cdot \text{Temp})))}$$

DOCUMENT: The steady-state ratio of aqueous formate to aqueous hydrogen ($\mu\text{mol}/\mu\text{mol}$).

$$\text{H}_2\text{_atm} = \text{Cg_H}_2 \cdot R^2 \cdot \text{Temp} / 1\text{E6} \text{ {atm}}$$

$$\text{H}_2\text{_per_DCE_Dechlorinated} = 1 \text{ {}\mu\text{mol Hydrogen/}\mu\text{mol DCE converted to VC}}$$

$$\text{H}_2\text{_per_PCE_Dechlorinated} = 1 \text{ {}\mu\text{mol Hydrogen/}\mu\text{mol PCE converted to TCE}}$$

$$\text{H}_2\text{_per_TCE_Dechlorinated} = 1 \text{ {}\mu\text{mol Hydrogen/}\mu\text{mol TCE converted to DCE}}$$

$$\text{H}_2\text{_per_VC_Dechlorinated} = 1 \text{ {}\mu\text{mol Hydrogen/}\mu\text{mol VC Dechlorinated to ETH}}$$

$$\text{Inflow_Zero_Hydrogen} = \text{IF}(\text{Day_4_Purge_H}_2 > 0) \text{ THEN } (0) \text{ ELSE } (1)$$

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 0 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

$$\text{Ks_H}_2\text{_methane} = 0.5$$

DOCUMENT: Half-velocity coefficient for hydrogen conversion to methane.

An average value of $0.96 \mu\text{mol}/\text{L}$ was reported by Smatlak, 1995; however, a slightly lower value was used for modeling.

$$\text{k_H}_2\text{_methane} = 40 \text{ {}\mu\text{mol H}_2/\text{mg VSS-hr}}$$

DOCUMENT: Rate of hydrogen conversion to methane estimated from this study, Young, 1997; and Smatlak, 1995.

$$\text{Pulse_Value_Hydrogen} = 0$$

DOCUMENT: This is the amount of hydrogen fed (μmol) at each pulse beginning at 0 hr and occurring every 48 hr.

HYDROGENOTROPHIC METHANOGENS

$$\text{X_Hydrogenotrophic_Methanogens}(t) =$$

$$\text{X_Hydrogenotrophic_Methanogens}(t - dt) +$$

$$(\text{Biomass_Growth_Hydrogenotrophic_Methanogens} -$$

Biomass_Decay_Hydrogenotrophic_Methanogens -
 Day_4_Waste_X_Hydrogenotrophic_Methanogens) * dt
 INIT X_Hydrogenotrophic_Methanogens =
 Initial_X_Hydrogenotrophic_Methanogens {mg VSS}
 DOCUMENT: Reservoir representing hydrogenotrophic methanogen biomass {mg VSS}.

INFLOWS:

Biomass_Growth_Hydrogenotrophic_Methanogens =
 Y_Hydrogenotrophic_Methanogens*H2_For_Methanogenesis
 {mg VSS/hr}

OUTFLOWS:

Biomass_Decay_Hydrogenotrophic_Methanogens =
 Decay_Hydrogenotrophic_Methanogens*
 X_Hydrogenotrophic_Methanogens {mg VSS/hr}

Day_4_Waste_X_Hydrogenotrophic_Methanogens =
 PULSE(X_Hydrogenotrophic_Methanogens*Liquid_Waste_Rate,
 Waste_Pulse_Time,Waste_Increment_Time)
 {mg VSS wasted every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and replaced with 10 mL of fresh basal medium every 4 days (96 hr). This decreases the amount of soluble constituents by 10 percent. Volatile constituents are not affected since they are purged out after each 96 hr period.

Decay_Hydrogenotrophic_Methanogens = 0.001

DOCUMENT: 0.001/hr. Generic number.

Initial_X_Hydrogenotrophic_Methanogens = 0

DOCUMENT: Initial amount of hydrogenotrophic methanogen biomass present (mg VSS).

X_Cw_Hydrogenotrophic_Methanogens =
 X_Hydrogenotrophic_Methanogens/Vw {mg VSS/L}

DOCUMENT: Converter to allow reporting of hydrogenotrophic methanogen biomass as a concentration {mg VSS/L}.

$X_{Mt_Hydrogenotrophic_Methanogens} =$
 $X_{Hydrogenotrophic_Methanogens} \{mg \text{ VSS}\}$
 $Y_{Hydrogenotrophic_Methanogens} = 0.00143$
 DOCUMENT: Yield for hydrogen-using methanogens (0.00143 mg VSS/ μ mol H₂)
 Weimer and Zeikus, 1978.

LACTIC ACID

$Mt_Lactate(t) = Mt_Lactate(t - dt) + (Lactic_Acid_Feeding -$
 $Day_4_Waste_Lactate - Lactate_Fermented_to_Acetate -$
 $Lactate_Fermented_to_Propionate) * dt$
 $INIT \text{ } Mt_Lactate = Initial_Lactate \{ \mu mol \}$
 DOCUMENT: Reservoir representing lactic acid (μ mol).

INFLOWS:

$Lactic_Acid_Feeding = PULSE(Pulse_Value_Lactic_Acid,$
 $Feed_Pulse_Time_Donor, Feed_Increment_Time)$
 DOCUMENT: Pulse input of lactic acid (μ mol) beginning at time = 0 hr and
 occurring every 48 hr thereafter.

OUTFLOWS:

$Day_4_Waste_Lactate = PULSE(Mt_Lactate * Liquid_Waste_Rate,$
 $Waste_Pulse_Time, Waste_Increment_Time)$
 $\{ \mu mol \text{ wasted every } 96 \text{ hr} \}$

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and
 replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This
 decreases the amount of soluble constituents by 10 percent. Volatile constituents
 are not affected since they are purged out after each 96 hr period.

$Lactate_Fermented_to_Acetate =$
 $(k_{Lactate_to_Acetate} * X_{Mt_Lactate_to_Acetate_Fermenters} * Cw_Lactate * Thermo_Factor_{Lactate_to_Acetate}) /$
 $(Ks_{Lactate_to_Acetate} + Cw_Lactate)$

DOCUMENT: Lactate fermentation to acetate and hydrogen (μ mol/hr).

$Lactate_Fermented_to_Propionate =$
 $(k_{Lactate_to_Propionate} * X_{Mt_Lactate_to_Propionate_Fermenters} * Cw_Lactate * Thermo_Factor_{Lactate_to_Propionate}) /$
 $(Ks_{Lactate_to_Propionate} + Cw_Lactate)$

DOCUMENT: Lactate fermentation to propionate (μ mol/hr).

$$Cw_Lactate = Mt_Lactate/Vw \{ \mu\text{mol/L} \}$$

DOCUMENT: Converter to allow reporting of lactic acid as a concentration $\{ \mu\text{mol/L} \}$.

$$\text{Initial_Lactate} = 1\text{E-}20$$

DOCUMENT: The initial amount of lactate present (μmol).

$$Ks_Lactate_to_Acetate = 2.52$$

DOCUMENT: Half-velocity coefficient for lactate fermentation. $2.52 \mu\text{mol/L}$, Fennell, est., 1996.

$$Ks_Lactate_to_Propionate = 2.52$$

DOCUMENT: Same as for lactate to acetate.

$$k_Lactate_to_Acetate = 8.57$$

DOCUMENT: Apparent rate of lactate degradation was $2.67 \mu\text{mol lactate/mg VSS-hr}$, est., Fennell}.

This occurred under a thermodynamic ceiling (ave) of -50 kJ/mol lactate fermented (on average) for the 1:1 TISs and -50 kJ/mol for the 2:1 TISs. Delta G critical was set at -19 kJ/mol and the thermo factor was calculated for each condition. There is less lactate-degrader biomass than the total VSS measured. From my biomass estimates the lactate degraders make up 27.4 % for 1:1 TISs and 33.9 % for 2:1 TISs, of the total biomass in the bottle.

The rate that would be observed in the absence of a thermodynamic limit and accounting for the fraction of relevant biomass is:

rate = apparent rate/(thermo factor*fraction of relevant VSS).

$$\text{AVE } k = 8.57 \mu\text{mol/mg lactate VSS-hr}$$

$$k_Lactate_to_Propionate = 8.57$$

DOCUMENT: Same as for lactate to acetate.

$$\text{Pulse_Value_Lactic_Acid} = 0$$

DOCUMENT: This is the amount of lactic acid fed $\{ \mu\text{mol} \}$ at each pulse beginning at 0 hr and occurring every 48 hr.

METHANE FROM ACETATE

$Mt_Methane_From_Acetate(t) = Mt_Methane_From_Acetate(t - dt) +$
 $(Methane_Production_From_Acetate -$
 $Day_4_Purge_Methane_from_Acetate) * dt$

INIT $Mt_Methane_From_Acetate = 0$ { μ mol}

DOCUMENT: Reservoir representing methane formed by acetotrophic methanogens { μ mol}.

INFLOWS:

$Methane_Production_From_Acetate =$
 $Inflow_Zero_Methane_From_Acetate *$
 $Acetate_Conversion_to_Methane * fe_Acetate$

DOCUMENT: Methane production from acetate. Methane Produced = {HAc to Methane flow * fe}. Where fe is the fraction of the donor that is used for energy.

OUTFLOWS:

$Day_4_Purge_Methane_from_Acetate =$
 $PULSE((Mt_Methane_From_Acetate), Waste_Pulse_Time,$
 $Waste_Increment_Time)$

DOCUMENT: The pulse output simulates the purge of methane formed from acetate from the bottle every 4 days (96 hr).

$Cg_Methane_From_Acetate = Mt_Methane_From_Acetate /$
 $((Vw/Hc_CH4)+Vg)$ { μ mol/L}

$Cw_Methane_From_Acetate =$
 $Mt_Methane_From_Acetate / (Vw + (Hc_CH4 * Vg))$ { μ mol/L}
 $fe_Acetate = 0.9582$

DOCUMENT: fe -- fraction of acetate for energy

$Inflow_Zero_Methane_From_Acetate =$
 $IF(Day_4_Purge_Methane_from_Acetate > 0) THEN(0) ELSE(1)$

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 1E-20 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

METHANE FROM HYDROGEN

$Mt_Methane_From_H2(t) = Mt_Methane_From_H2(t - dt) +$
 $(Methane_Production_From_H2 - Day_4_Purge_Methane_From_H2) *$
 dt

INIT Mt_Methane_From_H2 = 0 { μ mol}

DOCUMENT: Reservoir representing the methane produced by hydrogenotrophic methanogens { μ mol}.

INFLOWS:

Methane_Production_From_H2 =

Inflow_Zero_Methane_From_H2*fe_H2*H2_For_Methanogenesis*
H2_To_CH4_Molar_Conversion_Factor { μ mol/hr}

DOCUMENT: Methane production from hydrogen. Methane Produced = {H2 for Methanogenesis * stoichiometric conversion (CH₄/H₂) * fe}. Where fe is the fraction of the donor that is used for energy.

OUTFLOWS:

Day_4_Purge_Methane_From_H2 = PULSE((Mt_Methane_From_H2),
Waste_Pulse_Time,Waste_Increment_Time)

DOCUMENT: The pulse output simulates the purge of methane formed from hydrogen from the bottle every 4 days (96 hr-dt).

Cg_Methane_from_H2 = Mt_Methane_From_H2/((Vw/Hc_CH4)+Vg)
{ μ mol/L}

Cw_Methane_From_H2 = Mt_Methane_From_H2/(Vw+(Hc_CH4*Vg))
{ μ mol/L}

fe_H2 = 0.8877

DOCUMENT: fe -- fraction of hydrogen used for energy

H2_to_CH4_Molar_Conversion_Factor = 0.25
{ μ mol CH₄ Formed per μ mol H₂}

Inflow_Zero_Methane_From_H2 =

IF(Day_4_Purge_Methane_From_H2>0)THEN(0)ELSE(1)

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 0 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

PROPIONIC ACID

Mt_Propionate(t) = Mt_Propionate(t - dt) + (Propionate_Production +
Propionic_Acid_Feeding - Day_4_Waste_Propionate -
Propionate_Fermented_to_Acetate) * dt

INIT Mt_Propionate = Initial_Propionate { μ mol}

DOCUMENT: Reservoir representing propionic acid { μmol }.

INFLOWS:

Propionate_Production =
 $\text{fe_Ethanol} * \text{Ethanol_Fermented_to_Propionate} * \text{Propionate_Formed_per_Ethanol} + \text{fe_Lactate} * \text{Lactate_Fermented_to_Propionate} * \text{Propionate_Formed_per_Lactate}$
 { $\mu\text{mol/hr}$ }

DOCUMENT: Propionate production from all donors. Propionate Produced =
 Sum of {donor fermentation flow * stoichiometric conversion (Prop/Donor) * fe}.
 Where fe is the fraction of the donor that is used for energy.

Propionic_Acid_Feeding =
 $\text{PULSE}((\text{Pulse_Value_Propionic_Acid} + \text{FYE_Propionate}), \text{Feed_Pulse_Time_Donor}, \text{Feed_Increment_Time})$
 DOCUMENT: Pulse input of propionic acid { μmol } beginning at time = 0 hr and
 occurring every 48 hr thereafter.

OUTFLOWS:

Day_4_Waste_Propionate =
 $\text{PULSE}(\text{Mt_Propionate} * \text{Liquid_Waste_Rate}, \text{Waste_Pulse_Time}, \text{Waste_Increment_Time})$ { μmol wasted every 96 hr}

DOCUMENT: Ten percent (10 mL) of the liquid from the culture is wasted and
 replaced with 10 mL of fresh basal medium every 4 days (96 hr-dt). This
 decreases the amount of soluble constituents by 10 percent. Volatile constituents
 are not affected because they are purged out after each 96 hr period.

Propionate_Fermented_to_Acetate =
 $(\text{k_Propionate} * \text{X_Mt_Propionate_Fermenters} * \text{Cw_Propionate} * \text{Thermo_Factor_Propionate}) / (\text{Ks_Propionate} + \text{Cw_Propionate})$
 { $\mu\text{mol/hr}$ }

$\text{Cw_Propionate} = \text{Mt_Propionate} / \text{Vw}$ { $\mu\text{mol/L}$ }

DOCUMENT: Converter to allow reporting of propionic acid as a concentration
 { $\mu\text{mol/L}$ }.

Initial_Propionate = 1E-20

DOCUMENT: Initial amount of propionate present (μmol).

$K_s_{\text{Propionate}} = 11.3$

DOCUMENT: Half-velocity coefficient for propionate. 11.3 $\mu\text{mol/L}$ Fennell, est. 1996.

$k_{\text{Propionate}} = 2.21$

DOCUMENT: Apparent rate of propionate degradation was 0.096 $\{\mu\text{mol propionate/mg VSS-hr, est., Fennell}\}$.

This occurred under a thermodynamic ceiling (ave) of -20 kJ/mol propionate fermented (on average) for the 1:1 TISs and -27 kJ/mol for the 2:1 TISs. Delta G critical was set at -19 kJ/mol and the thermo factor was calculated for each condition. There is less propionate-degrader biomass than the total VSS measured. From my biomass estimates the propionate degraders make up 6.2 % for 1:1 TISs and 6.5% for 2:1 TISs, of the total biomass in the bottle.

The rate that would be observed in the absence of a thermodynamic limit and accounting for the fraction of relevant biomass is:

rate = apparent rate/(thermo factor*fraction of relevant VSS).

AVE $k = 2.21 \mu\text{mol/mg propionate VSS-hr}$

$\text{Pulse_Value_Propionic_Acid} = 0$

DOCUMENT: This is the amount of propionic acid fed $\{\mu\text{mol}\}$ at each pulse beginning at 0 hr and occurring every 48 hr.

THERMODYNAMICS

$Cw_{\text{Hydrogen_Ion}} = 10^{-\text{pH}}$

$\text{delta_G_critical_Butyrate} = -19 \text{ \{kJ/mol butyrate\}}$

$\text{delta_G_critical_Ethanol_to_Acetate} = -19 \text{ \{kJ/mol ethanol\}}$

$\text{delta_G_critical_Ethanol_to_Propionate} = -19 \text{ \{kJ/mol ethanol\}}$

$\text{delta_G_critical_Lactate_to_Acetate} = -19 \text{ \{kJ/mol lactate\}}$

$\text{delta_G_critical_Lactate_to_Propionate} = -19 \text{ \{kJ/mol lactate\}}$

$\text{delta_G_critical_Propionate_to_Acetate} = -19 \text{ \{kJ/mol propionate\}}$

DOCUMENT: The maximum value that delta G can acquire that still provides the organism with enough energy to make ATP. Analysis of butyrate data--degradation proceeds at delta G values of -20 kJ/mol butyrate. Arbitrarily used a value 5 % higher.

$$\begin{aligned} \text{delta_G_rxn_Butyrate} = & \text{delta_G_zero_Butyrate} + \\ & (R * \text{Temp} * \text{LOGN}((g_ionic_Z1 * Cw_Hydrogen_Ion * (g_ionic_Z1 * \\ & Cw_Acetate / 1E6)^2 * (g_H2 * Cw_H2 / 1E6)^2) / (g_ionic_Z1 * Cw_Butyrate / \\ & 1E6))) \end{aligned}$$

$$\begin{aligned} \text{delta_G_rxn_Ethanol_to_Acetate} = & \text{delta_G_zero_Ethanol_to_Acetate} + \\ & R * \text{Temp} * \text{LOGN}((g_ionic_Z1 * Cw_Hydrogen_Ion * g_ionic_Z1 * \\ & Cw_Acetate / 1E6 * (g_H2 * Cw_H2 / 1E6)^2) / (g_ionic_Z1 * Cw_Ethanol / 1E6)) \end{aligned}$$

$$\begin{aligned} \text{delta_G_rxn_Ethanol_to_Propionate} = & \\ & \text{delta_G_zero_Ethanol_to_Propionate} + R * \text{Temp} * \text{LOGN}(((g_ionic_Z1 * \\ & Cw_Hydrogen_Ion)^{(1/3)} * (g_ionic_Z1 * Cw_Acetate / 1E6)^{(1/3)} * \\ & (g_ionic_Z1 * Cw_Propionate / 1E6)^{(2/3)}) / (g_ionic_Z1 * Cw_Ethanol / \\ & 1E6 * (g_ionic_Z1 * Cw_Bicarbonate)^{(2/3)})) \end{aligned}$$

$$\begin{aligned} \text{delta_G_rxn_Lactate_to_Acetate} = & \text{delta_G_zero_Lactate_to_Acetate} + \\ & R * \text{Temp} * \text{LOGN}((g_ionic_Z1 * Cw_Hydrogen_Ion * g_ionic_Z1 * \\ & Cw_Bicarbonate * g_ionic_Z1 * Cw_Acetate / 1E6 * (g_H2 * Cw_H2 / 1E6)^2) / \\ & (g_ionic_Z1 * Cw_Lactate / 1E6)) \end{aligned}$$

$$\begin{aligned} \text{delta_G_rxn_Lactate_to_Propionate} = & \\ & \text{delta_G_zero_Lactate_to_Propionate} + R * \text{Temp} * \text{LOGN}(((g_ionic_Z1 * \\ & Cw_Hydrogen_Ion)^{(1/3)} * (g_ionic_Z1 * Cw_Acetate / 1E6)^{(1/3)} * \\ & (g_ionic_Z1 * Cw_Bicarbonate)^{(1/3)} * (g_ionic_Z1 * Cw_Propionate / 1E6)^{(2/3)}) / \\ & (g_ionic_Z1 * Cw_Lactate / 1E6)) \end{aligned}$$

$$\begin{aligned} \text{delta_G_rxn_Propionate} = & \text{delta_G_zero_Propionate} + R * \text{Temp} * \\ & \text{LOGN}((g_ionic_Z1 * Cw_Acetate / 1E6 * (g_H2 * Cw_H2 / 1E6)^3 * g_ionic_Z1 * \\ & Cw_Hydrogen_Ion * g_ionic_Z1 * Cw_Bicarbonate) / (g_ionic_Z1 * \\ & Cw_Propionate / 1E6)) \end{aligned}$$

$$\text{delta_G_zero_Butyrate} = 123.16 \text{ {kJ/mol}}$$

$$\text{delta_G_zero_Ethanol_to_Acetate} = 84.85 \text{ {kJ/mol}}$$

$$\text{delta_G_zero_Ethanol_to_Propionate} = -26.41 \text{ {kJ/mol}}$$

$$\text{delta_G_zero_Lactate_to_Acetate} = 71.01 \text{ {kJ/mol}}$$

$$\text{delta_G_zero_Lactate_to_Propionate} = -40.26 \text{ {kJ/mol}}$$

$$\text{delta_G_zero_Propionate} = 166.9 \text{ {kJ/mol}}$$

DOCUMENT: Estimation

```
one_minus_expGRT_Butyrate =
1-EXP((delta_G_rxn_Butyrate-delta_G_critical_Butyrate)/(R*Temp))
```

```
one_minus_expGRT_Ethanol_to_Acetate =
1-EXP((delta_G_rxn_Ethanol_to_Acetate-
delta_G_critical_Ethanol_to_Acetate)/(R*Temp))
```

```
one_minus_expGRT_Ethanol_to_Propionate =
1-EXP((delta_G_rxn_Ethanol_to_Propionate-
delta_G_critical_Ethanol_to_Propionate)/(R*Temp))
```

```
one_minus_expGRT_Lactate_to_Acetate =
1-EXP((delta_G_rxn_Lactate_to_Acetate-
delta_G_critical_Lactate_to_Acetate)/(R*Temp))
```

```
one_minus_expGRT_Lactate_to_Propionate =
1-EXP((delta_G_rxn_Lactate_to_Propionate-
delta_G_critical_Lactate_to_Propionate)/(R*Temp))
```

```
one_minus_expGRT_Propionate =
1-EXP((delta_G_rxn_Propionate-
delta_G_critical_Propionate_to_Acetate)/(R*Temp))
```

```
Thermo_Factor_Butyrate =
IF(one_minus_expGRT_Butyrate>=0)
THEN(one_minus_expGRT_Butyrate)ELSE(0)
```

```
Thermo_Factor_Ethanol_to_Acetate =
IF(one_minus_expGRT_Ethanol_to_Acetate>=0)
THEN(one_minus_expGRT_Ethanol_to_Acetate)ELSE(0)
```

```
Thermo_Factor_Ethanol_to_Propionate =
IF(one_minus_expGRT_Ethanol_to_Propionate>=0)
THEN(one_minus_expGRT_Ethanol_to_Propionate)ELSE(0)
```

```
Thermo_Factor_Lactate_to_Acetate =
IF(one_minus_expGRT_Lactate_to_Acetate>=0)
THEN(one_minus_expGRT_Lactate_to_Acetate)ELSE(0)
```

Thermo_Factor_Lactate_to_Propionate =
 IF(one_minus_expGRT_Lactate_to_Propionate>=0)
 THEN(one_minus_expGRT_Lactate_to_Propionate)ELSE(0)

Thermo_Factor_Propionate =
 IF(one_minus_expGRT_Propionate>=0)
 THEN(one_minus_expGRT_Propionate)ELSE(0)

Not In a Sector

Acetate_Formed_per_Butyrate = 2
 { μmol Acetate Formed/ μmol Butyrate Fermented to Acetate}
 Acetate_Formed_per_Ethanol = 1
 { μmol Acetate formed/ μmol Ethanol Fermented to Acetate}
 Acetate_Formed_per_Lactate = 1
 { μmol Acetate formed/ μmol Lactate Fermented to Acetate}
 Acetate_Formed_per_Lactate_to_Propionate = (1/3)
 { μmol Acetate formed/ μmol Lactate Fermented to Propionate }
 Acetate_Formed_per_Propionate = 1
 { μmol acetate formed/ μmol propionate}
 Acetate_Per_Ethanol_to_Propionate = (1/3)
 { μmol Acetate formed/ μmol Ethanol Fermented to Propionate}

fe_Butyrate = 0.9753

DOCUMENT: fe -- the fraction of the donor butyrate that is used for energy

fe_Ethanol = 0.9708

DOCUMENT: fe -- fraction of ethanol used for energy

fe_Lactate = 0.9482

DOCUMENT: fe -- fraction of lactate used for energy

fe_Propionate = 0.9818

DOCUMENT: fe -- fraction of propionate used for energy

H2_per_Butyrate_Fermented_to_Acetate = 2

{ μmol H2/ μmol Butyrate Fermented To Acetate}

H2_per_Ethanol_Fermented_to_Acetate = 2

{ μmol H2/ μmol Ethanol Fermented to Acetate}

$$\text{H2_per_Lactate_Fermented_to_Acetate} = 2$$

{ $\mu\text{mol H}_2/\mu\text{mol Lactate Fermented to Acetate}$ }

$$\text{H2_per_Propionate_Fermented_to_Acetate} = 3$$

{ $\mu\text{mol H}_2/\mu\text{mol Propionate Fermented to Acetate}$ }

$$\text{Propionate_Formed_per_Ethanol} = (2/3)$$

{ $\mu\text{mol Propionate}/\mu\text{mol Ethanol Converted to Propionate}$ }

$$\text{Propionate_Formed_per_Lactate} = (2/3)$$

{ $\mu\text{mol Propionate}/\mu\text{mol Lactate converted to Propionate}$ }

$$\text{Cg_Total_Methane} =$$

$$\text{Cg_Methane_from_H}_2 + \text{Cg_Methane_From_Acetate}$$

$$\text{Cw_Total_Methane} =$$

$$\text{Cw_Methane_From_H}_2 + \text{Cw_Methane_From_Acetate}$$

$$\text{Mt_Total_Methane} =$$

$$\text{Mt_Methane_From_H}_2 + \text{Mt_Methane_From_Acetate}$$

APPENDIX IV

JUSTIFICATION OF EQUILIBRIUM ASSUMPTION FOR MODELING H₂

A4.1. *Comparison of Equilibrium and Non-Equilibrium Model*

Equilibrium between the gas and liquid phases was assumed for H₂ and CH₄ in the version of the model used for all the simulations. This assumption was made because the inclusion of gas-liquid partitioning for H₂ and CH₄ in the model required extremely small time steps (dt) to adequately capture the transfer of very small "packets" of these sparingly soluble gases from one phase to another. To verify that this assumption was valid, a version of the model was prepared that was exactly the same as that used for all the simulations, except that the H₂ module was modified to include gaseous (Mg_{H2}) and aqueous (Mw_{H2}) stocks, and transfer between these two stocks to simulate volatilization and dissolution. The module is shown in Section A4.2. The equation for volatilization and dissolution was as Equation 5.1. The concentration of H₂ in the gaseous phase was computed via Equation A4.1.

$$C_{gH2} = \frac{M_{gH2}}{V_g} \quad (A4.1)$$

The concentration of H₂ in the aqueous phase was calculated assuming that the aqueous-phase stock included not only H₂ but also formate as in Equation A4.2.

$$C_{wH_2} = \frac{M_{wH_2}}{V_w + \left(\frac{\text{formate(aq)}}{H_2(\text{aq})} \times V_w \right)} \quad (\text{A4.2})$$

Simulations were run for an ethanol-fed culture and a butyric-acid-fed culture at a 1:1 ratio of donor to PCE using the version of the model with the equilibrium assumption and using the version of the model that includes the non-equilibrium module. H_2 in the gaseous phase and aqueous phase were tracked, as were PCE disappearance and donor degradation which are both controlled by the aqueous H_2 concentration. These comparisons are shown in Figures A4.1 and A4.2. In both cases, the equilibrium version of the model predicted that the gaseous-phase H_2 concentration would peak earlier and at a slightly higher concentration than the model that used the non-equilibrium H_2 module. The aqueous H_2 concentration, however, was predicted to be very similar for both versions of the model. Furthermore, donor degradation and PCE dechlorination—both of which are governed by the aqueous H_2 concentration—were practically identical for both versions of the model. This comparison shows that the equilibrium assumption was acceptable in terms of aqueous H_2 concentrations. The equilibrium assumption may, however, have predicted gaseous concentrations that peaked too quickly and slightly too high.

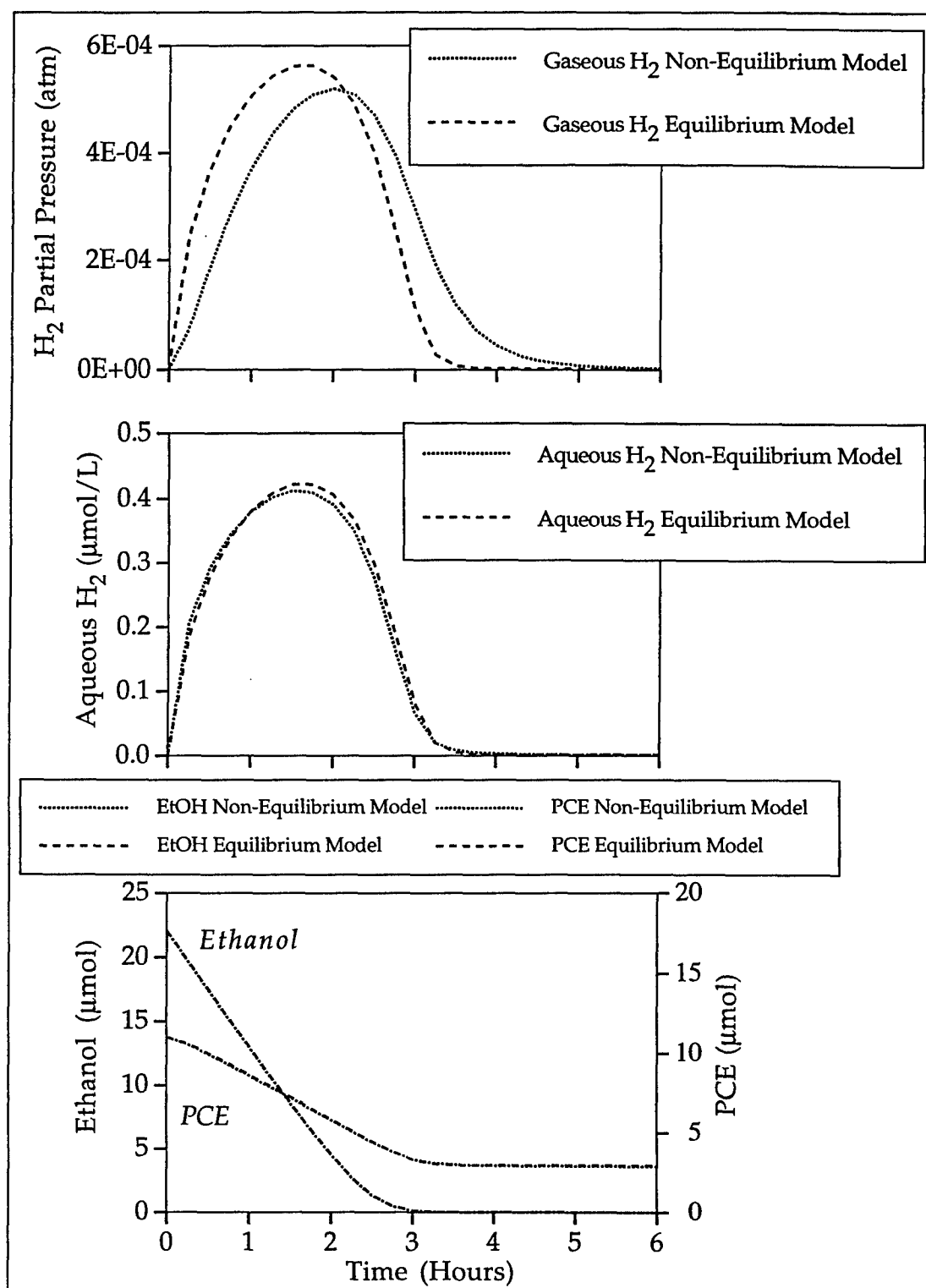


Figure A4.1. Comparison of simulations of ethanol-amended culture run with model using an assumption of gas-liquid equilibrium and model with a non-equilibrium module.

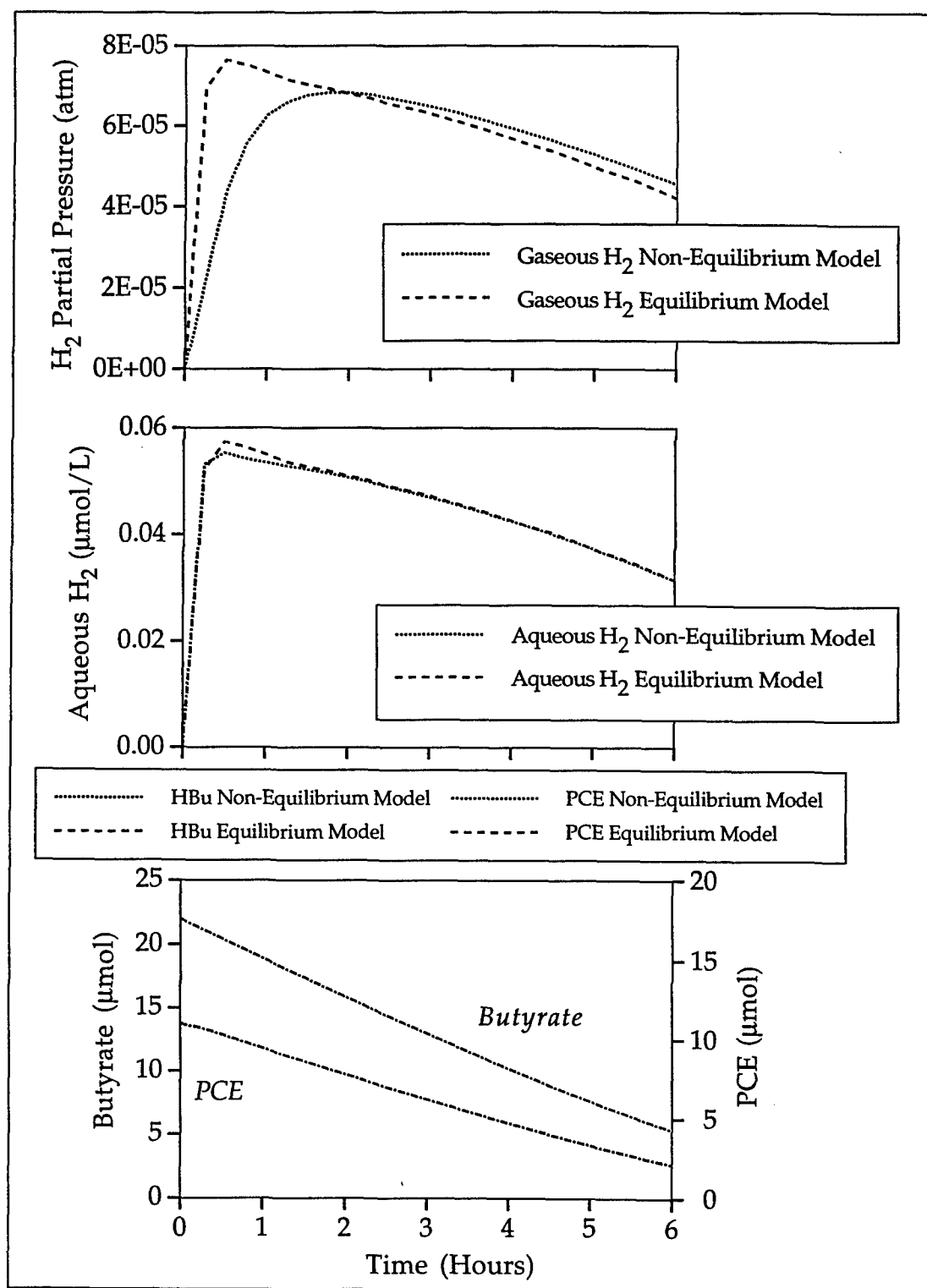
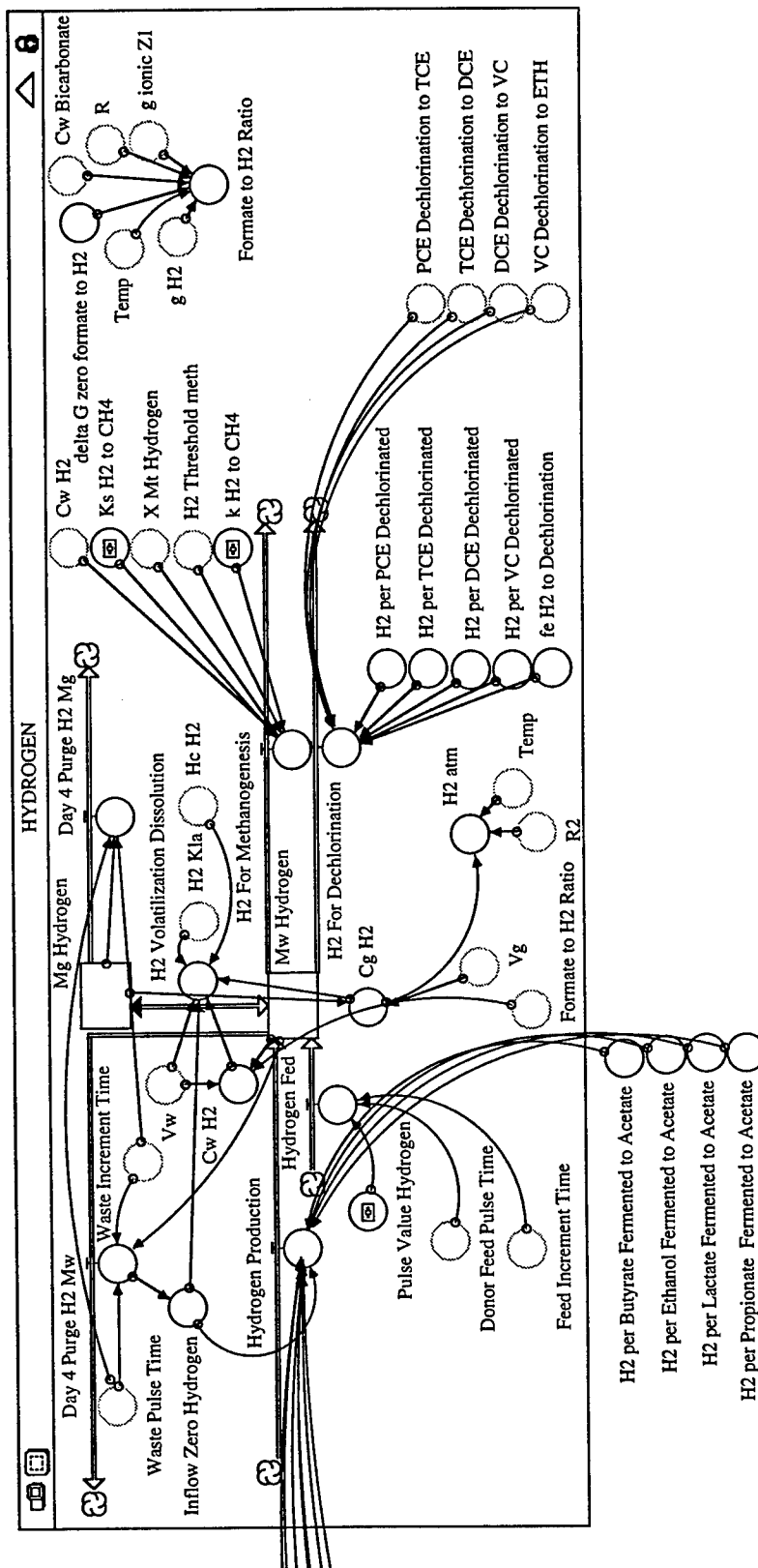


Figure A4.2. Comparison of simulations of butyric-acid-amended culture run with model using an assumption of gas-liquid equilibrium and model with a non-equilibrium module.

A4.2. Hydrogen Non-Equilibrium Module

This section contains a printout of the model construction layer and the equations for the non-equilibrium module for hydrogen.



STELLA EQUATIONS FOR NON-EQUILIBRIUM HYDROGEN MODULE

HYDROGEN

$Mg_Hydrogen(t) = Mg_Hydrogen(t - dt) + (- Day_4_Purge_H2_Mg - H2_Volatilization_Dissolution) * dt$

INIT $Mg_Hydrogen = 1E-20$

OUTFLOWS:

$Day_4_Purge_H2_Mg =$

$PULSE((Mg_Hydrogen), Waste_Pulse_Time, Waste_Increment_Time)$

$H2_Volatilization_Dissolution = Inflow_Zero_Hydrogen * Vw * H2_Kla * ((Cg_H2 / Hc_H2) - Cw_H2) \text{ } \{\mu\text{mol/hr}\}$

$Mw_Hydrogen(t) = Mw_Hydrogen(t - dt) + (Hydrogen_Production + Hydrogen_Fed + H2_Volatilization_Dissolution - H2_For_Dechlorination - H2_For_Methanogenesis - Day_4_Purge_H2_Mw) * dt$

INIT $Mw_Hydrogen = 1E-20 \text{ } \{\mu\text{mol}\}$

DOCUMENT: The Mw Hydrogen Stock represents all aqueous hydrogen plus the aqueous formate that is in equilibrium with the aqueous hydrogen.

INFLOWS:

$Hydrogen_Production = Inflow_Zero_Hydrogen * (fe_Propionate * Propionate_Fermented_to_Acetate * H2_per_Propionate_Fermented_to_Acetate + fe_Butyrate * Butyrate_Fermented_to_Acetate * H2_per_Butyrate_Fermented_to_Acetate + fe_Ethanol * Ethanol_Fermented_to_Acetate * H2_per_Ethanol_Fermented_to_Acetate + fe_Lactate * Lactate_Fermented_to_Acetate * H2_per_Lactate_Fermented_to_Acetate)$

DOCUMENT: Hydrogen production from all donors.

Hydrogen Produced = Sum of {donor fermentation flow * stoichiometric conversion (H2/Donor) * fe}. Where fe is the fraction of the donor that is used for energy.

$Hydrogen_Fed = PULSE(Pulse_Value_Hydrogen,$

$Donor_Feed_Pulse_Time, Feed_Increment_Time)$

$H2_Volatilization_Dissolution = Inflow_Zero_Hydrogen * Vw * H2_Kla * ((Cg_H2 / Hc_H2) - Cw_H2) \text{ } \{\mu\text{mol/hr}\}$

$$H2_Kla*((Cg_H2/Hc_H2)-Cw_H2) \text{ } \{\mu\text{mol/hr}\}$$

OUTFLOWS:

$$H2_For_Dechlorination = \\ (PCE_Dechlorination_to_TCE*H2_per_PCE_Dechlorinated + \\ TCE_Dechlorination_to_DCE*H2_per_TCE_Dechlorinated + \\ DCE_Dechlorination_to_VC*H2_per_DCE_Dechlorinated + \\ VC_Dechlorination_to_ETH*H2_per_VC_Dechlorinated)/ \\ fe_H2_to_Dechlorination \text{ } \{\mu\text{mol/hr}\}$$

$$H2_For_Methanogenesis = (k_H2_to_CH4*X_Mt_Hydrogen* \\ (Cw_H2-H2_Threshold_meth))/(Ks_H2_to_CH4 + \\ (Cw_H2-H2_Threshold_meth)) \text{ } \{\mu\text{mol/hr}\}$$

$$Day_4_Purge_H2_Mw = \\ PULSE((Mw_Hydrogen),Waste_Pulse_Time,Waste_Increment_Time) \\ \text{DOCUMENT: The pulse output simulates the purge of hydrogen from the bottle} \\ \text{every 4 days (96 hours-dt).}$$

$$Cg_H2 = Mg_Hydrogen/Vg \text{ } \{\mu\text{mol/L}\}$$

$$Cw_H2 = Mw_Hydrogen/(Vw+Formate_to_H2_Ratio*Vw) \text{ } \{\mu\text{mol/L}\}$$

$$\text{delta_G_zero_formate_to_H2} = 19.03 \text{ } \{\text{kJ/mol rxn}\}$$

DOCUMENT: delta G rxn at 35 C for $\text{HCOO}^- + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}_2 \text{ (aq)}$

$$fe_H2_to_Dechlorination = 0.9023$$

DOCUMENT: fe -- fraction of hydrogen used for energy

$$\text{Formate_to_H2_Ratio} = g_H2*g_ionic_Z1*Cw_Bicarbonate / \\ (g_ionic_Z1*EXP(-\text{delta_G_zero_formate_to_H2}/(R*Temp)))$$

DOCUMENT: The steady-state ratio of aqueous formate to aqueous hydrogen ($\mu\text{mol}/\mu\text{mol}$).

$$H2_atm = Cg_H2*R2*Temp/1E6 \text{ } \{\text{atm}\}$$

$$H2_per_DCE_Dechlorinated = 1 \text{ } \{\mu\text{mol Hydrogen}/\mu\text{mol DCE converted to VC}\}$$

$$H2_per_PCE_Dechlorinated = 1 \text{ } \{\mu\text{mol Hydrogen}/\mu\text{mol PCE converted to TCE}\}$$

$$H2_per_TCE_Dechlorinated = 1 \text{ } \{\mu\text{mol Hydrogen}/\mu\text{mol TCE converted to DCE}\}$$

$H2_per_VC_Dechlorinated = 1 \text{ } \{\mu\text{mol Hydrogen}/\mu\text{mol VC converted to ETH} \}$

$Inflow_Zero_Hydrogen = IF(Day_4_Purge_H2_Mw > 0) THEN(0) ELSE(1)$

DOCUMENT: This converter takes a value of 1 if there is no purge occurring. It takes a value of 0 if there IS a purge event. The purpose of the converter is to zero the flow so that the stock is fully purged.

$Ks_H2_to_CH4 = 0.5$

DOCUMENT: half-velocity coefficient for hydrogen conversion to methane

$k_H2_to_CH4 = 40$

DOCUMENT: rate of hydrogen conversion to methane

$Pulse_Value_Hydrogen = 0$

APPENDIX V

DETERMINATION OF ACTIVITY COEFFICIENTS

Activity coefficients were required for all pertinent species for free-energy calculations. These were determined from the ionic strength of the basal salts medium described in Table 3.4. The ionic strength was determined by analyzing the ionic species initially present upon preparation (Table A5.1), assuming pH 7, and with a further assumption that the iron added precipitated an equivalent amount of the added sulfide. Using the ionic strength, the Guntelburg Approximation (Equation A5.1) was used to estimate activity coefficients for the mono-charged ionic species H^+ , HCO_3^- , acetate $^-$, butyrate $^-$, lactate $^-$, and propionate $^-$.

$$\log \gamma_i = -\frac{1}{2} Z^2 \frac{\sqrt{I}}{1 + \sqrt{I}} \quad (\text{A5.1})$$

Where γ_i is the activity coefficient of constituent i , Z is the charge on the ion, and I is the ionic strength. The activity coefficient was determined to be 0.771 (Table A5.1).

An activity coefficient for the non-charged species, H_2 , was calculated from Equation A5.2.

$$\log \gamma_i = k_c * I \quad (\text{A5.2})$$

where γ_i is the activity coefficient of constituent i , k_c is the salting-out coefficient, and I is the ionic strength. For H_2 , a salting-out coefficient of 0.102 was used [272] and γ was determined to be 1.02.

Table A5.1. Ionic strength and activity calculation for basal salts medium.

Compound	Concentration (eq/L)
H ⁺	0.0000001
K ⁺	0.00128
Mg ⁺²	0.00197
Na ⁺	0.075596
NH ₄ ⁺	0.00374
Cl ⁻	0.00672
HCO ₃ ⁻	0.0714
HPO ₄ ⁻²	0.000877
H ₂ PO ₄ ⁻	0.00404
OH ⁻	0.0000001
S ⁻²	0.00316
<i>Ionic Strength</i>	<i>0.08557</i>
<i>Activity Coefficient (Z=1)</i>	<i>0.771</i>

APPENDIX VI

CALCULATION OF FREE ENERGIES AT 35°C

$\Delta G^\circ_{35^\circ}$ was defined as the standard free energy of reaction for a temperature of 35°C, with unit activity of all solutes, including H^+ and H_2 (as an aqueous component rather than a gaseous one). The values reported for free energies elsewhere [233], are for 25°C, pH 7, and with H_2 expressed as a gaseous component. Modeling was carried out with pH as a variable and temperature at 35°C, therefore, calculations of $\Delta G^\circ_{35^\circ}$ were needed.

The $\Delta G^\circ_{35^\circ}$ values were calculated starting with basic values—the standard free energy of formation, $\Delta G^\circ_{f25^\circ}$, and the standard enthalpy of formation, $\Delta H^\circ_{f25^\circ}$, from the elements at 25°C—for each compound of interest. The free energy of formation, $\Delta G^\circ_{f25^\circ}$, for each compound was first converted to a value for 35°C using the van't Hoff Equation, Equation A6.1.

$$\ln \left[\frac{K_{35}}{K_{25}} \right] = \left[\frac{\Delta H^\circ_{f25^\circ}}{R} \right] \left(\frac{1}{T_{25}} - \frac{1}{T_{35}} \right) \quad (\text{A6.1})$$

Where:

- R = 0.00831441 kJ/°K-mol;
- T_{25} = 298.15 °K;
- T_{35} = 308.15 °K;
- K_{25} = the equilibrium constant at 25°C; and
- K_{35} = the equilibrium constant at 35°C.

First, K_{25} was calculated for each compound of interest from values for $\Delta G^\circ_{f25^\circ}$ using Equation A6.2.

$$\Delta G^\circ_{f25^\circ} = -RT \ln K_{25} \quad (\text{A6.2})$$

$$\text{or} \quad K_{25} = \exp \left[\frac{-\Delta G^\circ_{f25^\circ}}{RT} \right] .$$

K_{35} was then calculated using the van 't Hoff Equation, K_{25} , and the $\Delta H^\circ_{f25^\circ}$ values. Over temperature ranges of 20°C or less, it is generally agreed that the $\Delta H^\circ_{f25^\circ}/R$ value can be assumed to be approximately constant.

$$K_{35} = K_{25} \times \exp \left\{ \frac{\Delta H^\circ_{f25^\circ}}{R} \left(\frac{1}{T_{25}} - \frac{1}{T_{35}} \right) \right\}$$

$\Delta G^\circ_{f35^\circ}$ was then calculated: $\Delta G^\circ_{f35^\circ} = -RT \ln K_{35}$.

The standard free energy of formation, $\Delta G^\circ_{f25^\circ}$, the standard enthalpy of formation, $\Delta H^\circ_{f25^\circ}$, and the calculated free energy of formation $\Delta G^\circ_{f35^\circ}$ for each compound are shown in Table A6.1.

Obtaining values for $\Delta G^\circ_{f25^\circ}$ and $\Delta H^\circ_{f25^\circ}$ for propionate (aq) proved difficult. Thauer *et al.* [233] list a value for $\Delta G^\circ_{f25^\circ}$ for propionate. The original source for this listed value (-86.3 kcal/mol or -361.08 kJ/mol) is Stadtman *et al.* [215]. While this value is quoted often and is used almost exclusively in the literature, it is an estimate! Quoting from Stadtman *et*

al., "Although no free energy data are available for propionate⁻, the $-\Delta F'_f$ for propionate⁻ can be assumed to be 86.6, which is intermediate between the $-\Delta F'_f$ for acetate⁻ (88.99) and butyrate⁻ (84.28)." Why the value listed in Thauer *et al.* is slightly different than that in the Stadtman paper is not clear.

McCarty [153] lists a $\Delta G^\circ_{f, 25^\circ}$ for propionate of -87.47 kcal/mol. His value was obtained by modifying a value for $\Delta G^\circ_{f, 25^\circ}$ for propionic acid (l). If one follows the group contribution method [149] to calculate a $\Delta G^\circ_{f, 25^\circ}$ for propionate (aq), a value of -88.1 kcal/mol is obtained. All of these values are fairly close, so any one of them could probably be used with fairly good confidence. The Thauer *et al.* value was used in this study. However, to correct *this* value to 35°C, a $\Delta H^\circ_{f, 25^\circ}$ was also needed. This value also proved difficult to locate.

The value used for $\Delta H^\circ_{f, 25^\circ}$ for propionate (aq) used in this study was obtained by modifying $\Delta H^\circ_{f, 25^\circ}$ for propionic acid (l).

The first step in the modification was to correct for dissolution:

Propionic Acid (l, 298.15°K, 1 atm) →

Propionic Acid (aq, 298.15°K, 1 atm).

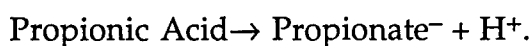
To get a value for the aqueous standard state, the heat exchange with the environment that accompanies the solution of 1 mole of propionic acid in an infinite amount of water, ΔH°_S , was added to the $\Delta H^\circ_{f, 25^\circ}$ of propionic acid (l). The $\Delta H^\circ_{f, 25^\circ}$ for propionic acid (l) is -510.7 kJ/mol [136]. A heat of solution, ΔH°_S , of -13.45 kcal/mol (-56.27 kJ/mol) was found for propionic acid [2].

ΔH_{f25}° propionic acid (aq) =
 ΔH_{f25}° propionic acid (l) + $\Delta H_{\text{os}}^{\circ}$ (heat of solution of 1 mole of
 propionic acid (l) in an infinite amount of water).

$$= -510.7 \text{ kJ/mol} + -56.27 \text{ kJ/mol}$$

$$= -566.97 \text{ kJ/mol}$$

This value was then corrected for ionization:



The heat of ionization for propionic acid ionizing to propionate in dilute aqueous systems at 25°C is -0.14 kcal/mol (-0.586 kJ/mol) [41].

$$\Delta H_{\text{rxn}}^{\circ} = \Sigma \Delta H_{\text{f}}^{\circ} \text{ products} - \Sigma \Delta H_{\text{f}}^{\circ} \text{ reactants}$$

$$\Delta H_{\text{rxn}}^{\circ} = -0.586 \text{ kJ/mol}$$

and,

$$\Delta H_{f25}^{\circ} \text{ propionate (aq)} = \Delta H_{25}^{\circ} \text{ for propionic acid (aq)}$$

$$- \Delta H_{f25}^{\circ} \text{ for H}^{+} - \Delta H_{\text{rxn}}^{\circ}$$

$$= -566.97 - 0 - (-0.586)$$

$$= -566.38 \text{ kJ/mol}$$

Table A6.1 shows standard values for compounds of interest that were used for the calculations and the values computed for ΔG_{f35}° . The complete fermentation reactions of interest to this study may be found in Table A6.2.

ΔG_{25}° and ΔG_{35}° were calculated from the $\Delta G_{\text{f}}^{\circ}$ values of relevance to the reaction and at the appropriate temperature, Equation A6.3.

$$\Delta G^{\circ} = \Sigma \Delta G_{\text{f}}^{\circ} \text{ products} - \Sigma \Delta G_{\text{f}}^{\circ} \text{ reactants} \quad (\text{A6.3})$$

Table A6.1. Thermodynamic values for pertinent compounds.

Compound	ΔG°_f at 25°C (kJ/mol)	ΔH_f at 25°C (kJ/mol)	ΔG°_f at 35°C (kJ/mol)
Acetate (aq)	-369.41 ^a	-486 ^d	-373.24
Butyrate (aq)	-352.63 ^a	-535.55 ^b	-346.49
Ethanol (aq)	-181.75 ^a	-288.3 ^d	-177.39
Lactate (L (+) ion, aq)	-517.81 ^a	-686.64 ^b	-511.02
Propionate	-361.08 ^a	-566.38 ^c	-354.19
Hydrogen (aq)	17.57 ^b	-4.18 ^b	18.3
Hydrogen Ion (aq)	0 ^a	0 ^b	0
Bicarbonate Ion (aq)	-586.85 ^a	-691.99 ^b	-583.32
Formate (aq)	-351.04 ^a	-425.6 ^d	-348.5
Water (l)	-237.18 ^a	-285.83 ^b	-235.55

^a Thauer et al. [233]; ^b Wilhoit [266]; ^c calculated as explained above;

^d [225]. Values converted where necessary from these references using 1 kcal = 4.184 kJoules

The overall standard free energies of reaction are shown for each fermentation at 25°C and 35°C in Table A6.2.

Table A6.2. ΔG° values for the fermentations of interest at 25°C and at 35°C.

Fermentation	ΔG°_{25} (kJ/mol)	ΔG°_{35} (kJ/mol)
$\text{Butyrate}^- + 2 \text{H}_2\text{O} \rightarrow 2 \text{Acetate}^- + \text{H}^+ + 2 \text{H}_2$	123.31	123.16
$\text{Ethanol} + \text{H}_2\text{O} \rightarrow \text{Acetate}^- + \text{H}^+ + 2 \text{H}_2$	84.66	84.85
$\text{Lactate}^- + 2 \text{H}_2\text{O} \rightarrow \text{Acetate}^- + \text{HCO}_3^- + \text{H}^+ + 2 \text{H}_2$	71.05	71.01
$\text{Propionate}^- + 3 \text{H}_2\text{O} \rightarrow$ $\text{Acetate}^- + \text{HCO}_3^- + \text{H}^+ + 3 \text{H}_2$	169.07	166.9
1 Ethanol + 2/3 $\text{HCO}_3^- \rightarrow$ 2/3 $\text{Propionate}^- + 1/3 \text{Acetate}^- + 1/3 \text{H}^+ + 1 \text{H}_2\text{O}$	-28.05	-26.41
1 $\text{Lactate}^- \rightarrow$ 1/3 $\text{Acetate}^- + 2/3 \text{Propionate}^- +$ 1/3 $\text{HCO}_3^- + 1/3 \text{H}^+$	-41.66	-40.26
$\text{HCOO}^- (\text{aq}) + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}_2 (\text{aq})$	18.94	19.03

APPENDIX VII

ESTIMATION OF KINETIC PARAMETERS FOR DONOR FERMENTATION

The results of donor degradation progress curves during time-intensive studies were analyzed for the purpose of estimating k , the maximum specific rate of substrate utilization, and K_S , the half-velocity coefficient for each donor. The parameters were estimated using a non-linear regression program developed by Bagely [11]. The regression program was written in ThinkPascal (Symantec) and was run on a PowerMac 7500.

The half-velocity coefficient, K_S , was calculated for all available data since it is not directly dependent upon the biomass concentration. Tables A7.1, A7.2, A7.3, and A7.4 show the runs that were analyzed and the parameters that were obtained. The maximum specific rate, k , could only be estimated for runs which had measurements of biomass. Values of k_{apparent} (based on the total biomass VSS) were corrected for both the thermodynamic limitation under which the specific test was operated and for the estimated fraction of the biomass that was actually responsible for degrading the donor (see Equation A7.1). For this calculation the thermodynamic factor, Φ , was calculated from $\Delta G_{\text{critical}}$ and the ΔG_{rxn} under which the test operated (as determined from the free energy analysis for each TIS). The biomass fractions were calculated from the hypothetical biomass distributions shown in Table 5.14. Values for k are shown in Tables A7.5, A7.6, A7.7, A7.8, A7.9, and A7.10.

$$k = \frac{k_{\text{apparent}}}{\Phi \times \frac{\text{donor degrader biomass}}{\text{total biomass}}} \quad (\text{A7.1})$$

k values for propionic acid and butyric acid were calculated both for a $\Delta G_{\text{critical}}$ of -19 kJ/mol donor and for $\Delta G_{\text{critical}}$ of -14.25 kJ/mol donor so that some model comparisons could be made assuming each of these values. The average k value for each substrate was used in the model. The k values for butyric acid fermentation are lower than those found in the literature (see Table A1.1), and the values obtained for propionic acid were somewhere within the wide range of the k values found in the literature (see Table A1.4). The average K_S for butyric acid, 34 μM , was somewhat lower than the range reported, 57 to 470 μM (see Table A1.1). The average value for K_S for propionic acid, 11.3 μM was also lower than the range reported, 38 to 4520 μM . Large confidence intervals were associated with many of the determinations of kinetic parameters from this study. The values are within an order-of-magnitude of the literature values in most cases.

Table A7.1. K_S values obtained from non-linear regression analysis of butyric acid fermentation progression curves.

Run	Donor:PCE Ratio	K_S (μM)
BUTYRIC ACID		
TC1-2-3B	2:1	56.6
TC1-F-3A	2:1	150.6
PCE Sens Test Bottle 2 Initial	2:1	9.8
PCE Sens Test Bottle 1 Initial	2:1	18.2
PCE Sens Test Bottle 2 Final	2:1	80.3
PCE Sens Test Bottle 1 Final	2:1	5.0
Fair ED I TIS 1 HBu 1	2:1	19.4
Fair ED I TIS 1 HBu 2	2:1	66.1
Fair ED I TIS 3 HBu 1	2:1	86.5
Fair ED I TIS 3 HBu 2	2:1	44.8
Fair ED II TIS 2 HBu	1:1	20.9
Fair ED II TIS 3 HBu	1:1	0.1
Fair ED II TIS 4 HBu	2:1	25.9
AVE \pm 95% CI		34.3 \pm 20.5

Table A7.2. K_S values obtained from non-linear regression analysis of ethanol fermentation progression curves.

Run	Donor:PCE Ratio	K_S (μM)
ETHANOL		
Fair ED II TIS 1 EtOH	1:1	60.5
Fair ED II TIS 2 EtOH	1:1	1.0
Fair Ed II TIS 4 EtOH	2:1	0.1
TIS I EtOH/FYE (Bottle 4)	2:1	0.6
TIS I EtOH Only (Bottle 7)	2:1	21.4
TIS I EtOH + FYE (Bottle 9)	2:1	0.1
TIS II EtOH + FYE (Bottle 2)	2:1	50.5
TIS II EtOH only (Bottle 6)	2:1	1.0
TIS II EtOH + SFYE (Bottle 9)	2:1	17.4
AVE \pm 95% CI		17 \pm 18

Table A7.3. K_S values obtained from non-linear regression analysis of lactic acid fermentation progression curves.

Run	Donor:PCE Ratio	K_S (μM)
LACTIC ACID		
Fair Ed II TIS 1 Lac	1:1	0.04
Fair Ed II TIS 5 Lac	2:1	5.0
AVE \pm 95% CI		2.5 \pm 31.5

Table A7.4. K_S values obtained from non-linear regression analysis of propionic acid fermentation progression curves.

Run	Donor:PCE Ratio	K_S (μM)
PROPIONIC ACID		
Fair Ed I TIS 4 Prop 1	1:1	6.7
Fair Ed I TIS 4 Prop 2	1:1	0.2
Fair Ed I TIS 5 Prop 1	1:1	0.4
Fair Ed I TIS 5 Prop 2	1:1	0.05
Fair Ed II TIS 1 Prop	1:1	0.4
Fair Ed II TIS 4 Prop	2:1	60.0
AVE \pm 95% CI		11.3 \pm 25.2

Table A7.7. Rate of ethanol fermentation estimated from donor degradation progress curves generated during time-intensive studies and using a $\Delta G_{\text{critical}}$ of -19 kJ/mol donor.

Run	Donor:PCE Ratio	Biomass (mg VSS/L)	Φ	Estimated Fraction of Donor Degraders	k_{apparent} ($\mu\text{mol/mg VSS}\cdot\text{hr}$)	k ($\mu\text{mol/mg VSS}\cdot\text{hr}$)
Fair ED II TIS 1 EtOH	1:1	32.34	0.8	0.1615	3.09	23.9
Fair ED II TIS 2 EtOH	1:1	32.34	0.8	0.1615	2.19	17
Fair Ed II TIS 4 EtOH	2:1	35.48	1	0.1751	7.44	42.5
TIS I EtOH/FYE (Bottle 4)	2:1	41.99	1	0.2079	2.24	10.8
TIS I EtOH Only (Bottle 7)	2:1	40.39	1	0.2079	4.38	21.1
TIS I EtOH + FYE (Bottle 9)	2:1	41.85	1	0.2079	2.17	10.5
TIS II EtOH + FYE (Bottle 2)	2:1	46.55	1	0.2079	6.2	29.8
TIS II EtOH only (Bottle 6)	2:1	40.35	1	0.2079	3.78	18.2
TIS II EtOH + SFYE (Bottle 9)	2:1	39.94	1	0.2079	4.9	23.6
AVE \pm 95% CI						21.9 \pm 7.6

Table A7.10. Rate of propionic acid fermentation estimated from donor degradation progress curves generated during time-intensive studies and using a $\Delta G_{\text{critical}}$ of -14.25 kJ/mol donor.

Run	Donor:PCE Ratio	Biomass (mg VSS/L)	Φ	Estimated Fraction of Donor Degraders	k_{apparent} ($\mu\text{mol}/\text{mg}$ VSS-hr)	k ($\mu\text{mol}/\text{mg}$ VSS-hr)
Fair Ed II TIS 1 Prop	1:1	22.78	0.89	0.0624	0.0395	0.7
Fair Ed II TIS 4 Prop	2:1	65.25	1	0.0651	0.1526	2.3
AVE \pm 95% CI						1.5 ± 10.37

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