1

PM-1 3 1/2 x 4 PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

**INTRACELLULAR REDUCING POWER APPLICATIONS
TO DUAL SUBSTRATE COMETABOLIC
DEGRADATION MODELLING**

**BY
PASCALE CHAMPAGNE-HARTLEY**

**A Thesis presented to
the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements
for the degree of
Master of Engineering*
Department of Civil and Environmental Engineering
Carleton University
Ottawa, Ontario
Canada**

© PASCALE CHAMPAGNE-HARTLEY, APRIL 1996

*** The Master of Engineering program is a joint program with the University of Ottawa
administered by the Ottawa-Carleton Institute for Civil Engineering**

**Author's Library
of Canada**

**Author's Library
of Canada**

**Author's Library
of Canada**

**Bibliothèque nationale
du Canada**

**Bibliothèque nationale du Canada
et
Bibliothèque de la Bibliothèque nationale**

**Bibliothèque nationale
du Canada**

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-612-19489-2

Canada

Name Carol Campbell

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

SUBJECT TERM

0645

U-M-I

SUBJECT CODE

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS

Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION

General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523

Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS

Language 0679
General 0289
Ancient 0290
Linguistics 0291
Modern 0291
Literature 0401
General 0294
Classical 0295
Comparative 0297
Medieval 0298
Modern 0316
African 0591
American 0305
Asian 0352
Canadian (English) 0355
Canadian (French) 0593
English 0311
Germanic 0312
Latin American 0315
Middle Eastern 0313
Romance 0314
Slavic and East European 0370

PHILOSOPHY, RELIGION AND THEOLOGY

Philosophy 0424
Religion 0318
General 0321
Biblical Studies 0319
Clergy 0320
History of 0322
Philosophy of 0464
Theology 0323

SOCIAL SCIENCES

American Studies 0324
Anthropology 0326
Archaeology 0327
Cultural 0310
Physical 0272
Business Administration 0270
General 0454
Accounting 0338
Banking 0385
Management 0501
Marketing 0502
Canadian Studies 0503
Economics 0504
General 0505
Agricultural 0506
Commerce Business 0507
Finance 0508
History 0509
Labor 0510
Theory 0511
Folklore 0358
Geography 0359
Gerontology 0351
History 0476
General 0466

Ancient 0512
Medieval 0513
Modern 0514
Black 0128
African 0131
Asia, Australia and Oceania 0132
Canadian 0133
European 0134
Latin American 0135
Middle Eastern 0136
United States 0137
History of Science 0585
Law 0198
Political Science 0615
General 0616
International Law and Relations 0617
Public Administration 0814
Recreation 0452
Social Work 0626
Sociology 0627
General 0938
Criminology and Penology 0631
Demography 0628
Ethnic and Racial Studies 0629
Individual and Family Studies 0630
Industrial and Labor Relations 0631
Public and Social Welfare 0632
Social Structure and Development 0700
Theory and Methods 0701
Transportation 0702
Urban and Regional Planning 0703
Women's Studies 0451

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES

Agriculture 0473
General 0285
Agronomy 0475
Animal Culture and Nutrition 0476
Animal Pathology 0359
Food Science and Technology 0478
Forestry and Wildlife 0479
Plant Culture 0480
Plant Pathology 0817
Plant Physiology 0777
Range Management 0746
Wood Technology 0306
Biology 0287
General 0308
Anatomy 0309
Biostatistics 0379
Botany 0329
Cell 0353
Ecology 0369
Entomology 0793
Genetics 0410
Limnology 0307
Microbiology 0317
Molecular 0416
Neuroscience 0433
Oceanography 0821
Physiology 0778
Radiation 0472
Veterinary Science 0786
Zoology 0760
Biophysics 0425
General 0996
Medical 0996

Geodesy 0370
Geology 0372
Geophysics 0388
Hydrology 0411
Mineralogy 0345
Paleobotany 0426
Paleoecology 0418
Paleontology 0985
Paleozoology 0427
Palynology 0368
Physical Geography 0415
Physical Oceanography 0768

HEALTH AND ENVIRONMENTAL SCIENCES

Environmental Sciences 0566
Health Sciences 0300
General 0992
Audiology 0567
Chemotherapy 0350
Dentistry 0769
Education 0758
Hospital Management 0982
Human Development 0564
Immunology 0347
Medicine and Surgery 0569
Mental Health 0570
Nursing 0380
Nutrition 0354
Obstetrics and Gynecology 0381
Occupational Health and Therapy 0571
Ophthalmology 0419
Pathology 0572
Pharmacology 0382
Pharmacy 0573
Physical Therapy 0574
Public Health 0575
Radiology 0575
Recreation 0575

Speech Pathology 0467
Toxicology 0381
Home Economics 0386

PHYSICAL SCIENCES

Pure Sciences 0484
Chemistry 0485
General 0486
Agriculture 0487
Analytical 0488
Biochemistry 0489
Inorganic 0490
Nuclear 0491
Organic 0492
Pharmaceutical 0493
Physical 0494
Polymer 0495
Radiation 0496
Mathematics 0497
Physics 0498
General 0499
Acoustics 0500
Astronomy and Astrophysics 0501
Atmospheric Science 0502
Atomic 0503
Electronics and Electricity 0504
Elementary Particles and High Energy 0505
Fluid and Plasma 0506
Molecular 0507
Nuclear 0508
Optics 0509
Radiation 0510
Solid State 0511
Statistics 0512
Applied Sciences 0513
Applied Mechanics 0514
Computer Science 0515

Engineering 0516
General 0517
Aerospace 0518
Agricultural 0519
Automotive 0520
Biomedical 0521
Chemical 0522
Civil 0523
Electronics and Electrical 0524
Heat and Thermodynamics 0525
Hydraulic 0526
Industrial 0527
Marine 0528
Materials Science 0529
Mechanical 0530
Metallurgy 0531
Mining 0532
Nuclear 0533
Packaging 0534
Petroleum 0535
Sanitary and Municipal 0536
System Science 0537
Technology 0538
Operations Research 0539
Plastic Technology 0540
Textile Technology 0541

PSYCHOLOGY

General 0542
Behavioral 0543
Clinical 0544
Developmental 0545
Experimental 0546
Industrial 0547
Personality 0548
Physiological 0549
Psychobiology 0550
Psychometrics 0551
Spiral 0552

The undersigned recommend to the Faculty of Graduate Studies and Research, acceptance of the Thesis Entitled ***Intracellular Reducing Power Applications to Dual Substrate Comatabolic Degradation Modelling*** submitted by Pascale Champagne-Hartley in partial fulfillment of the requirements for the Degree of Master of Engineering.

Professor W.J. Parker, Thesis co-supervisor

Professor P.J. Van Geel, Thesis co-supervisor

Professor J.L. Humar, Chair
Department of Civil and Environmental Engineering

Carleton University
May 2, 1996

ABSTRACT

A computer model able to account for dual substrate limitation and cosubstrate utilization in suspended biomass and biofilm systems was developed. The model can predict unsteady state variations in electron donor, electron acceptor and secondary substrate concentrations, as well as changes in active biomass within a mixed reactor (CSTR). The model can also simulate unsteady state substrate concentration profiles and active biomass within a biofilm.

The performance of the model was verified by comparing program simulations to existing models. Single substrate limitation, dual substrate limitation and cometabolic substrate utilization cases tested the suspended biomass system, whereas, steady state and unsteady state single substrate limitation cases provided a comparison for the biofilm system. In general, COMDEG model predictions were found to adequately reproduce existing model results.

A sensitivity analysis revealed that neither of the systems was sensitive to variations in initial parameter values. Suspended biomass electron donor, electron acceptor, secondary substrate and biomass concentrations were most affected by changes in donor maximum specific utilization rate, acceptor maximum specific utilization rate, reducing power (M) coefficient and yield coefficient, respectively. In the biofilm system, electron donor and electron acceptor concentrations were most affected by changes in donor maximum specific utilization rate, whereas the reducing power (M) coefficient had the greatest impact on secondary substrate removal.

Case studies demonstrated that decreases in electron donor and secondary substrate removal with decreasing aeration would be expected in both systems. The biofilm system was found to be sensitive to fluctuations in organic loading when compared to the suspended biomass system, in part due to the assumption that suspended growth contributions were negligible in the fixed-film system. The biofilm system was able to handle lower HRT values, whereas the suspended biomass system remained more stable under fluctuations at higher HRT values.

ACKNOWLEDGMENTS

I would like to thank my supervisors, Dr W Parker and Dr P Van Geel, for their support and interest throughout this project. Their comments and observations kept me on the alert and were greatly appreciated.

The Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged for its funding during the course of this project.

Many thanks to my family and friends for their continued support and encouragement. Special thanks to my husband Joel and daughters, Alexandra and Rachel, whose love and devotion have helped me keep sight of my goals throughout my studies.

TABLE OF CONTENTS

ABSTRACT	iii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	vi
LIST OF FIGURES	vii
SYMBOLS AND NOMENCLATURE	xviii
CHAPTER 1 INTRODUCTION	1
1.1 Overview	1
1.2 Objectives	2
CHAPTER 2 BACKGROUND	3
2.1 Multiple Substrate Interactions	3
2.1.1 Substrate Definitions	3
2.1.1.1 Electron Donor	3
2.1.1.2 Electron Acceptor	4
2.1.1.3 Secondary Substrate	4
2.1.2 Concept of Cosubstrate Utilization	5
2.1.2.1 Halogenated Secondary Substrate Degradation Reactions	6
2.1.2.1.1 Hydrolytic Reactions	8
2.1.2.1.2 Oxygenolytic Reactions	8
2.1.2.1.3 Reductive Dehalogenation Reactions	9
2.1.2.2 Cosubstrate Utilization	10
2.1.2.3 Experimental Work Involving Cosubstrate Utilization	13
2.1.3 Concept of Dual Substrate Limitation	17

2.1.3.1	Single Substrate Limitation	17
2.1.3.2	Dual Substrate Limitation	18
2.1.3.3	Experimental Work Involving Dual Substrate Limitation	20
2.2	Bacterial Physiological Responses	22
2.2.1	Bacterial Metabolic Functions	23
2.2.1.1	Transport	23
2.2.1.2	Catabolic Reactions	24
2.2.1.3	Respiration	28
2.2.1.4	Biosynthesis	30
2.2.1.5	Metabolic Function Interactions	31
2.2.2	Responses to Electron Acceptor Limitations	31
2.2.3	Responses to Electron Donor Limitations	33
2.3	Biological System Modelling	34
2.3.1	Biological Process Modelling Overview	34
2.3.2	Limitations of Current Modelling Approaches	36
2.3.2.1	Transient vs Steady State Models	36
2.3.2.2	Substrate Utilization Kinetics	37
CHAPTER 3. MODEL DEVELOPMENT		40
3.1	Substrate Utilization Kinetics	40
3.1.1	Electron Donor Utilization Rate	40
3.1.2	Electron Acceptor Utilization Rate	42
3.1.3	Secondary Substrate Utilization Rate	43
3.1.3.1	Reductive Dehalogenation	45
3.1.3.2	Oxygenation Reaction	47

3.1.4 Substrate Utilization in Biological Systems	48
3.1.4.1 Suspended Biomass Systems	48
3.1.4.2 Biofilm Systems	50
3.2 Biological Growth Kinetics	55
3.2.1 Suspended Biomass Growth	55
3.2.2 Biofilm Growth	56
3.3 Numerical Approach	57
3.3.1 Initial and Boundary Conditions	57
3.3.1.1 Suspended Biomass System	57
3.3.1.2 Biofilm System	57
3.3.2 Numerical Approximation Technique	60
3.3.2.1 Suspended Biomass System	61
3.3.2.2 Biofilm System	61
3.3.2.2.1 Intermediate Layer Substrate Utilization	62
3.3.2.2.2 Biofilm/Support Interface Layer Substrate Utilization	62
3.3.2.2.3 Biofilm/Liquid Interface Layer Substrate Utilization	63
3.3.2.2.4 Biofilm Layer Growth	64
3.3.2.2.5 Bulk Phase Substrate Utilization	66
3.4 Splitting and Joining of Biofilm Layers	66
3.4.1 Layer Splitting Feature	66
3.4.2 Layer Joining Feature	67
CHAPTER 4: MODEL SIMULATION	69
4.1 Model Assumptions	69
4.1.1 General Assumptions	69

4.1.2	Suspended Biomass System Assumptions	70
4.1.3	Biofilm System Assumptions	70
4.2	Simulation Overview	71
4.2.1	Suspended Biomass System	71
4.2.2	Biofilm System	71
4.3	Iterative vs. EFD Approach	75
4.3.1	Iterative Approach	75
4.3.2	EFD Approach	76
4.3.3	Comparison and Justification of Use	76
CHAPTER 5	MODEL VERIFICATION	81
5.1	Numerical Verification	81
5.2	Spatial and Temporal Discretization	81
5.2.1	Time Step	82
5.2.2	Biofilm Layer Thickness	82
5.2.2.1	Minimum Layer Thickness	82
5.2.2.2	Maximum Layer Thickness	83
5.3	Dynamic Biofilm Model Responses	86
5.3.1	Start-Up Biofilm Profile	88
5.3.2	Bulk Phase and Biofilm Layer Concentration Fluctuations	88
5.4	Model Comparison to Previous Studies	100
5.4.1	Suspended Biomass System	100
5.4.1.1	Primary Single Substrate Limitation Case	100
5.4.1.2	Primary Dual Substrate Limitation Case	107
5.4.1.3	Cometabolic Dual Substrate Limitation Case	113

5.4.2 Biofilm System	119
5.4.2.1 Steady State Single Substrate Limitation Cases	124
5.4.2.2 Transient Single Substrate Limitation Case	128
CHAPTER 6 SENSITIVITY ANALYSIS	138
6.1 Base Cases	138
6.1.1 Suspended Biomass System	138
6.1.2 Biofilm System	142
6.2 Sensitivity to Initial Condition Values	146
6.2.1 Suspended Biomass System	146
6.2.2 Biofilm System	153
6.3 Normalized Sensitivity Analysis	162
6.4 Results of Sensitivity Analysis	163
6.4.1 Suspended Biomass System	163
6.4.2 Biofilm System	165
6.4.3 Suspended Biomass System vs Biofilm System	168
CHAPTER 7. CASE STUDIES	169
7.1 Case 1: System Aeration Variations	169
7.1.1 Case 1 Suspended Biomass System Results and Discussion	169
7.1.2 Case 1 Biofilm System Results and Discussion	176
7.2 Case 2: Organic Loading Variations	188
7.2.1 Case 2 Suspended Biomass System Results and Discussion	188
7.2.2 Case 2 Biofilm System Results and Discussion	193
7.3 Case 3: Hydraulic Retention Time Variations	204
7.3.1 Case 3 Suspended Biomass System Results and Discussion	204

7 3 2 Case 3 Biofilm System Results and Discussion	209
7 4 Suspended Biomass System vs Biofilm System	220
CHAPTER 8 CONCLUSIONS AND RECOMMENDATIONS	222
8.1 Conclusions	222
8 2 Recommendations	224
BIBLIOGRAPHY	225
APPENDIX A: FINITE DIFFERENCE EQUATIONS	233
APPENDIX B SOURCE CODE	237

LIST OF TABLES

Table 2.1: Summary of biological systems modelling	38
Table 3.1: Secondary substrate utilization rate summary	46
Table 4.1: Biofilm input parameters for approximation technique comparison	77
Table 5.1: Secondary substrate biokinetic parameters	89
Table 5.2: Growth parameters from batch reactor data (Shamat and Maier, 1980)	102
Table 5.3: Experimental dual substrate limitation kinetic parameter values (Sinclair and Ryder, 1975)	108
Table 5.4: Dual substrate limitation model kinetic parameter values (Bae, 1992)	118
Table 5.5: Biofilm kinetic parameter values (Rittmann and McCarty, 1980, Saez and Rittmann, 1988)	125
Table 5.6: Transient model kinetic parameter values (Skowlund, 1990)	129
Table 6.1: Suspended biomass system base case kinetic and operational parameters	139
Table 6.2: Biofilm system base case kinetic and operational parameters	143
Table 6.3: Normalized sensitivity coefficient: suspended biomass system	164
Table 6.4: Normalized sensitivity coefficient: biofilm system	166

M		Reducing power constant
M_a		Mass of electron acceptor
M_d		Mass of electron donor
M_e		Mass of enzyme
M_s		Mass of substrate
M_x	=	Mass of biomass
M_2	=	Mass of secondary substrate
MLSS	=	Mixed liquor suspended solids
NAD	=	Nicotinamide adenine dinucleotide - oxidized state
NADH	=	Nicotinamide adenine dinucleotide - reduced state
NADP	=	Nicotinamide adenine dinucleotide phosphate - oxidized state
NADPH	=	Nicotinamide adenine dinucleotide phosphate - reduced state
q_a	=	Electron acceptor specific utilization rate ($M_a M_e^{-1} T^{-1}$)
q_d	=	Electron donor specific utilization rate ($M_d M_e^{-1} T^{-1}$)
q_{ma}	=	Electron acceptor apparent maximum specific utilization rate ($M_a M_e^{-1} T^{-1}$)
q_{md}	=	Electron donor apparent maximum specific utilization rate ($M_d M_e^{-1} T^{-1}$)
q_{md}^1	=	Electron donor maximum specific utilization rate ($M_d M_e^{-1} T^{-1}$)
q_{mea}	=	Enzyme specific electron acceptor maximum utilization rate ($M_a M_e^{-1} T^{-1}$)
q_{med}	=	Enzyme specific electron donor maximum utilization rate ($M_d M_e^{-1} T^{-1}$)
q_{me2}	=	Enzyme specific secondary substrate maximum utilization rate ($M_2 M_e^{-1} T^{-1}$)
q_{m2}	=	Secondary substrate apparent maximum specific utilization rate ($M_2 M_e^{-1} T^{-1}$)
Q	=	Reactor volumetric flow rate ($L^3 T^{-1}$)
r_a	=	Electron acceptor utilization rate by respiratory enzymes ($M_a L^{-3} T^{-1}$)
r_b	=	Bulk phase substrate utilization or biomass growth rate ($M_x L^{-3} T^{-1}$)

Figure 5.10: Biofilm/liquid interface and bulk phase electron acceptor fluctuations due to layer splitting	97
Figure 5.11: Biofilm/liquid interface and bulk phase secondary substrate fluctuations due to layer splitting	98
Figure 5.12: Total biofilm thickness fluctuations due to layer splitting	99
Figure 5.13: Chlorinated substrate remaining in batch reactors after inoculation with MLSS (Shamat & Maier, 1980)	103
Figure 5.14: Chlorinated substrate remaining in batch reactors after inoculation with MLSS (COMDEG)	104
Figure 5.15: Effluent chlorinated substrate concentrations for different residence time values (Shamat and Maier, 1980)	105
Figure 5.16: Effluent chlorinated substrate concentrations for different residence time values (COMDEG)	106
Figure 5.17: Experimental and interacting kinetic model results for 0.2 kg/m ³ glycerol concentration (Sinclair and Ryder, 1975)	109
Figure 5.18: Experimental and interacting kinetic model results for 1.0 kg/m ³ glycerol concentration (Sinclair and Ryder, 1975)	110
Figure 5.19: Experimental and interacting kinetic model results for 2.0 kg/m ³ glycerol concentration (Sinclair and Ryder, 1975)	111
Figure 5.20: Experimental and interacting kinetic model results for 5.0 kg/m ³ glycerol concentration (Sinclair and Ryder, 1975)	112
Figure 5.21: Numerical model results for 0.2 kg/m ³ glycerol concentration (COMDEG)	114
Figure 5.22: Numerical model results for 1.0 kg/m ³ glycerol concentration (COMDEG)	115
Figure 5.23: Numerical model results for 2.0 kg/m ³ glycerol concentration (COMDEG)	116
Figure 5.24: Numerical model results for 5.0 kg/m ³ glycerol concentration (COMDEG)	117
Figure 5.25: Removal of secondary substrate undergoing an oxygenase reaction, $K_{so} = 0.06$ mg DO/L (Bae, 1992)	120
Figure 5.26: Removal of secondary substrate undergoing an oxygenase reaction, $K_{so} = 0.019$ mg DO/L (Bae, 1992)	121
Figure 5.27: Removal of secondary substrate undergoing an oxygenase reaction, $K_{so} = 0.06$ mg DO/L (COMDEG)	122

Figure 5.28: Removal of secondary substrate undergoing an oxygenase reaction, $K_{\omega} = 0.019$ mg DO/L (COMDEG)	123
Figure 5.29: Steady state biofilm thickness predictions using the Rittmann and McCarty (1980), Sáez and Rittmann (1988) and COMDEG models	126
Figure 5.30: Steady state substrate flux into a biofilm predictions using the Rittmann and McCarty (1980), Sáez and Rittmann (1988) and COMDEG models	127
Figure 5.31: Total and active biofilm growth using different inactivation coefficients in an unlimited biofilm (Skowlund, 1990)	130
Figure 5.32: Total and active biofilm growth using different inert material retention coefficients in an unlimited biofilm (COMDEG)	131
Figure 5.33: Active fraction profile vs. biofilm depth at five different times (Skowlund, 1990)	134
Figure 5.34: Substrate concentration profile vs. biofilm depth at five different times (Skowlund, 1990)	135
Figure 5.35: Active fraction profile vs. biofilm depth at five different times (COMDEG)	136
Figure 5.36: Substrate concentration profile vs. biofilm depth at five different times (COMDEG) ...	137
Figure 6.1: Primary and secondary bulk phase substrate concentrations under base case conditions .	140
Figure 6.2: Suspended biomass growth under base case conditions	141
Figure 6.3: Biofilm bulk phase substrate concentrations under base case conditions	144
Figure 6.4: Total and active biofilm growth under base case conditions	145
Figure 6.5: Biofilm substrate concentration profiles under base case conditions	147
Figure 6.6: Biofilm steady state active fraction profile under base case conditions	148
Figure 6.7: Suspended biomass system: electron donor concentration response to initial biomass concentrations	149
Figure 6.8: Suspended biomass system: electron acceptor concentration response to initial biomass concentrations	150
Figure 6.9: Suspended biomass system: secondary substrate concentration response to initial biomass concentrations	151
Figure 6.10: Suspended biomass system: biomass growth response to initial biomass concentrations	152

Figure 6.11: Biofilm system: bulk phase electron donor concentration response to initial biofilm thickness and active fraction conditions	154
Figure 6.12: Biofilm system: bulk phase electron acceptor concentration response to initial biofilm thickness and active fraction conditions	155
Figure 6.13: Biofilm system: bulk phase secondary substrate concentration response to initial biofilm thickness and active fraction conditions	156
Figure 6.14: Biofilm system: biofilm thickness response to initial biofilm thickness and active fraction conditions	157
Figure 6.15: Biofilm system: electron donor profile at steady state for various initial biofilm thickness and active fraction conditions	158
Figure 6.16: Biofilm system: electron acceptor profile at steady state for various initial biofilm thickness and active fraction conditions	159
Figure 6.17: Biofilm system: secondary substrate profile at steady state for various initial biofilm thickness and active fraction conditions	160
Figure 6.18: Biofilm system: active fraction profile at steady state for various initial biofilm thickness and active fraction conditions	161
Figure 7.1: Suspended biomass system: steady state electron donor concentration with variations in $k_L a$	171
Figure 7.2: Suspended biomass system: steady state electron acceptor concentration with variations in $k_L a$	172
Figure 7.3: Suspended biomass system: steady state secondary substrate concentration with variations in $k_L a$	173
Figure 7.4: Suspended biomass system: steady state biomass concentration with variations in $k_L a$	174
Figure 7.5: Suspended biomass system: secondary substrate removal as a function of primary substrate concentrations	175
Figure 7.6: Biofilm system: steady state bulk phase electron donor concentration with variations in $k_L a$	177
Figure 7.7: Biofilm system: steady state bulk phase electron acceptor concentration with variations in $k_L a$	178
Figure 7.8: Biofilm system: steady state bulk phase secondary substrate concentration with variations in $k_L a$	179
Figure 7.9: Biofilm system: steady state biofilm thickness with variations in $k_L a$	180

Figure 7.10: Biofilm system: bulk phase secondary substrate removal as a function of primary substrate concentrations	182
Figure 7.11: Biofilm system: steady state electron donor profile with variations in $k_L a$	184
Figure 7.12: Biofilm system: steady state electron acceptor profile with variations in $k_L a$	185
Figure 7.13: Biofilm system: steady state secondary substrate profile with variations in $k_L a$	186
Figure 7.14: Biofilm system: steady state active fraction profile with variations in $k_L a$	187
Figure 7.15: Suspended biomass system: steady state electron donor concentration with variations in S_{di}	189
Figure 7.16: Suspended biomass system: steady state electron acceptor concentration with variations in S_{di}	190
Figure 7.17: Suspended biomass system: steady state secondary substrate concentration with variations in S_{di}	191
Figure 7.18: Suspended biomass system: steady state biomass concentration with variations in S_{di}	192
Figure 7.19: Biofilm system: steady state bulk phase electron donor concentration with variations in S_{di}	195
Figure 7.20: Biofilm system: steady state bulk phase electron acceptor concentration with variations in S_{di}	196
Figure 7.21: Biofilm system: steady state bulk phase secondary substrate concentration with variations in S_{di}	197
Figure 7.22: Biofilm system: steady state biofilm thickness with variations in S_{di}	198
Figure 7.23: Biofilm system: steady state electron donor profile with variations in S_{di}	200
Figure 7.24: Biofilm system: steady state electron acceptor profile with variations in S_{di}	201
Figure 7.25: Biofilm system: steady state secondary substrate profile with variations in S_{di}	202
Figure 7.26: Biofilm system: steady state active fraction profile with variations in S_{di}	203
Figure 7.27: Suspended biomass system: steady state electron donor concentration with variations in HRT	205
Figure 7.28: Suspended biomass system: steady state electron acceptor concentration with variations in HRT	206
Figure 7.29: Suspended biomass system: steady state secondary substrate concentration with variations in HRT	207

Figure 7.30: Suspended biomass system: steady state biomass concentration with variations in HRT.....	208
Figure 7.31: Biofilm system: steady state bulk phase electron donor concentration with variations in HRT.	211
Figure 7.32: Biofilm system: steady state bulk phase electron acceptor concentration with variations in HRT.....	212
Figure 7.33: Biofilm system: steady state bulk phase secondary substrate concentration with variations in HRT.....	213
Figure 7.34: Biofilm system: steady state biofilm thickness with variations in HRT	214
Figure 7.35: Biofilm system: steady state electron donor profile with variations in HRT	215
Figure 7.36: Biofilm system: steady state electron acceptor profile with variations in HRT	216
Figure 7.37: Biofilm system: steady state secondary substrate profile with variations in HRT	217
Figure 7.38: Biofilm system: steady state active fraction profile with variations in HRT	218

SYMBOLS AND NOMENCLATURE

a	=	Biofilm specific area ($L_f^2 L^{-3}$)
A	=	Biofilm cross-sectional area (L_f^2)
$ADP \cdot P_i$	=	Adenosine diphosphate + inorganic phosphate
ATP	=	Adenosine triphosphate
b	=	Endogenous decay coefficient (T^{-1})
B	=	Contois constant (dimensionless)
C_s	=	Oxygen saturation concentration ($M_s L^{-3}$)
COD	=	Chemical oxygen demand
$CSTR$	=	Continuous stirred tank reactor
D_s	=	Electron acceptor diffusion coefficient through water ($L^2 T^{-1}$)
D_{af}	=	Electron acceptor diffusion coefficient through biofilm ($L_f^2 T^{-1}$)
D_d	=	Electron donor diffusion coefficient through water ($L^2 T^{-1}$)
D_{df}	=	Electron donor diffusion coefficient through biofilm ($L_f^2 T^{-1}$)
D_f	=	Diffusion coefficient through biofilm ($L_f^2 T^{-1}$)
D_2	=	Secondary substrate diffusion coefficient through water ($L^2 T^{-1}$)
D_{2f}	=	Secondary substrate diffusion coefficient through biofilm ($L_f^2 T^{-1}$)
DO	=	Dissolved oxygen
$[E_T]$	=	Total specified enzyme content ($M_e M_x^{-1}$)
f	=	Active biomass fraction ($M_x M_x^{-1}$)
f_a	=	New active biomass fraction ($M_x M_x^{-1}$)
f_o	=	Initial active biomass fraction ($M_x M_x^{-1}$)
FAD	=	Flavin adenine dinucleotide - oxidized state
$FADH_2$	=	Flavin adenine dinucleotide - reduced state

I_d	=	Electron donor interaction coefficient ($M_d M_2^{-1}$)
I_2	=	Secondary substrate interaction coefficient ($M_2 M_d^{-1}$)
J	=	Substrate flux into biofilm ($M_s L^{-2} T^{-1}$)
J_a	=	Electron acceptor flux into biofilm ($M_a L^{-2} T^{-1}$)
J_d	=	Electron donor flux into biofilm ($M_d L^{-2} T^{-1}$)
J_2	=	Secondary substrate flux into biofilm ($M_2 L^{-2} T^{-1}$)
$k_L a$	=	Oxygen/aeration mass transfer coefficient (T^{-1})
k_m	=	Substrate effective diffusion layer mass transfer coefficient (LT^{-1})
k_{ma}	=	Electron acceptor effective diffusion layer mass transfer coefficient (LT^{-1})
k_{md}	=	Electron donor effective diffusion layer mass transfer coefficient (LT^{-1})
k_{m2}	=	Secondary substrate effective diffusion layer mass transfer coefficient (LT^{-1})
k_i	=	Inert material retention coefficient ($M_i M_r^{-1}$)
K_i	=	Skowlund inactivation coefficient (T^{-1})
K_{id}	=	Electron donor inhibition constant ($M_d L^{-3}$)
K_{i2}	=	Secondary substrate inhibition constant ($M_2 L^{-3}$)
K_{sa}	=	Electron acceptor half maximum rate concentration ($M_a L^{-3}$)
K_{sd}	=	Electron donor half maximum rate concentration ($M_d L^{-3}$)
K_{so}	=	Oxygenase half maximum rate concentration for dissolved oxygen ($M_a L^{-3}$)
K_{s2}	=	Secondary substrate half maximum rate concentration ($M_2 L^{-3}$)
L	=	Effective diffusion-resistance layer thickness (L)
L	=	Reactor length unit
L_f	=	Total biofilm layer thickness (L_f)
L_f	=	Biofilm length unit
L_{f0}	=	Initial biofilm layer thickness (L_f)

M	Reducing power constant
M_a	Mass of electron acceptor
M_d	Mass of electron donor
M_e	Mass of enzyme
M_s	Mass of substrate
M_b	Mass of biomass
M_2	Mass of secondary substrate
MLSS	Mixed liquor suspended solids
NAD	Nicotinamide adenine dinucleotide - oxidized state
NADH	Nicotinamide adenine dinucleotide - reduced state
NADP	Nicotinamide adenine dinucleotide phosphate - oxidized state
NADPH	Nicotinamide adenine dinucleotide phosphate - reduced state
q_a	= Electron acceptor specific utilization rate ($M_a M_e^{-1} T^{-1}$)
q_d	= Electron donor specific utilization rate ($M_d M_e^{-1} T^{-1}$)
q_{ma}	= Electron acceptor apparent maximum specific utilization rate ($M_a M_e^{-1} T^{-1}$)
q_{md}	= Electron donor apparent maximum specific utilization rate ($M_d M_e^{-1} T^{-1}$)
q_{md}^1	= Electron donor maximum specific utilization rate ($M_d M_e^{-1} T^{-1}$)
q_{mea}	= Enzyme specific electron acceptor maximum utilization rate ($M_a M_e^{-1} T^{-1}$)
q_{med}	= Enzyme specific electron donor maximum utilization rate ($M_d M_e^{-1} T^{-1}$)
q_{me2}	= Enzyme specific secondary substrate maximum utilization rate ($M_2 M_e^{-1} T^{-1}$)
q_{m2}	= Secondary substrate apparent maximum specific utilization rate ($M_2 M_e^{-1} T^{-1}$)
Q	= Reactor volumetric flow rate ($L^3 T^{-1}$)
r_a	= Electron acceptor utilization rate by respiratory enzymes ($M_s L^{-3} T^{-1}$)
r_b	= Bulk phase substrate utilization or biomass growth rate ($M_s L^{-3} T^{-1}$)

r_d	=	Electron donor utilization rate by catabolic enzymes ($M_d L^{-3} T^{-1}$)
r_{db}	=	Bulk phase electron donor utilization rate by catabolic enzymes ($M_d L^{-3} T^{-1}$)
r_{df}	=	Biofilm electron donor utilization rate by catabolic enzymes ($M_d L_f^{-3} T^{-1}$)
r_f	=	Biofilm substrate utilization rate ($M_s L_f^{-3} T^{-1}$)
r_g	=	Biofilm generation rate ($M_s L_f^{-3} T^{-1}$)
r_i	=	Biofilm decay rate ($M_s L_f^{-3} T^{-1}$)
r_s	=	Suspended biomass growth rate ($M_s L^{-3} T^{-1}$)
r_{sf}	=	Biofilm secondary substrate utilization rate ($M_s L_f^{-3} T^{-1}$)
r_{so}	=	Secondary substrate utilization rate via oxygenation reaction ($M_s L^{-3} T^{-1}$)
r_{srd}	=	Secondary substrate utilization rate via reductive dehalogenation ($M_s L^{-3} T^{-1}$)
R_p	=	Reducing power factor
RBC	=	Rotating biological contactor
sl	=	Sloughing coefficient (T^{-1})
S_a	=	Electron acceptor concentration ($M_a L^{-3}$)
S_{ab}	=	Bulk phase electron acceptor concentration ($M_a L^{-3}$)
S_{abi}	=	Bulk phase influent electron acceptor concentration ($M_a L^{-3}$)
S_{abo}	=	Bulk phase initial electron acceptor concentration ($M_a L^{-3}$)
S_{af}	=	Biofilm electron acceptor concentration ($M_a L_f^{-3}$)
S_{as}	=	Biofilm/liquid interface electron acceptor concentration ($M_a L_f^{-3}$)
S_b	=	Bulk phase substrate concentration ($M_s L^{-3}$)
S_{bi}	=	Bulk phase influent substrate concentration ($M_s L^{-3}$)
S_d	=	Electron donor concentration ($M_d L^{-3}$)
S_{db}	=	Bulk phase electron donor concentration ($M_d L^{-3}$)
S_{di}	=	Bulk phase influent electron donor concentration ($M_d L^{-3}$)

S_{db}	Bulk phase initial electron donor concentration ($M_d L^{-3}$)
S_{df}	Biofilm electron donor concentration ($M_d L_f^{-3}$)
S_{di}	Biofilm/liquid interface electron donor concentration ($M_d L_f^{-3}$)
S_f	Biofilm substrate concentration ($M_s L_f^{-3}$)
S_{fn}	New biofilm substrate concentration ($M_s L_f^{-3}$)
S_i	Biofilm/liquid interface substrate concentration ($M_s L_f^{-3}$)
S_2	Secondary substrate concentration ($M_2 L^{-3}$)
S_{2b}	Bulk phase secondary substrate concentration ($M_2 L^{-3}$)
S_{2bi}	Bulk phase influent secondary substrate concentration ($M_2 L^{-3}$)
S_{2bo}	Bulk phase initial secondary substrate concentration ($M_2 L^{-3}$)
S_{2f}	Biofilm secondary substrate concentration ($M_2 L_f^{-3}$)
S_{2i}	Biofilm/liquid interface secondary substrate concentration ($M_2 L_f^{-3}$)
t	Time (T)
T	Time unit
T_2	Electron donor transformation capacity ($M_2 M_d^{-1}$)
μ	Specific growth rate ($M_r M_r^{-1} T^{-1}$)
μ_m	Maximum specific growth rate ($M_r M_r^{-1} T^{-1}$)
v_{ad}	Electron acceptor consumption/electron donor consumption coefficient ($M_s M_d^{-1}$)
v_{ax}	Electron acceptor consumption/cell decay coefficient ($M_s M_r^{-1}$)
V	Reactor volume (L^3)
X	Bulk phase biomass concentration ($M_r L^{-3}$)
X_f	Biofilm biomass density ($M_r L_f^{-3}$)
X_i	Bulk phase influent biomass concentration ($M_r L^{-3}$)
X_m	Bulk phase maximum achievable biomass concentration ($M_r L^{-3}$)

X_0	=	Bulk phase initial biomass concentration (M, L^{-3})
Y	=	Biomass yield coefficient (M, M_d^{-1})
z	=	Biofilm layer thickness (L_t)
z_a	=	Active biofilm layer thickness (L_t)
z_i	=	Inactive biofilm layer thickness (L_t)
z_l	=	Minimum biofilm layer thickness (L_t)
z_m	=	Maximum biofilm layer thickness (L_t)

CHAPTER 1

INTRODUCTION

1.1 OVERVIEW

Ecological and public health considerations have resulted in great attention being given to the microbial degradation of synthetic organic compounds. When these compounds are introduced in a soil, water or wastewater treatment system, microorganisms present in such environments should, in theory, be able to convert the organic chemicals to their inorganic products. Although mineralization is thought to eventually occur for most organics, some may persist for some time and, consequently, accumulate in the environment and migrate up the food chain.

Research has shown that multiple substrate systems often provide growth enhancement by concurrent utilization of more than one substrate as an energy source which can result in a more efficient metabolism. This can translate into a more rapid and successful degradation of hazardous compounds of interest (Kim and Maier, 1986, Klecka and Maier, 1988). It has also been demonstrated that external electron donor and electron acceptor concentrations will strongly affect the removal efficiency of hazardous compounds (Bae, 1992, Bae and Rittmann, 1994). As such, the effects of dual substrate limitation and cosubstrate utilization should be addressed in the modelling of biological systems.

Since the mid 1970's, biological treatment research has focused on the development of kinetic models to simulate and predict primary and secondary substrate removal in suspended and fixed-film biological systems. Previous modelling efforts have primarily examined zero or first order kinetics in steady state and, in some cases, unsteady state environments. However, these previous efforts have not considered the impact of dual substrate limitation and cosubstrate

interaction on secondary substrate removal in unsteady state suspended biomass and biofilm systems

1.2 OBJECTIVES

In this research, it is intended to develop a computer model based on modified Monod kinetics which will account for dual substrate limitation and cosubstrate utilization in suspended and fixed-film biological systems. The model will simulate and predict unsteady state variations in electron donor (primary substrate), secondary substrate and electron acceptor concentrations, as well as changes in active biomass concentrations within a continuously stirred tank reactor (CSTR). The computer model will also predict unsteady state substrate concentration profiles, as well as, fluctuations in biofilm thickness and active biomass within a biofilm.

The specific undertakings include the development of the numerical model using a set of non-linear, unsteady state, differential equations based on works by Kissel (1984), Wanner and Gujer (1984, 1986), Skowlund (1990) and Bae (1992). This model will be capable of simulating primary and secondary substrate removal under single and dual substrate limitation conditions in both suspended biomass and biofilm systems.

The second undertaking will involve the verification of the numerical model response using existing numerical models and experimental data. Next, a sensitivity analysis will be performed to enable the identification of system parameters which can most affect simulation results, and must, therefore, be evaluated with the greatest accuracy.

The final undertaking will be the application of the numerical model to three theoretical case studies. The purpose is to test the response of the suspended biomass and biofilm systems under aeration variations, fluctuating organic loadings and fluctuating flow rate conditions.

CHAPTER 2

BACKGROUND

2.1 MULTIPLE SUBSTRATE INTERACTIONS

Multiple substrate systems often provide growth enhancement through concurrent utilization of more than one substrate as an energy source which results in a more efficient metabolism. This can translate into a more rapid and successful degradation of certain hazardous compounds of interest (Kim and Maier, 1986; Klecka and Maier, 1988). Various pathways have been suggested to describe the degradation of halogenated secondary substrates. It has been demonstrated that the internal reducing power provided by external electron donor and electron acceptor concentrations can strongly affect the removal efficiency of these halogenated compounds via reductive dehalogenation and oxygenation reactions (Bae, 1992; Bae and Rittmann, 1994).

2.1.1 SUBSTRATE DEFINITIONS

The main requirement in biological processes is the growth of cells to a density which will allow for the degradation of one or many target compounds at an acceptable rate. To sustain a suitable density, the cells must utilize electron donors, energy sources and electron acceptors at rates which result in a net positive growth.

2.1.1.1 ELECTRON DONOR

A growth substrate is an electron donor that provides reducing power and energy for cell growth and maintenance; whereas, an energy substrate is an electron donor that provides reducing power and energy, but does not support growth by itself. The oxidation of an electron donor, also referred to as primary substrate, provides energy and electrons for the cell synthesis and growth of chemotrophic microorganisms. In biological treatment processes, the primary substrate is usually

an organic compound which can be easily metabolized by the inherent biomass (Grady, 1985; Rittmann *et al.*, 1988; Criddle, 1993). In order to facilitate the comparison of various electron donors, substrate concentrations are generally expressed in terms of mg COD/L equivalents.

2.1.1.2 ELECTRON ACCEPTOR

For chemotrophic microorganisms, electrons are transferred from an electron donor to an electron acceptor releasing free energy which can be captured and used for the synthesis of new cell material. Typical electron acceptors include oxygen (O_2) in aerobic environments, nitrate (NO_3^-) and nitrite (NO_2^-) in nitrifying/denitrifying systems, and sulfate (SO_4^{2-}) and bicarbonate (HCO_3^-) in certain anaerobic environments. These compounds are reduced upon acceptance of electrons from the electron donor (Gaudy and Gaudy, 1980). For comparative purposes, the concentrations of electron acceptors are converted to units of mg DO/L equivalents because it is this dissolved oxygen equivalent which will affect the respiratory subsystem of the microorganisms.

2.1.1.3 SECONDARY SUBSTRATE

A secondary substrate is a compound whose transformation and/or oxidation does not supply significant energy for microbial growth. This can occur for one of two reasons. (1) The substrate is present in very low concentrations where the contribution of electrons and energy due to oxidation is insignificant compared to that of the primary substrate. (2) The substrate can be transformed only as a cometabolite which yields no energy or electrons because the compound is either only partially transformed or the energy and electrons are not captured for use (Rittmann *et al.*, 1988).

A class of compounds which are of particular interest as secondary substrates are xenobiotic (synthetic organic) compounds. These xenobiotic compounds are often persistent in

natural systems and toxic to humans and/or the environment at high concentrations. However, they are generally present at low concentrations in industrial and municipal wastewaters.

The secondary substrate type treated in this thesis is defined as being present in trace amounts and, as such, does not contribute significantly to microbial growth, does not exert an electron acceptor demand and does not significantly inhibit or affect microbial degradation functions.

2.1.2 CONCEPT OF COSUBSTRATE UTILIZATION

In the metabolism of multiple substrates, the term cometabolism is often used interchangeably for degradation patterns such as diauxie, simultaneous utilization and competitive inhibition. In order to avoid confusion, these terms are defined in this section.

Diauxie or sequential utilization generally results from catabolite repression or catabolite inhibition. Only one substrate can be consumed until its supply is exhausted before the consumption of the second substrate is initiated. The presence of the first substrate represses the degradation of the second substrate. Simultaneous utilization refers to the degradation of two or more substrates by a microbial system and may or may not result in growth from all substrates being degraded. Competitive inhibition occurs when two growth substrates are consumed by microorganisms at a higher or lower rate than if they were present as the sole growth substrate. Cometabolism refers to the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound and can also include transformations by resting cells if no growth results (Chang *et al.*, 1993). The latter definition of the term cometabolism will be utilized herein.

2.1.2.1 HALOGENATED SECONDARY SUBSTRATE DEGRADATION REACTIONS

As a group, halogenated organic compounds constitute a majority of the currently listed priority pollutants. Chlorinated organics are the most prevalent and best documented, although the presence of bromo-, iodo- and fluoro-substituents has been reported (Knackmuss, 1981; Vogel *et al.*, 1987; Vogel, 1988; Weightman and Slater, 1979; Neilson, 1990). In order to be successful, the biodegradation of halogenated aliphatics and aromatics must be preceded or accompanied by the dehalogenation of the parent compound (Kobayashi and Rittmann, 1982).

In general, one of three types of enzyme mediated reaction mechanisms are involved in the dehalogenation of aliphatic and aromatic compounds; namely, hydrolytic oxidation, oxygenolytic oxidation or reductive dehalogenation (Colwell and Saylor, 1978). The most common biodehalogenation reactions are summarized in Figure 2.1. These biologically mediated reactions often require external energy in the form of electron acceptors (e.g. NAD, NADP or FAD) and donors (e.g. NADH, NADPH or FADH₂). The type of reaction that occurs will depend upon the structure of the compound and the ambient environmental conditions (Vogel *et al.*, 1987).

Halogenated organic compounds are often found in the aqueous phase at relatively low concentrations and must be accumulated intracellularly to induce the enzyme activity required for biodegradation. Energetically, the accumulation of the secondary substrate and its initial biodegradation are considered fortuitous because no ATP is generated as a result of the breakdown of the secondary substrate. Thus, the energy must be obtained from the metabolism of the primary electron donor and electron acceptor. However, certain aliphatic end products may eventually be formed which can be shunted to energy-generating pathways such as the tricarboxylic acid or glyoxylate cycles (Colwell and Saylor, 1978; Neilson, 1990).

Hydrolytic Reactions

Oxygenolytic Reactions

Reductive Dehalogenation Reactions

Figure 2-1: Common biodehalogenation reactions.
(X: halogen constituent, R: aliphatic or aromatic carbon structure, A: reduced compound)

2.1.2.1.1 HYDROLYTIC REACTIONS

Hydrolytic dehalogenation involves the displacement of a halogen substituent with a hydroxyl group employing a series of substrate-specific dehalogenase enzymes. Experiments aimed at clarifying the dehalogenation process have revealed that the reaction utilizes water, as opposed to molecular oxygen, as the source of the hydroxyl group.

The reaction is generally specific to halogenated aliphatics, although it has been reported as a mechanism in some aromatics. The enzyme appears to be more specific toward *para*-oriented insertions in aromatic compounds (Colwell and Saylor, 1978; Vogel *et al.*, 1987; Reineke and Knackmuss, 1988). Although these reactions are formally represented as simple nucleophilic displacements of the chlorine atoms by hydroxyl groups, some enzymes have been shown to be glutathione-dependent and to belong to the glutathione S-transferase supergene family which would suggest that consecutive nucleophilic reactions must be involved (Neilson, 1990).

2.1.2.1.2 OXYGENOLYTIC REACTIONS

In the presence of molecular oxygen, dehalogenation can be achieved by the displacement of the halogen substituent by oxygen via non-specific, mixed-function monooxygenases or dioxygenases. The reaction involves the addition of molecular oxygen prior to substrate oxidation or aromatic ring cleavage and, in general, is not specific for the halogenated organic. The reaction leads to one of two substitution products; alcohols or diols in the case of halogenated aromatics. In addition, the elimination of the halogen substituent in aromatic compounds can proceed prior to or following ring cleavage. Oxygenase systems are employed extensively in the degradation of non-halogenated aromatic ring systems (Colwell and Saylor, 1978; Reineke and Knackmuss, 1988, Neilson, 1990).

The oxygenolytic process introduces the oxygen as a hydroxyl group where the hydrogen is ultimately obtained from the reduced cofactor, NADH. The oxygenase system of most bacteria

generally include cytochrome or iron-sulfur proteins that can mediate oxidation via the electron transport chain. The hydrogen required to complete the oxidation is transported from the NADH via the electron transport chain of the respiratory subsystem where it combines with an oxygen molecule to form the hydroxyl group. Thus, molecular oxygen, as well as the Fe^{2+} and NADH cofactors, are essential in order to obtain efficient oxygenase enzyme activity (Vogel *et al.*, 1987).

The mediation of oxygenolytic oxidation via the respiratory subsystem and metabolic cofactor production allows for the modelling of this dehalogenation reaction since both the respiratory and metabolic subsystems can be controlled by external electron donor and electron acceptor concentrations.

2.1.2.1.3 REDUCTIVE DEHALOGENATION REACTIONS

Out of necessity, microorganisms have evolved different mechanisms for biodegradation in the absence of molecular oxygen. Many of the exact pathways and enzymes involved are still unclear, but the breakdown of organics in the absence of oxygen is now known to proceed under at least five different situations: through anaerobic photometabolism, under nitrate-reducing conditions, with sulfate as the electron acceptor, through fermentation and through fermentation coupled to methanogenesis (Reineke and Knackmuss, 1988).

Most reduction reactions require the use of transition metals or transition metal complexes as intermediates in the transfer of electrons. The initial step involves the transfer of a single electron resulting in the formation of an alkyl radical and the oxidation of a metal complex. The alkyl radical then scavenges a hydrogen atom from the immediate surrounding matrix or, most likely, from the transition metal complex itself (Vogel *et al.*, 1987).

The formation of the alkyl radical involves either the transfer of an electron to the reduced transition metal complex, with a standard reduction potential as the driving force, or the transfer of a halogen atom, which is a process governed by carbon-halogen bond energies. Regardless of the

exact mechanism, compounds with higher standard reduction potentials or smaller carbon-halogen bond energies will be more conducive to faster one electron reductions. More highly halogenated organics which have higher relative standard reduction potentials than their less halogenated counterparts, will tend to be dehalogenated more quickly and release more energy upon reduction. This is consistent with a hypothesis that reductive dehalogenation is an energy-producing reaction coupled to the metabolism of the primary electron donor (Vogel *et al.*, 1987, Neilson, 1990).

Research has implicated cytochrome as an active reducing agent in the reductive dehalogenation process. Reduction results when the halogenated organic compound outcompetes oxygen (when present) for the electrons supplied by the NADH via the electron transport chain of the respiratory subsystem (Vogel *et al.*, 1987). As with the oxygenolytic oxidation process, reductive dehalogenation involves mediation by cofactors and the respiratory subsystem which should be accounted for in the modelling of such systems.

2.1.2.2 COSUBSTRATE UTILIZATION

Cometabolism refers to the transformation of a compound (xenobiotic) by metabolic reactions which do not contribute to the growth of the microorganism. In some cases, it is believed to result from the lack of specificity of enzymes and cofactors. A key factor influencing cometabolic reactions is the need for a cosubstrate (primary substrate) to support the growth of cometabolic organisms because the cometabolic utilization of xenobiotic compounds is not related to their use as nutrient or energy sources (Weightman and Slater, 1979, Criddle, 1993).

Many mathematical models that attempt to simulate secondary substrate utilization have been developed. These have included zero growth saturation kinetics, logistic growth models, transformation capacity models and inhibition/interaction models.

When metabolizing a secondary compound, with growth dependent upon a primary compound, the rate of transformation of the secondary compound is likely to follow some form of

saturation kinetics. The saturation kinetics are essentially the same as Monod kinetics except that the secondary substrate does not contribute to growth (Namkung *et al.*, 1983, Bouwer, 1985; Schmidt *et al.*, 1985, Grady, 1990, Criddle, 1993). Hence the rate of secondary substrate utilization is defined as.

$$-\frac{dS_2}{dt} = q_{m2}X\left(\frac{S_2}{K_{s2} + S_2}\right) \quad (2.1)$$

where S_2 is the secondary substrate concentration (M_2L^{-3}), K_{s2} is the half maximum rate concentration of the secondary substrate (M_2L^{-3}), q_{m2} is the maximum specific substrate utilization rate of the secondary substrate in the absence of the primary substrate ($M_2M_1^{-1}T^{-1}$), X is the biomass density (M_1L^{-3}) and t is time (T). This model does not account for any loss of transformation activity in the absence of the growth substrate or for the loss of transformation activity due to other factors such as product toxicity and inhibition.

Relationships that describe the growth of active organisms have been developed to model the degradation of compounds that do not contribute appreciably to growth. The most general expression of growth as a function of time that is utilized is the logistic equation which represents the growth of a population when there is an upper limit to the population density (Schmidt *et al.*, 1985; Hess *et al.*, 1990).

$$-\frac{dS_2}{dt} = q_{m2}\left(\frac{S_2}{K_{s2} + S_2}\right)\left[\frac{X_m}{1 + ((X_m + X_0)\exp(-\mu_m t)) / X_0}\right] \quad (2.2)$$

where X_m is the maximum biomass density achievable (M_1L^{-3}), X_0 is the initial biomass density (M_1L^{-3}) and μ_m is the maximum specific growth rate ($M_1M_1^{-1}T^{-1}$). The greatest advantage of the logistic equation over the Monod equation is that the logistic equation can be solved analytically to obtain an expression for population density as a function of time. The Monod equation must generally be solved numerically. One disadvantage of the logistic growth model is that it does not account for the possible loss of transformation activity.

Inhibition/interaction between primary and secondary substrates has been accounted for with the following relationships (Papanastasiou and Maier, 1982, Klecka and Maier, 1988, Gantzer, 1989, Grady, 1990, Chang *et al* , 1993, Sáez and Rittmann, 1994)

$$-\frac{dS_d}{dt} = q_{md} \left[\frac{S_d}{K_{s,d} + S_d + (S_d^2 / K_{i,d}) + (S_2 / I_2)} \right] X$$

and

(2.3)

$$-\frac{dS_2}{dt} = q_{m2} \left[\frac{S_2}{K_{s,2} + S_2 + (S_2^2 / K_{i,2}) + (S_d / I_d)} \right] X$$

where $K_{s,d}$ is the half maximum rate concentration of the electron donor ($M_d L^{-3}$) and S_d is the electron donor concentration ($M_d L^{-3}$), q_{md} is the maximum specific electron donor utilization rate ($M_d M_x^{-1} T^{-1}$), $K_{i,2}$ and $K_{s,d}$ are the substrate inhibition constants of the secondary substrate ($M_2 L^{-3}$) and electron donor ($M_d L^{-3}$) and I_2 and I_d are the interaction coefficients for the secondary substrate ($M_2 M_d^{-1}$) and electron donor ($M_d M_2^{-1}$). Competitive inhibition is not expected if the natural substrate for a constitutive enzyme is not present. Also, inhibitory and interactive effects of the secondary substrate on electron donor utilization rates are generally considered negligible when the secondary substrate is present in significantly lower concentrations than the electron donor (Criddle, 1993).

The transformation capacity model is based on the assumption that the secondary substrate utilization rate depends on two factors: (1) the utilization rate of the primary substrate and (2) the maximum rate at which the microorganisms can transform the secondary substrate in the absence of the primary substrate (Chang *et al* , 1993; Criddle, 1993).

$$-\frac{dS_2}{dt} = (T_2 q_d + q_{m2}) \left(\frac{S_2}{K_{s,2} + S_2} \right) X \quad (2.4)$$

where T_2 is the primary substrate (electron donor) transformation capacity ($M_2 M_d^{-1}$) and q_d is the specific utilization rate of the electron donor ($M_d M_x^{-1} T^{-1}$)

In a similar type of derivation, Bae and Rittmann (1994) presented a relationship which directly links secondary substrate utilization to electron donor utilization, consequently relating cosubstrate metabolism to external electron donor and electron acceptor concentrations through the NADH/NAD cofactor ratio. As was discussed in the previous section, enzyme mediated oxidation and reduction reactions require energy in the form of NADH in order to proceed. Thus, the relationship between electron donor, electron acceptor and secondary substrate concentrations is expressed through a reducing factor, R_p

$$-\frac{dS_2}{dt} = q_{m_{e2}}[E_T]R_p\left(\frac{S_2}{K_{s2} + S_2}\right)X \quad (2.5)$$

where $q_{m_{e2}}$ is the secondary substrate maximum specific utilization rate by a specified enzyme ($M_2M_c^{-1}T^{-1}$), $[E_T]$ is the total specified enzyme content per unit cell mass ($M_cM_c^{-1}$) and R_p is the reducing power factor which is a function of electron donor and electron acceptor concentrations which ultimately control the NADH/NAD ratio.

2.1.2.3 EXPERIMENTAL WORK INVOLVING COSUBSTRATE UTILIZATION

Papanastasiou and Maier (1982) conducted batch experiments with a 2,4-dichlorophenoxyacetate (2,4-D) acclimated wastewater mixed liquor suspended solids (MLSS) culture. It was populated predominantly by *Pseudomonas* species and capable of metabolizing glucose and 2,4-D as target substrates under aerobic conditions. The microorganisms were fed glucose and 2,4-D alone or as mixed substrates. Papanastasiou and Maier found that the degradation of glucose (84 mg/L) followed typical Monod kinetics in the absence of 2,4-D. Substrate utilization of 2,4-D (95 mg/L) alone followed inhibition kinetics which were believed to result from product toxicity. When both glucose (46 mg/L) and 2,4-D (56 mg/L) were available, mutual inhibition was observed. However, the effect on 2,4-D degradation was apparently masked by the fact that larger concentrations of active biomass were produced when glucose was available.

The concurrent utilization and substrate interaction demonstrated in this experiment resulted in a substantial reduction of the mean cell residence time for CSTR systems. They were also thought to extend the area of stability of the process into higher dilution rates and higher influent concentrations.

The secondary utilization of various trace halogenated organic compounds by aerobic and anaerobic biofilm consortia was examined by Bouwer (1985). Using acetate (1 mg/L and 100 mg/L) as a primary substrate, it was concluded that the Monod kinetics that were developed for a compound that was present as a primary substrate were also applicable when the compound was present as a secondary substrate at low concentrations ($<29 \mu\text{g/L}$). The biofilm thickness (biomass growth) was controlled solely by the degradation of the primary substrate. However, Bouwer did not examine the possible loss of secondary substrate transformation in the absence of the primary substrate.

Kim and Maier (1986) examined the aerobic degradation of 2,4-D and 3,5-dichlorobenzoate (3,5-DCB) by 2,4-D and 3,5-DCB acclimated MLSS cultures in the presence or absence of a nutrient broth. They found that at lower 2,4-D (1 mg/L and $140 \mu\text{g/L}$) and 3,5-DCB (1 mg/L and $120 \mu\text{g/L}$) concentrations, the presence of the nutrient broth (100 mg/L) as the primary substrate tended to inhibit the rate of degradation of 2,4-D and 3,5-DCB.

The presence of relatively high concentrations of non-growth secondary substrates, such as 3,5,6-trichloro-2-pyridinol (500-2000 $\mu\text{g/L}$) and 2,4-D (500-2000 $\mu\text{g/L}$), in a pentachlorophenol acclimated MLSS culture was found to decrease the rate of aerobic degradation of the primary or growth substrate, pentachlorophenol (500 $\mu\text{g/L}$), by Klecka and Maier (1988). They also observed that the addition of a second growth substrate, such as phenol (500-2000 $\mu\text{g/L}$) and 2,4,5-trichlorophenol (500-2000 $\mu\text{g/L}$), could accelerate the utilization rate of pentachlorophenol (500

$\mu\text{g/L}$) due to an increase in cell mass concentration that resulted from simultaneous growth on both substrates. These effects were shown to be predictable via an inhibition/interaction model.

Nies and Vogel (1990) performed tests to determine the effects of various primary substrates (acetate (7.8 g/L), acetone (4.0 g/L), glucose (5.6 g/L) and methanol (4.0 g/L)) on the dechlorination of polychlorinated biphenyls (PCBs) in anaerobic river sediments. They concluded that primary substrates such as acetate, acetone, glucose and methanol accelerate the dechlorination of PCBs (300 ppm). They also found a relationship between substrate type and the rate and extent of dechlorination (acetate < acetone < glucose < methanol). The most probable source of electrons for the dechlorination reactions were the primary substrates. The reduction state of the primary substrate impacted upon the availability of electrons, with alcohol > aldehyde > ketone > carboxylic acid.

Hughes and Parkin (1992) examined the effect of primary electron donor (acetate) loading on the anaerobic biotransformation of chlorinate aliphatics at high concentrations. They demonstrated that higher acetate loading rates (1000 mg/L) in anaerobic fill-draw reactors could increase the transformation of three chlorinated aliphatics; chloroform (1 mg/L), dichloromethane (5 mg/L) and 1,1,1-trichloroethane (2 mg/L).

Cometabolism kinetics of benzene, toluene and *p*-xylene were studied for two *Pseudomonas* species (strains B1 and X1) in an aerobic activated carbon fluidized bed reactor by Chang *et al* (1993). They observed that strain B1 was able to metabolize the non-growth substrate *p*-xylene (5 mg/L) in the presence of toluene (5 mg/L). They established that this cometabolic transformation could be quantified mathematically by coupling the *p*-xylene transformation to the consumption of toluene during growth and to the loss of biomass during the decay phase through a transformation capacity term.

Sez and Rittmann (1994) utilized *Pseudomonas putida* in steady state aerobic chemostats to examine substrate interactions between a primary substrate, phenol (1000 mg/L), and a

secondary substrate, 4-chlorophenol (50 mg/L). It was found that the cometabolic degradation of 4-chlorophenol was proportional to phenol oxidation and that 4-chlorophenol inhibited the oxidation of the primary substrate, phenol. Sáez and Rittmann found that these phenomena could be predicted with an interaction/inhibition model. However, their report did not state whether or not 4-chlorophenol could be metabolized by the *Pseudomonas putida* as a growth substrate which could be an important factor in explaining the systemic mechanisms involved in the degradation of this substrate.

The degradation of carbon tetrachloride (18.6 µg/L and 737 µg/L) in denitrifying biofilms was examined by Bae and Rittmann (1990, 1994). Experiments were performed using acetate (7.5 mg COD/L) as the primary electron donor and nitrate (stoichiometrically sufficient concentration) as a primary electron acceptor. They concluded that the secondary substrate biodegradation could be accelerated significantly through the manipulation of the primary electron donor and electron acceptor in a manner consistent with predictions based on the internal reducing power model.

Based on the multiple substrate degradation terminology defined previously, works by Papanastasiou and Maier (1982), Kim and Maier (1986), Klecka and Maier (1988) and Hughes and Parkin (1992) involved simultaneous utilization and competitive inhibition between the substrates of interest. The biodegradability of 4-chlorophenol (5 mg COD/L) as a sole substrate was demonstrated by Lu and Chen (1992), thereby qualifying the report by Sáez and Rittmann (1994) as simultaneous utilization. On the other hand, cometabolic degradation patterns were clearly reported in experiments by Bouwer (1985), Nies and Vogel (1990), Chang *et al.* (1993) and Bae and Rittmann (1994). Secondary substrate utilization from these experiments should be predictable using an internal reducing power model which will be presented in the following sections.

2.1.3 CONCEPT OF DUAL SUBSTRATE LIMITATION

In the previous section, it has been found that the rate of primary substrate utilization and the type of electron acceptor can affect secondary substrate degradation. Dual substrate limitation which controls the rate of primary substrate utilization will influence the generation of reducing power that is required for reductive dehalogenation or oxygenase reactions to proceed. Thus, the effects of dual substrate limitation, as opposed to single substrate limitation, should be incorporated into the suspended biomass and biofilm models since the secondary substrate utilization rate can be limited by either or both primary substrates.

2.1.3.1 SINGLE SUBSTRATE LIMITATION

Several single substrate limitation growth models have been presented in the literature. In general, they represent a modified form of Monod kinetics. The Monod expression is a hyperbolic equation relating specific cell growth rate to the rate limiting substrate concentration.

$$\mu = \mu_m \left(\frac{S_d}{K_{sd} + S_d} \right) \quad (2.6)$$

where μ is the specific cell growth rate ($M_x M_x^{-1} T^{-1}$). This equation is often modified to express the specific substrate utilization rate.

$$-q_d = q_{md} \left(\frac{S_d}{K_{sd} + S_d} \right) \quad (2.7)$$

where $-q_{md} = \mu_{md}/Y$ ($M_d M_x^{-1} T^{-1}$) and Y is the yield coefficient ($M_x M_d^{-1}$). In single limitation, only one compound limits the overall growth rate, while all other substrates and nutrients are assumed to be present in concentrations, S , significantly greater than K_s . Having a concentration, S , much greater than K_s , essentially saturates the enzyme allowing for the maximum cell growth, μ_m , or substrate utilization, q_{md} , rates to occur (Bae, 1992).

2.1.3.2 DUAL SUBSTRATE LIMITATION

During growth, the rate of cell synthesis is proportional to the consumption of all necessary substrates and nutrients. Under dual substrate limiting conditions, both the electron donor and electron acceptor are considered to have concentrations within the rate limiting range, thereby, simultaneously affecting the growth rate of the biological system. Two schools of thought exist to describe the effects of dual limitation; namely the non-interactive model and the multiplicative model.

A non-interactive model implies that the growth rate of the microorganisms can only be limited by one substrate at a time. For a growth rate based on Monod kinetics, the expression could be written as (Bader, 1978)

$$\mu = \mu_m \left(\frac{S_a}{K_{sa} + S_a} \right); \quad \text{when} \quad \frac{S_a}{K_{sa}} \leq \frac{S_d}{K_{sd}}$$

and

(2.8)

$$\mu = \mu_m \left(\frac{S_d}{K_{sd} + S_d} \right); \quad \text{when} \quad \frac{S_d}{K_{sd}} \leq \frac{S_a}{K_{sa}}$$

where S_a is the electron acceptor concentration ($M_e L^{-3}$) and K_{sa} is the half maximum rate concentration of the electron acceptor ($M_e L^{-3}$). This model has been used to describe dual substrate limitation by Sykes (1973), Williamson and McCarty (1976a; 1976b), Rittmann and Dovantzis (1983), Kissel *et al.* (1984) and Odencrantz *et al.* (1990). One of the major criticisms of the non-interactive model is that it assumes that the microbial metabolic subsystems are independent of each other, when in reality, all subsystems are interactive by communication through intermediate metabolites and cofactors (Bae, 1992).

A multiplicative model is based upon the assumption that if both substrates are present in subsaturating concentrations, then both affect the growth rate of the microorganisms. The model is constructed by combining two single substrate limiting expressions together (Bader, 1978),

$$\mu = \mu_m \left(\frac{S_d}{K_{sd} + S_d} \right) \left(\frac{S_a}{K_{sa} + S_a} \right) \quad (2.9)$$

This double Monod equation has been adopted by various researchers to simulate dual substrate limitation conditions (Sinclair and Ryder, 1975, Howell and Atkinson, 1976; Kissel *et al.*, 1984; Lau *et al.*, 1984; Barton and McKeown, 1986; Borden and Bedient, 1986, Malmstead *et al.*, 1995). The multiplicative model has been criticized for two reasons. First, it tends to yield unrealistic results at low substrate concentrations. Second, it assumes that the dual limitation of the electron donor and the electron acceptor resembles two substrates reacting together at one single point along the metabolic pathway. Therefore, it does not take into account the many reaction steps, intermediates and cofactors along the pathway.

In more recent work, Bae (1992) presented an interactive dual substrate limitation expression which accounts for variations in metabolic responses due to changes in electron donor and/or electron acceptor concentrations. This was achieved through the introduction of an intracellular reducing power variable.

$$-q_d = q_{med}[E_T] \log([NAD/NADH]^\alpha [ATP/ADP \cdot pi]^\beta) \left(\frac{S_d}{K_{sd} + S_d} \right)$$

and (2.10)

$$-q_a = q_{mea}[E_T] \log([NAD/NADH]^\alpha [ATP/ADP \cdot pi]^\beta) \left(\frac{S_a}{K_{sa} + S_a} \right)$$

where q_{med} and q_{mea} are the maximum specific rates of electron donor ($M_d M_e^{-1} T^{-1}$) and electron acceptor ($M_a M_e^{-1} T^{-1}$) utilization by a specified enzyme, NAD/NADH and ATP/ADP·Pi are the nicotinamide adenine dinucleotide and the adenosine triphosphate to adenosine diphosphate + inorganic phosphate intracellular cofactor ratios, and α and β are constants. The relationship is considered interactive, and does eventually reduce to the multiplicative expression, because the $\log([NAD/NADH]^\alpha [ATP/ADP \cdot Pi]^\beta)$ value is dependent upon both the electron donor and electron

acceptor concentrations within the system. The biochemical principles involved in the development of this expression are discussed further in section 2.2.

2.1.3.3 EXPERIMENTAL WORK INVOLVING DUAL SUBSTRATE LIMITATION

Various authors have presented mathematical models describing non-interactive and multiplicative multiple substrate situations in both suspended biomass (Sykes, 1973, Sinclair and Ryder, 1975; Bader, 1978; Lau *et al.*, 1984) and biofilm (Howell and Atkinson, 1976, Williamson and McCarty, 1976b; Rittmann and Dovantzis, 1983; Kissel *et al.*, 1984; Bae, 1992; Malmstead *et al.*, 1995) systems. However, few experimental reports present conclusive information supporting microbial reaction rates under dual limitation conditions despite the theoretical and practical significance of this concept.

Sinclair and Ryder (1975) demonstrated that concentration changes of oxygen and glycerol at different dilution rates could be successfully predicted using the multiplicative kinetic model. The tests were performed on a series of chemostats under oxygen and glycerol limiting conditions for a pure *Candida utilis* yeast culture. Most of the experiments were performed with either electron donor or electron acceptor concentrations that were near saturation which essentially yielded a series of experiments testing single substrate limitation. As such, the validity of the multiplicative model could not be verified from those results because dual substrate limiting conditions were never truly evaluated.

Williamson and McCarty (1976b) performed experiments that evaluated dual substrate limitation in downflow columns packed with plastic beads and supporting enrichment cultures of *Nitrosomonas-Nitrobacter* (ammonia fed) and *Nitrobacter* (nitrite fed) nitrifying bacteria. They found that electron donor (NH_4^+ and NO_2^-) and electron acceptor (O_2) concentrations, under flux and substrate limiting conditions could be predicted using the non-interactive model. However, as with Sinclair and Ryder (1975) they also utilized electron donor and electron acceptor

concentrations near their saturation concentrations which reduced their experiments to tests of single substrate limitation.

The growth kinetics of a consortia in a chemostat under subsaturating electron donor and electron acceptor concentrations were studied by Lau *et al.* (1984). A constant, limiting glucose concentration was maintained in the reactor, but the oxygen concentration was varied through changes in stirring rates. At relatively low bulk dissolved oxygen concentrations (0.5 mg/L) the dissolved oxygen profile within a 200 μm -diameter floc was shown to drop very sharply (< 0.1 mg/L) within the first 25 μm . The corresponding glucose concentration profile for a bulk glucose concentration of 20 mg/L showed a mild gradient caused by the reduction in biochemical activity which occurs when the dissolved oxygen is depleted. At higher bulk dissolved oxygen concentrations, steeper glucose concentration gradients were observed. Lau *et al.* found that the growth kinetics of the consortia could be predicted using the multiplicative model.

In a further analysis of the results presented by Lau *et al.*, Bae (1992) argued that the results could not conclusively support a multiplicative interaction between the electron donor and electron acceptor because the $\left(\frac{S_d}{K_{sd} + S_d}\right)\left(\frac{S_a}{K_{sa} + S_a}\right)$ value, which is equivalent to the μ/μ_m ratio (see equation (2.9)), was shown to decrease under dual subsaturation concentrations. The ratio should have remained constant, because the control of the glucose dilution rate would yield a constant μ . If reaction kinetics obey a multiplicative model, then the concentration of one substrate should, in theory, change if the concentration of the other substrate should fall within its rate limiting range. On the other hand, Bae did conclude that the reaction kinetics did not obey a non-interactive model since the substrate concentration fluctuated when electron acceptor concentrations fell within the rate limiting range.

Odencrantz *et al.* (1990) examined the interaction between acetate and nitrate in a porous medium through the application of nitrate at two injection ports. They concluded that the limiting

substrate (NO_3^- before the second injection port and acetate after the second injection port) profiles could be obtained using the non-interactive model. As in previous experiments, however, the non-limiting substrates (acetate before the second injection port and NO_3^- after the second injection port) were present in near saturating concentrations, thus, the multiplicative model could not be excluded as a possible predictive tool.

Bae (1992) performed experiments under varied acetate and oxygen limiting conditions using pure cultures of *Pseudomonas putida* in a special chemostat apparatus. Using an expression accounting for variations in metabolic responses due to changes in electron donor and electron acceptor concentrations (equation (2.10)) as his theoretical basis, an extensive analysis of his results showed, conclusively, that an interactive multiplicative model was necessary to describe primary substrate utilization under dual substrate limiting conditions. The model was also found to adequately describe electron donor utilization rates under single substrate limitation conditions and was developed further to describe cometabolic secondary substrate biodegradation.

2.2 BACTERIAL PHYSIOLOGICAL RESPONSES

Secondary substrate biodegradation is believed to be dependent upon the response of microorganisms to the primary substrates. As such, a description of the metabolic subsystems, physiological responses to substrate depletion and their effects are presented in the following sections.

Microorganisms are often subjected to environmental stresses such as temperature, pH and changes in nutrient availability. As such, they have developed the ability to adapt to their changing environment. Adverse environmental changes will stress the microorganism which then responds through alterations in the genetic constitution of the cell or phenotypic adaptation (Harder and Dijkhuizen, 1983).

When dual substrate limitation occurs, depletions in electron donor and electron acceptor concentrations become the stress element. Electron donor depletion stresses the electron donor metabolizing subsystem, while electron acceptor depletion stresses the respiratory subsystem. Each individual subsystem will respond to the stress and will, consequently, affect the other subsystem because the subsystems are linked by common electron carriers (Bae, 1992).

The biodegradation of a cometabolic secondary substrate is a consequence of adaptations in bacterial physiology. Cometabolism likely results from the lack of specificity of enzymes and cofactors (Criddle, 1993). It does not stress the bacterial system unless it is present in high concentrations which can be toxic to the microorganism. Biodegradation is able to occur due to fortuitous mechanisms or the evolution of alternate pathways which utilize the non-specific enzymes and cofactors. The energy or reducing power (mainly in the form of NADH) required in reductive dehalogenation or oxygenase reactions is provided from the catabolic breakdown of the electron donor which is supplied to sustain microbial growth (Nies and Vogel, 1990).

2.2.1 BACTERIAL METABOLIC FUNCTIONS

The elementary bacterial metabolic functions for cell growth and maintenance are divided into four independent subsystems; namely, transport, catabolic reactions, respiration and biosynthesis (Mandelstam *et al.*, 1982).

2.2.1.1 TRANSPORT

The transport subsystem takes up necessary substrates and nutrients from the environment and brings them inside the cell. Four possible mechanisms are responsible for electron donor and electron acceptor transport; facilitated diffusion, group translocation, active transport and passive transport (Brock and Madigan, 1988). Organic substrates (electron donor and secondary substrates) can be transported by one of the first three mechanisms. Facilitated diffusion is a mass

action or concentration gradient mediated process where the substrate (e.g. glycerol) is carried in by a specific transport protein. Group translocation is an energy-linked process where a substance (e.g. glucose and many other sugars) is transported while being simultaneously converted into a derivative by chemical modification such as phosphorylation. Active transport also requires energy and is the process by which the substance (e.g. acetate and many amino acids) can accumulate to high concentrations in the cytoplasm in chemically unaltered form. The fourth mechanism, passive transport, applies to water and oxygen and does not require any energy input.

2.2.1.2 CATABOLIC REACTIONS

Catabolism is the process by which the electron donor substrate is oxidized, thereby releasing energy through the production of adenine triphosphate (ATP), reduced intracellular electron carriers and 12 precursor metabolites for other cell functions. The reduced/oxidized pair of intracellular electron carriers exist in the form of nicotinamide adenine dinucleotide (NADH/NAD), nicotinamide adenine dinucleotide phosphate (NADPH/NADP) and flavin adenine dinucleotide (FADH₂/FAD). The respiratory subsystem utilizes the NADH and FADH₂ electron carriers in the production of energy, while NADPH is employed for reducing power in biosynthesis (Lehninger, 1982; Bae, 1992).

Most electron donors provided for biomass growth must usually be modified to a product (e.g. glucose, acetate, pyruvate) which can be shunted to one of the major energy producing pathways, namely, glycolysis or the tricarboxylic acid (TCA) cycle. The upstream degradation of these electron donors is accomplished through various enzymatic reactions, the sequence of which is dependent upon the type and complexity of the electron donor. For instance, monosaccharides can be transformed through phosphorylation reactions which allows them to enter the glycolytic sequence. Disaccharides and polysaccharides must first be hydrolyzed to yield hexose units and subsequently be phosphorylated.

Four important transformations occur during glycolysis. (1) the degradation of the glucose skeleton yielding 2 pyruvate which can then enter the TCA cycle; (2) the phosphorylation of 2 ADP to 2 ATP by high-energy phosphate compounds, (3) the transfer of electrons forming 2 NADH, and (4) the generation of six biosynthetic precursors throughout the pathway for each molecule of glucose (Lehninger, 1982; Bae, 1992). Figure 2.2 illustrates the glycolysis pathway (Brock and Madigan, 1988).

For aerobic bacteria, the TCA cycle is an important means of generating energy and reduced intracellular electron carriers. The reactions involved in the TCA cycle when pyruvate or acetate are fed as the electron donor substrate can be found in Figure 2.3. For each molecule of pyruvate consumed, the TCA cycle generates, 3 CO₂, 3 NADH, 1 NADPH, 1 FADH₂, and 1 ATP (or guanosine triphosphate - GTP in some bacteria) as well as some cycle intermediates serving as biosynthetic precursors (Lehninger, 1982; Brock and Madigan, 1988). The NADH and NADPH are essentially free molecules that can be catalyzed by any NADH dehydrogenase. On the other hand, FADH₂ can only be utilized in the respiratory subsystem for ATP generation because it is tightly bound to succinate dehydrogenase which is directly linked to the electron transport chain (Lehninger, 1982; Bae, 1992).

The utilization of two- or three-carbon acids as carbon sources cannot occur by means of the TCA cycle alone. This cycle can continue to operate only if the four-carbon acid, oxaloacetate, is regenerated at every turn. Any removal of carbon compounds for biosynthetic reactions could, thus, prevent the completion of the cycle. When cells grow on acetate or fatty acids, because these compounds can not be converted directly to pyruvate, the glyoxylate cycle must be utilized to produce the oxaloacetate required to complete the cycle. As can be seen in Figure 2.3, this cycle employs most of the TCA cycle reactions. By avoiding the decarboxylation reactions present in the TCA cycle, the glyoxylate cycle can replenish the supply of TCA cycle intermediates (Brock and Madigan, 1988; Bae, 1992).

Figure 2-2: Glycolysis

Figure 2-3: Tricarboxylic acid and glyoxylate cycles

The relative importance of each catabolic pathway, glycolysis, the TCA cycle and the glyoxylate cycle will depend mainly upon the electron donor substrate supplied to the microbial system. For example, if glucose was used as the primary substrate, then glycolysis and the TCA cycle would be involved. Whereas, if carbon source was pyruvate then the TCA alone would be predominant. On the other hand, if acetate was the sole electron donor, both the TCA cycle and the glyoxylate cycle would be required (Bae, 1992)

2.2.1.3 RESPIRATION

Respiration, also referred to as oxidative phosphorylation, oxidizes NADH and FADH₂ and transfers the electrons to the final electron acceptor, producing ATP in the process. As can be seen from Figure 2.4 (SD succinate dehydrogenase, UQ ubiquinone, C cytochrome), the respiratory subsystem is located in the cellular membrane and is composed of two parts, the respiratory chain and the ATP synthesizing system (ATPase) (Bae, 1992)

The electron transport systems, or respiratory chain, are composed of membrane-associated electron carriers. These systems have two basic functions, to accept electrons from an electron donor (NADH or FADH₂) and transfer them to a terminal electron acceptor, in this case oxygen. Several types of oxidation-reduction enzymes and electron transport proteins are involved in electron transport. These include NADH dehydrogenase which transfers hydrogen atoms from NADH, riboflavin-containing electron carriers called flavoproteins such as FAD, non-heme iron-sulfur proteins, cytochromes which are proteins containing an iron-porphyrin ring called heme, and quinones which are non-protein electron carriers sometimes referred to as coenzyme Q (Brock and Madigan, 1988).

During electron transfer, the protons are translocated outside the cellular membrane, thereby creating a proton gradient. This proton gradient creates an energized state of the cellular membrane which is called a proton motive force (PMF). The energy provided by the PMF can be

Figure 2-4: Respiratory subsystem

used directly to do work such as ion transport or drive the formation of high-energy phosphate bonds in ATP (Brock and Madigan, 1988, Bae, 1992)

In the ATPase part of the respiratory subsystem, electrons are able to flow back and forth from inside and outside the cell. The direction of proton flow is based on the magnitude of the PMF and the phosphorylation potential (ATP/ADP-Pi). ATP is synthesized when protons flow from outside to inside the cell, whereas ATP is hydrolyzed when the flow is from inside to outside the cell. The cellular metabolic functions of growing cells generally require the constant synthesis of ATP which implies a fairly large PMF and a low phosphorylation potential (Bae, 1992)

The respiratory subsystem requires four inputs: (1) the electron acceptor supplied externally, (2) NADH or FADH₂ which is produced from electron donor oxidation in the catabolic subsystem, (3) ADP which is regenerated from ATP in biosynthesis, and (4) inorganic phosphorous (Pi) which is either obtained externally or recycled metabolically.

2.2.1.4 BIOSYNTHESIS

Biosynthesis is the synthetic phase of metabolism. Small precursor molecules, including amino acids, sugars, fatty acids and nitrogenous bases, are synthesized into the large macromolecular components of the cell such as proteins, polysaccharides, lipids and nucleic acids (Lehninger, 1982)

The increased size and complexity of structures resulting from biosynthesis requires free energy which is furnished by the breakdown of ATP, produced mainly by respiration, to ADP and inorganic phosphate. The biosynthesis of some cell components also requires reducing hydrogen ions which are donated by NADPH produced from the TCA cycle and the pentose-phosphate pathway, or NADH from the pyridine-nucleotide transhydrogenase (Lehninger, 1982)

A portion of the energy produced by the respiratory subsystem is devoted to cell maintenance functions such as cell motility and resynthesis of degraded proteins. These

maintenance functions are lumped into the biosynthesis subsystem because they represent an energy sink (Bae, 1992).

2.2.1.5 METABOLIC FUNCTION INTERACTIONS

The metabolic subsystems communicate through the recycling of cofactors such as NADH/NAD and ATP/ADP·Pi. The electron donor (S_d) enters the cell via the transport subsystem and is supplied to the catabolic subsystem. Catabolic reactions generate reduced cofactors including NADH, NADPH and $FADH_2$ and precursor metabolites. The NADH and $FADH_2$ cofactors are sent to the respiratory subsystem, while the precursor metabolites and NADPH cofactor are supplied to the biosynthesis subsystem. The respiratory subsystem oxidizes the NADH and $FADH_2$ cofactors to NAD and FAD which are returned to the catabolic subsystem. In doing so, the PMF is created and ATP can, consequently, be generated. Some of the PMF is utilized in transport functions, while most of the ATP is consumed by the biosynthetic subsystem producing ADP and Pi which are recycled to the respiratory subsystem (Bae, 1992). Figure 2.5 illustrates the general interactions between the four main cellular metabolic subsystems.

2.2.2 RESPONSES TO ELECTRON ACCEPTOR LIMITATIONS

As the electron acceptor becomes limiting, the microorganism will exhibit at least two types of physiological responses. The first is a relatively rapid adaptation which involves the adjustment of intracellular cofactor concentrations. The second response is the induction of respiratory enzyme synthesis, and this is achieved less rapidly. Each response essentially allows the cell to adjust to lower electron acceptor concentration by minimizing the impact of the change in concentration.

In aerobic systems, organisms can adjust NAD and NADH concentrations depending upon the availability of external oxygen. The NAD/NADH ratio decreases as oxygen concentrations are

Figure 2-5: Metabolic subsystem interactions

depleted, and the ratio increases again with higher oxygen concentrations. Consequently, the electron transfer from NADH to the respiratory chain increases as the NAD/NADH ratio decreases such that the impact of oxygen depletion on the overall cell metabolism is minimized (Bae, 1992).

The ATP/ADP·Pi ratio also decreases as oxygen concentrations become depleted. This results in the following cellular phenomena. First, an internal compensation mechanism is triggered to enhance the cell's ability to take up oxygen. Second, the rate of respiration increases, thereby, generating more ATP (Bae, 1992).

Long-term adaptations to electron acceptor depletion involve a change in respiratory enzyme composition. It has been speculated that the adaptation is exhibited through an increase in total cytochrome content including a shift to cytochromes with a higher affinity for oxygen (Bae, 1992).

2.2.3 RESPONSES TO ELECTRON DONOR LIMITATIONS

When the electron donor becomes limiting, the microorganism will also respond through changes in cofactor concentrations and enzyme synthesis. Electron donor depletion will result in lower intermediate metabolite concentrations which must be compensated for by an increase in catabolic enzyme activity to maximize the growth rate (Bae, 1992).

As electron donor concentrations decrease an adverse affect on the growth of the organism is expected due to the lower production of intermediate metabolites required in the catabolic pathways. The cell compensates with an increase in the NAD/NADH ratio which helps sustain respiration and biosynthesis (or growth) despite the electron donor limitation.

The ATP/ADP·Pi will increase as the electron donor becomes depleted. These changes in the ATP/ADP·Pi ratio under depletion conditions have conflicting effects which are attributed to transport mechanisms which operate best under high phosphorylation potentials, and the catabolic subsystem TCA cycle which is allosterically inhibited by high ATP concentrations (Bae, 1992).

2.3 BIOLOGICAL SYSTEM MODELLING

Biological treatment systems are often too complex to be understood by purely qualitative measures. Mathematical modelling, an intellectual tool which allows one to integrate and prioritize various processes, has become essential for developing and applying biotreatment. Bacterial system modelling requires the formulation and solving of mass-balance equations for key biological and chemical species (Rittmann and Sáez, 1994).

2.3.1 BIOLOGICAL PROCESS MODELLING OVERVIEW

The modelling of biological processes has been the subject of much attention since the 1970s. Modellers of biological systems have attempted to develop systems of equations that reflect the physical and chemical characteristics of these systems. Biological systems have been broadly categorized as suspended or fixed growth. In their numerical and experimental work, researchers have addressed issues such as saturation kinetics, variable biomass and cometabolism of secondary substrates in steady and unsteady state systems. In addition, active fraction determination and mass transport across a liquid diffusion-resistance layer have been examined in fixed systems. A summary of research involving biofilm and suspended system models is presented in Table 2.1.

Prediction of growth and substrate utilization in biofilms is often difficult due to the complexity of the system which requires considerations such as the diffusion of substrates through a liquid diffusion-resistance layer and the biofilm, mass transfer limitations, as well as concurrent changes in biofilm thickness, active fraction and substrate concentration profiles. The system is generally described by a set of second order non-linear partial differential equations which must be solved numerically. In contrast, suspended biomass systems are usually easier to model because they generally represent a simplified case of the attached system without the mass transfer, active fraction and substrate concentration profile components. This reduces the number of equations

describing the system and simplifies the numerical analysis since the system of equations involves first order non-linear partial differential equations.

Lawrence and McCarty (1970) presented a steady state model that described the behavior of suspended biomass in wastewater treatment processes when substrate utilization followed Monod kinetics. Early biofilm work (Atkinson and Davies, 1974; Williamson and McCarty, 1976a) focused on primary substrate utilization and transport to and within the biofilm. Subsequent reports dealt with primary substrate utilization in single- (Rittmann and McCarty, 1978, 1980, 1981) or multi-species (Rittmann and Manem, 1992) biofilms under steady state conditions. However, the assumption of steady state has been found to have restricted ranges of applicability in limiting case solutions (Sáez and Rittmann, 1990; Skowlund, 1990).

Unsteady state models are required to describe fluctuations in active biomass fractions, biomass growth and substrate concentrations within a biotreatment process. Unsteady state expressions describing growth and substrate utilization were described by Sáez *et al.* (1984) for both attached and suspended systems, and by Wanner and Gujer (1984, 1986), Annachatre and Khanna (1987), and Skowlund (1990) for biofilms. Wanner and Gujer (1984, 1986) presented a very comprehensive numerical approach to transient multispecies biofilm modelling. However, they neglected to include the effects of the effective diffusion layer in their development. The presence of this layer may reduce the overall substrate flux into a biofilm thereby producing incorrect model predictions (Sáez *et al.*, 1984).

Very few models have incorporated the concept of dual limitation kinetics. Rittmann and Dovantzis (1983) studied a non-interactive dual substrate limitation biofilm model under steady state conditions. A numerical approach for an unsteady state multiplicative dual limitation model was presented by Kissel *et al.* (1984), while Barton and McKeown (1986) examined the use of a steady state multiplicative model in a suspended biomass system. Bae (1992) developed a general dual substrate limitation model which could account for electron donor and electron acceptor

interactions through internal reducing power in suspended biomass processes. Bae's principles were later applied by Malmstead *et al.* (1995) in a steady state biofilm model

By expanding internal reducing power concepts, Bae (1992) generated a model which can predict general cometabolic secondary substrate utilization. The equations derived from his research were applied to steady state biofilms by Bae and Rittmann (1994). They found that substrate utilization rates could be predicted accurately using Bae's system of equations under steady state conditions.

2.3.2 LIMITATIONS OF CURRENT MODELLING APPROACHES

Comprehensive models of biological processes need to account for the growth and maintenance of active biomass, which requires the consumption of electron donors and acceptors to gain energy for cell synthesis, as well as account for the cometabolic utilization of secondary substrates. As such, a comprehensive model should consider active biomass growth rates, electron donor and electron acceptor utilization rates including the concepts of dual substrate limitation and secondary substrate utilization rate dependence on electron donor and electron acceptor concentrations (Rittmann and Sáez, 1994).

From the review of biological systems modelling and Table 2.1, it is apparent that current models have not addressed the case of primary substrate interactions in the cometabolism of secondary substrates in unsteady state suspended growth and biofilm systems. Thus, the detailed description and development of such a model and a sensitivity analysis of key model parameter variations is warranted.

2.3.2.1 STEADY STATE VS. UNSTEADY STATE MODELS

To reduce or simplify the system of kinetic equations, many current models have assumed the biomass growth component to be negligible or at steady state in both biofilm and suspended

systems. However, this assumption can not be made when attempting to model start-up conditions or fluctuations in input parameters. This is especially true in biofilm systems where the steady state biofilm thickness, bulk phase substrate concentrations and biofilm active biomass and substrate concentration profiles are developed concurrently. The biomass growth rate is dependent on primary bulk substrate concentrations and biomass loss (death + sloughing). The assumption of steady state implies that the biomass growth equals the net biomass loss.

A steady state assumption is also often applied to develop biofilm substrate concentration profiles implying that the substrate diffusion into the biofilm is equal to the microbial substrate utilization. However, as was discussed previously, the assumption of steady state can only be made for restricted ranges of applicability in limiting case solutions.

On the other hand, transient models can predict and simulate steady state conditions once these have been established within the system. Therefore, for the sake of completeness, it is proposed to develop unsteady state models in this thesis to enable the examination of biological processes under a variety of system conditions.

2.3.2.2 SUBSTRATE UTILIZATION KINETICS

Substrate utilization kinetics are often reduced to zero order or first order expressions to arrive at analytical solutions. Once again, these model simplifications can only be applied to certain ranges of substrate concentrations, thereby, limiting the use of the model.

Few biological models combining dual substrate limitation and secondary substrate utilization have been presented in the literature. The system of equations developed by Bae (1992) is believed to be the most advanced and comprehensive approach to represent substrate utilization kinetics involving dual limitation and cometabolism. As such, the expressions derived by Bae (1992) will be incorporated into transient state suspended biomass and biofilm models and will form the basis of this thesis.

Table 2-1: Summary of Biological Systems Modelling

Author(s)	Unsteady State	Suspended System	Fixed System	Dual Limitation	Cometabolism	Variable Biomass	Diffusion Layer	Active Fraction	Saturation Kinetics	Numerical Approach	Experimental
Lawrence and McCarty, 1970		X							X		
Atkinson and Davies, 1974			X						X		
Williamson and McCarty, 1976a			X				X		X		
Williamson and McCarty, 1976b			X				X		X		X
Rittmann and McCarty, 1978			X				X		X		
Rittmann and McCarty, 1980			X			X	X		X		
Shamat and Maier, 1980		X							X		
Rittmann and McCarty, 1981			X			X	X		X		X
Trulcar and Characklis, 1982			X			X	X		X		X
Namkung <i>et al.</i> , 1983			X		X		X		X		X
Rittmann and Dovantzis, 1983			X	X			X		X		
Kissel <i>et al.</i> , 1984	X		X	X		X	X	X	X		
Rittmann, 1984			X				X		X		X
Saer <i>et al.</i> , 1984	X	X	X				X		X	X	
Wanner and Gujer, 1984	X		X				X		X	X	
Bouwer and McCarty, 1985	X		X		X				X		X
Schmidt <i>et al.</i> , 1985	X	X			X				X		
Suidan and Wang, 1985			X				X				
Barton and McKeown, 1986	X	X		X					X		X
Wanner and Gujer, 1986	X		X			X		X	X	X	X
Annachatre and Khanna, 1987	X		X			X	X				
Suidan <i>et al.</i> , 1987			X				X		X		
Belkhadir <i>et al.</i> , 1988	X		X			X					X
Capdeville <i>et al.</i> , 1988	X		X			X					X
Gantzer <i>et al.</i> , 1988			X		X		X				X
Saer and Rittmann, 1988			X			X	X		X		
Hamoda, 1989			X						X		X
Kim and Suidan, 1989			X				X		X		X
Saer and Rittmann, 1990			X				X		X		
Skowlund, 1990	X		X			X		X	X	X	

Author(s)	Unsteady State	Suspended System	Fixed System	Dual Limitation	Cometabolism	Variable Biomass	Diffusion Layer	Active Fraction	Saturation Kinetics	Numerical Approach	Experimental
Tabak <i>et al.</i> , 1990	X	X				X			X		X
Bae, 1992				X	X				X		X
Rittmann and Manem, 1992			X				X	X	X		X
Chang <i>et al.</i> , 1993	X	X			X	X			X		X
Criddle, 1993	X	X			X	X			X		X
Bae and Rittmann, 1994			X	X	X		X		X		X
Malmstead <i>et al.</i> , 1995			X	X	X	X	X		X		X

CHAPTER 3

MODEL DEVELOPMENT

The system of equations simulating growth and electron donor, electron acceptor and secondary substrate utilization in suspended biomass and biofilm systems are presented in the following sections. Two major numerical approximation techniques were required to solve (1) the unsteady state non-linear first order ordinary differential equations describing the suspended biomass system and bulk phase of the biofilm system, and (2) the unsteady state non-linear second order partial differential equations related to the more complex biofilm system.

The main objective of this section is to present mathematical expressions for growth and substrate utilization kinetics which incorporates multiple substrate interactions such as dual limitation and cometabolism in a transient system.

3.1 SUBSTRATE UTILIZATION KINETICS

Fixed and suspended biological systems must consume primary substrates to derive energy for crucial cellular functions such as cell maintenance, biosynthesis and motility. Thus, rate expressions for the utilization of both primary substrates, namely, the electron donor and the electron acceptor are essential. The occurrence of cometabolic reactions is also possible through one of several mechanisms depending upon underlying system conditions. This reaction can be modelled using a third rate expression for the secondary substrate of interest

3.1.1 ELECTRON DONOR UTILIZATION RATE

The electron donor utilization kinetics employed in the numerical model result from reactions taking place in the catabolic subsystem. If all catabolic enzymes are thought to behave as a single enzyme complex, then the kinetics for electron donor utilization, based on extracellular substrate concentrations, can be expressed in the following manner

$$-r_d = q_{md}^1 \log([NAD]/[NADH])^\alpha [ATP/ADP \cdot Pi]^\beta X f \frac{S_d}{K_{sd} + S_d} \quad (3.1)$$

where r_d is the electron donor utilization rate ($M_d L^{-3} T^{-1}$), q_{md}^1 is the maximum specific rate of electron donor utilization ($M_d M_x^{-1} T^{-1}$), X is the biomass concentration in the reactor ($M_x L^{-3}$), S_d is the electron donor concentration ($M_d L^{-3}$), K_{sd} is the half maximum rate concentration ($M_d L^{-3}$), α and β are constants and f is the active biomass fraction ($M_x M_x^{-1}$). The active fraction of biomass represents the portion of cells which contribute to physiological functions such as substrate consumption, cometabolic degradation, respiration and cell growth.

The behavior of the catabolic reactions are reflected by taking into account the electron donor, the reduced internal electron acceptors and, to a lesser extent, the ATP, ADP and Pi reaction variables. NAD is considered to be representative of internal electron acceptors (NAD, FAD and NADP) because (1) it is consumed in greater quantity and, (2) the FAD/FADH₂ and NADP/NADPH ratios are assumed to be functions of the NAD/NADH ratio. Bae (1992) demonstrated that the NAD/NADH and ATP/ADP·Pi ratios control the maximum rate of electron donor utilization, and that the logarithmic ratios could be applied to quantify these cofactor effects.

Bae (1992) concluded that systematic responses tend to maximize the overall growth rate under any given dual substrate conditions by accelerating the limiting reaction (e.g. catabolic) and slowing the non-limiting reaction (e.g. respiration) through changes in the NADH/NAD ratio. The linkage of systematic internal responses to external electron acceptor and electron donor concentrations reduces the overall reaction rate expression to the following multiplicative relationship.

$$-r_d = q_{md}^1 X f \left(\frac{S_d}{K_{sd} + S_d} \right) \left(\frac{S_d}{K_{sd} + S_d} \right) \quad (3.2)$$

The term $q_{md}^1 \log([NAD/NADH])^\alpha [ATP/ADP \cdot Pi]^\beta$ is essentially analogous to the apparent maximum specific rate of substrate utilization, q_{md} , in Monod kinetics. However, the intrinsic term

will vary with changes in primary substrate concentration, whereas the observed term employed in Monod kinetics is measured at saturating concentrations of electron donor and electron acceptor and is considered to remain constant. An expression relating the intrinsic parameters to Monod kinetics can be obtained by combining equations (3.1) and (3.2).

$$q_{md}^1 \log([NAD / NADH]^a [ATP / ADP \cdot Pi]^b) = q_{md} \left(\frac{S_a}{K_{sa} + S_a} \right) \quad (3.3)$$

Through the rearrangement of equation (3.3), it can be shown that the apparent maximum specific rate of electron donor utilization, q_{md} ($M_d M_r^{-1} T^{-1}$), is also related to the intrinsic parameter q_{md}^1 in the following manner

$$q_{md}^1 = \frac{q_{md}}{\log([NAD / NADH]_o^a [ATP / ADP \cdot Pi]_o^b)} \quad (3.4)$$

where the apparent maximum specific rate of electron donor utilization is measured at saturating concentrations of electron donor and electron acceptor denoted by the subscript o

3.1.2 ELECTRON ACCEPTOR UTILIZATION RATE

Similarly to electron donor utilization, the kinetic expression for electron acceptor utilization must take into account the variables which affect the reaction rate in the respiratory subsystem. The main reactants of this subsystem are the electron acceptor and intercellular electron donors, namely; NADH, $FADH_2$, ADP and Pi. Once again, NAD is considered the representative internal electron carrier variable because (1) the production of NADH from the catabolic subsystem is at least two times that of $FADH_2$ and (2) the redox potential of $FAD(H_2)$ is a function of NAD since the redox reactions of both cofactors are connected through the reversible electron-transport chain (Bae, 1992).

From his experiments, Bae (1992) found that the general responses of the $\log[NAD/NADH]$ and $\log [ATP/ADP \cdot Pi]$ ratios were inversely proportional to changes in electron

donor utilization rate (q_d). A relationship between electron donor and electron acceptor utilization rates was established and can be quantified using

$$-q_a = v_{a,d}(-q_d) + v_{a,x}b \quad (3.5)$$

where $v_{a,d}$ is a stoichiometric coefficient defined as the electron acceptor consumption per unit electron donor consumption ($M_a M_d^{-1}$), $v_{a,x}$ is a stoichiometric coefficient defined as the electron acceptor consumption per unit cell decay ($M_a M_x^{-1}$) which is generally 1.42 mg DO/mg cells (Metcalf and Eddy, 1991). The distribution of cell ages is such that not all the cells in the system are in a log growth phase at the same time. The expression for growth is corrected to account for the energy required for cell maintenance and other factors such as death and predation by incorporating a decay term which is generally identified as an endogenous decay coefficient (T^{-1}). Equation (3.7) is obtained by combining equations (3.2), (3.5) and the relation

$$-r_a = (-q_a)Xf \quad (3.6)$$

$$-r_a = (q_{ma}(\frac{S_d}{K_{sd} + S_d})(\frac{S_d}{K_{sd} + S_d}) + v_{a,x}b)Xf \quad (3.7)$$

where r_a is the electron acceptor utilization rate by respiratory enzymes ($M_a L^{-3} T^{-1}$) and q_{ma} is the maximum specific utilization rate for the electron acceptor ($M_a M_x^{-1} T^{-1}$).

3.1.3 SECONDARY SUBSTRATE UTILIZATION RATE

Internal reducing power is necessary for the biodegradation of certain xenobiotic compounds by reactions such as reductive dehalogenation and oxygenation. Reductive dehalogenation (e.g. degradation of hexachlorobenzene to pentachlorobenzene), is most common in anaerobic systems, but has been shown to occur under aerobic conditions (Bae and Rittmann, 1990). In this reaction, the substitution of a halogen atom with hydrogen is accompanied by the transfer of electrons during the reduction of the carbon atom. Oxygenase reactions (e.g. formation

of alcohols and diols during aromatic ring cleavage) consume electrons when incorporating one or two atoms of molecular oxygen, in the form of hydroxy groups, directly into an organic compound.

The electrons are often supplied by reduced intracellular electron carriers such as NADH. Since NADH is essentially used as a cosubstrate in these reactions, the reaction rate will be dependent on its concentration. As was discussed in sections 3.1.1 and 3.1.2, the NADH/NAD ratio is governed by the electron donor and electron acceptor concentrations.

Since the NADH/NAD ratio can be reasonably predicted from primary substrate concentrations, the biodegradation of a secondary substrate through reductive dehalogenation and oxygenation may also be controlled by them. Bae (1992) found that when the reactions were limited solely or partially by the electron donor, the ratio could be represented by equation (3.8)

$$\log(\text{NADH} / \text{NAD}) = 0.273(-q_d) - 1.11\left(\frac{S_a}{K_{s_d} + S_d}\right) - 0.717 \quad (3.8)$$

where q_d is defined by equation (3.2). However, it was found that equation (3.8) produced the wrong trend in the behavior of $\log(\text{NADH/NAD})$ ratio when the electron donor was not limiting. Instead, when the reactions were saturated with electron donor, thus, solely limited by the electron acceptor, the $\log(\text{NADH/NAD})$ could be represented by

$$\log(\text{NADH} / \text{NAD}) = 0.215(-q_d) - 0.717 \quad (3.9)$$

In general, when the reaction is at least partially limited by the electron donor, the $\log(\text{NADH/NAD})$ ratio increases as the reaction rate (q_d) and electron donor concentration increase, whereas, the ratio decreases as the reaction rate and electron acceptor concentration increase. As well, the ratio is more sensitive to electron acceptor control than to electron donor control under such conditions. On the other hand, if the reaction is solely limited by the electron acceptor, the ratio increases with increases in electron donor and/or electron acceptor concentrations.

3.1.3.1 REDUCTIVE DEHALOGENATION

The equation for secondary substrate utilization via reductive dehalogenation can be constructed from similar enzyme kinetic concepts as those presented for primary substrate utilization with one exception, the primary substrates must supply the reducing power (Bae, 1992).

$$-r_{2,d} = q_{m2} R_p X f \left(\frac{S_2}{K_{s2} + S_2} \right) \quad (3.10)$$

where $-r_{2,d}$ is the rate of secondary substrate utilization via reductive dehalogenation ($M_2 L^{-3} T^{-1}$), q_{m2} is the maximum specific rate of secondary substrate utilization ($M_2 M_x^{-1} T^{-1}$), S_2 is the secondary substrate concentration ($M_2 L^{-3}$), K_{s2} is the secondary substrate half maximum rate concentration ($M_2 L^{-3}$) and R_p is the reducing power which is a function of the NADH/NAD ratio.

$$R_p = \log(NADH / NAD) + M \quad (3.11)$$

The $\log(NADH/NAD)$ expression was defined by equations (3.8) or (3.9) depending upon primary substrates concentrations in the reactor. The M coefficient is a constant which maintains a positive value for $\{\log(NADH/NAD) + M\}$. The coefficient depends upon the NADH-dehydrogenation capability of the enzyme. If the capability is assumed to resemble that of respiratory dehydrogenase, such that the secondary utilization reaction ceases for the endogenous state ($\log(NADH/NAD) = -1.827$) when the electron donor is no longer available to supply the catabolic pathway and the electron acceptor is not limiting, then M must be 1.827.

The reducing power factor and secondary substrate utilization rates for extreme cases, where one or both primary substrates are either depleted or present in saturating concentrations, of reductive dehalogenation are summarized in Table 3.1. It can be expected that when the electron acceptor is present in saturating concentrations, the secondary substrate utilization rate increases with increasing electron donor concentration. When the electron donor has become depleted in the system, the rate of secondary substrate utilization represents the substrate removal by resting cells from which no growth will result. In this case, the secondary substrate utilization rate will

Table 3.1: Secondary substrate utilization rates summary.

Dual or Donor Limited				Acceptor Limited	
	$S_d \gg K_{sd}$	$S_d \text{ lim}$	$S_d = 0$	$S_d = 0$	$S_d \gg K_{sd}$
	$S_a \gg K_{sa}$	$S_a \gg K_{sa}$	$S_a \text{ lim}$	$S_a = 0$	$S_a = 0$
R_p	$0.273q_{md}$	$0.273q_{md}[S_d]$	0	1.11	$0.273q_{md}[S_d] + 1.11$
r_{20}	$R_p q_{m2} X f[S_2]$	$R_p q_{m2} X f[S_2]$	0	0	$R_p q_{m2} X f[S_2] [S_d]$
Note	$R_p q_{m2} = q_{m2}^I$	$r_{20} \uparrow \text{ as } S_d \uparrow$	$r_{20, \text{max}} \text{ at } S_d = K_{s2}$		$r_{20} \uparrow \text{ as } S_a \uparrow$
r_{2d}	$R_p q_{m2} X f[S_2]$	$R_p q_{m2} X f[S_2]$	0	$R_p q_{m2} X f[S_2]$	$R_p q_{m2} X f[S_2]$
Note	$R_p q_{m2} = q_{m2}^I$	$r_{2d} \uparrow \text{ as } S_d \uparrow$	$r_{2d} \uparrow \text{ as } S_a \downarrow$	$R_p q_{m2} = q_{m2}^{II}$	$R_p q_{m2} = q_{m2}^{II}$

$$*[S] = (S/K_s + S)$$

decrease with increasing electron acceptor concentration. However, when the system is solely limited by the electron acceptor, the reductive dehalogenation reaction rate will increase with increasing electron acceptor concentration

3.1.3.2 OXYGENATION REACTION

Oxygen plays an important role in secondary substrate utilization via monooxygenase or dioxygenase reactions. As such, equation (3.10) may be extended through a multiplicative relationship to account for oxygen effects

$$-r_{2o} = q_{m2} R_p X f \left(\frac{S_2}{K_{s2} + S_2} \right) \left(\frac{S_o}{K_{so}^I + S_o} \right) \quad (3.12)$$

where $-r_{2o}$ is the rate of secondary substrate utilization via oxygenation reaction ($M_2 L^{-3} T^{-1}$), K_{so}^I is the half maximum rate concentration for dissolved oxygen of the oxygenase ($M_2 L^{-3}$) and R_p is defined by equation (3.11). The secondary substrate utilization reaction is also considered to stop, or the oxygenase becomes inactive, under endogenous conditions yielding an M value of 1.827 as described in the previous section. As can be seen, the kinetics of the oxygenase resemble that of reductive dehalogenation, but it is further controlled by dissolved oxygen

The secondary substrate utilization rates for extreme cases, where one or both primary substrates are either depleted or present in saturating concentrations, of oxygenation reactions are summarized in Table 3.1 for a case where the oxygenase half maximum rate concentration for dissolved oxygen is equal to the electron acceptor half maximum rate concentration. In general, when the electron acceptor is present in saturating concentrations, the secondary substrate utilization rate increases with increasing electron donor concentration. When the electron donor has become depleted in the system, the rate of secondary substrate utilization represents the substrate removal by resting cells from which no growth will result. In this case, the secondary substrate utilization rate will increase with increasing electron acceptor concentration until an

optimum rate is reached and secondary substrate removal begins to decrease with increasing electron acceptor concentration. This phenomenon can be attributed to the fact that both the reducing power coefficient and the overall secondary substrate utilization rate are dependent upon the electron acceptor concentration. When the system is solely limited by the electron acceptor, however, the oxygenation reaction rate will increase with increasing electron acceptor concentration.

3.1.4 SUBSTRATE UTILIZATION IN BIOLOGICAL SYSTEMS

One goal of substrate utilization modelling is to predict and simulate the removal of compounds of interest in actual biological processes. These processes generally involve either suspended biomass or biofilm systems. In each case, the substrate utilization kinetics discussed in the previous sections must be applied to the overall reactor

3.1.4.1 *SUSPENDED BIOMASS SYSTEMS*

In suspended biomass systems, the substrate utilization rate equations ((3.2), (3.7), (3.10) and (3.12)) presented in the previous sections are applied to reactor bulk substrate concentrations. Thus, in the case of electron donor removal, equation (3.2) would be expressed as follows

$$-r_{db} = q_{md} X_f \left(\frac{S_{db}}{K_{sd} + S_{db}} \right) \left(\frac{S_{db}}{K_{sd} + S_{db}} \right) \quad (3.13)$$

where the subscript b refers to bulk phase substrate concentrations in the reactor. If this equation is applied to a completely mixed basin (CSTR) of constant volume and flow rate, as in Figure 3.1, the following transient state expression can be obtained

$$V \frac{dS_{db}}{dt} = Q(S_{dbi} - S_{db}) - Vr_{db} \quad (3.14)$$

Figure 3-1: Suspended biomass CSTR system.

where dS_{ab}/dt is the electron donor removal rate in the reactor, V is the reactor volume (L^3), Q is the reactor flow rate (L^3T^{-1}), the subscript i refers to substrate input concentrations to the reactor and t refers to the time domain (T).

Once equation (3.14) is expanded to include equation (3.13), the overall electron donor utilization rate in the reactor would be

$$\frac{dS_{ab}}{dt} = \frac{Q}{V}(S_{abi} - S_{ab}) - q_{md}Xf\left(\frac{S_{ab}}{K_{sa} + S_{ab}}\right)\left(\frac{S_{ab}}{K_{sd} + S_{ab}}\right) \quad (3.15)$$

Similarly, an expression for the overall bulk phase electron acceptor concentration can be obtained by using equation (3.7). However, a third term must be introduced to account for aeration within the reactor.

$$\frac{dS_{ab}}{dt} = \frac{Q}{V}(S_{abi} - S_{ab}) + k_L a(C_s - S_{ab}) - (q_{md}\left(\frac{S_{ab}}{K_{sa} + S_{ab}}\right)\left(\frac{S_{ab}}{K_{sd} + S_{ab}}\right) + v_{a,b})Xf \quad (3.16)$$

where $k_L a$ is the oxygen mass transfer rate (T^{-1}) and C_s is the oxygen saturation concentration ($M_s L^{-3}$).

Equations (3.10) and (3.12) can also be incorporated into mass balance equations (3.17) and (3.18) that describe the overall bulk phase secondary substrate removal rate via reductive dehalogenation and oxygenation respectively.

$$\frac{dS_{2b}}{dt} = \frac{Q}{V}(S_{2bi} - S_{2b}) - q_{m2}Xf[\log(NADH / NAD) + M]\left(\frac{S_{2b}}{K_{r2} + S_{2b}}\right) \quad (3.17)$$

$$\frac{dS_{2b}}{dt} = \frac{Q}{V}(S_{2bi} - S_{2b}) - q_{m2}Xf[\log(NADH / NAD) + M]\left(\frac{S_{2b}}{K_{r2} + S_{2b}}\right)\left(\frac{S_{ab}}{K_{ra} + S_{ab}}\right) \quad (3.18)$$

3.1.4.2 BIOFILM SYSTEMS

The development of equations describing substrate utilization in biofilm systems is more complex due to factors such as substrate diffusion through the biofilm and the presence of an effective diffusion-resistance layer.

The total rate of substrate accumulation within the biofilm is governed by two factors, the rate of substrate diffusion within the biofilm and the rate of substrate consumption by the active biomass. For the electron donor removal within the biofilm, Fick's law of diffusion is combined with the electron donor utilization rate presented in equation (3.2).

$$\frac{\partial S_{df}}{\partial t} = D_{df} \frac{\partial^2 S_{df}}{\partial z^2} - q_{md} f X_f \left(\frac{S_{df}}{K_{sa} + S_{df}} \right) \left(\frac{S_{df}}{K_{sd} + S_{df}} \right) \quad (3.19)$$

where the subscript f refers to conditions within the biofilm, X_f is the biomass concentration within the biofilm ($M_x L_f^{-3}$) which is assumed constant, D_{df} is the diffusion coefficient of the electron donor through the biofilm ($L_f^2 T^{-1}$) and z represents the depth dimension (L) measured from the biofilm/liquid interface. Diffusion coefficients through the biofilm were assigned a value 80% of the respective diffusion coefficients in water as recommended by Rittmann and McCarty (1981).

The substrate is made available to the cells within the biofilm through external mass transport from the bulk liquid to the film surface, across an effective diffusion-resistance layer. This phenomenon is described as follows for electron donor utilization.

$$J_d = k_{md} (S_{dh} - S_{ds}) = \frac{D_d}{L} (S_{dh} - S_{ds}) = D_d \frac{dS_{dh}}{dz} \quad (3.20)$$

where J_d is the electron donor flux ($M_d L_f^{-2} T^{-1}$), L is the effective diffusion-resistance layer thickness, D_d is the diffusion coefficient of the electron donor through water ($L^2 T^{-1}$), k_{md} is the effective diffusion-resistance layer mass transport coefficient of the electron donor ($L T^{-1}$) and S_{dh} is the electron donor concentration at the biofilm/liquid interface ($M_d L^{-3}$).

The rate of substrate removal and the substrate flux into the biofilm can be tied together through the conservation of mass principle. The mass of substrate leaving the bulk phase, which is represented by the flux term (equation (3.20)), is diffused, consumed or accumulated within the biofilm (equation (3.19)). It then follows that, the mass of substrate passing through the biofilm/liquid interface must also be diffused, consumed or accumulated within the biofilm. The

bulk phase and biofilm systems are connected physically through the biofilm/liquid interface at which point it is assumed that $S_{af} = S_{ab}$.

A biofilm immersed in a completely mixed basin, as might be seen in biological aerated filters and RBCs, is illustrated in Figure 3.2. In order to predict the bulk phase electron donor removal in a CSTR of constant volume and flow rate, an expression similar to that of equation (3.14) is applied. However, instead of using the electron donor consumption rate directly, the substrate flux expression must be incorporated into the relationship.

$$V \frac{dS_{ab}}{dt} = Q(S_{ab1} - S_{ab}) - AJ_d \quad (3.21)$$

where A is the cross-sectional area of the biofilm. The combination of equations (3.20) and (3.21) yields the final overall bulk phase electron donor removal rate within the CSTR

$$\frac{dS_{ab}}{dt} = \frac{Q}{V}(S_{ab1} - S_{ab}) - ak_{md}(S_{ab} - S_{as}) \quad (3.22)$$

where a is the specific area of the biofilm ($L^{-2}L^{-3}$). Electron acceptor accumulation within the biofilm can also be expressed by adding a diffusive flux term to equation (3.7)

$$\frac{\partial S_{af}}{\partial t} = D_{af} \frac{\partial^2 S_{af}}{\partial z^2} - (q_{ma}(\frac{S_{af}}{K_{sa} + S_{af}})(\frac{S_{df}}{K_{sd} + S_{df}}) + v_{ax}b) fX_f \quad (3.23)$$

where D_{af} is the diffusion coefficient of the electron acceptor through the biofilm (L^2T^{-1}). The overall bulk phase electron acceptor removal rate within the CSTR is very similar to the equation describing the bulk phase electron donor removal rate except for the aeration term

$$\frac{dS_{ab}}{dt} = \frac{Q}{V}(S_{ab1} - S_{ab}) + k_L a(C_s - S_{ab}) - ak_{ma}(S_{ab} - S_{as}) \quad (3.24)$$

where S_{as} is the electron acceptor concentration at the biofilm/liquid interface (M_eL^{-3}) and k_{ma} is the effective diffusion-resistance layer mass transport coefficient of the electron acceptor (LT^{-1})

Figure 3-2: Biofilm CSTR system.

Similar expressions for secondary substrate accumulation within the biofilm via reductive dehalogenation and oxygenation can be obtained by including the diffusive flux term to equations (3.10) and (3.12) respectively

$$\frac{\partial S_{2f}}{\partial t} = D_{2f} \frac{\partial^2 S_{2f}}{\partial z^2} - q_{m2} f X_f [\log(NADH / NAD)_f + M] \left(\frac{S_{2f}}{K_{s2} + S_{2f}} \right) \quad (3.25)$$

$$\frac{\partial S_{2f}}{\partial t} = D_{2f} \frac{\partial^2 S_{2f}}{\partial z^2} - q_{m2} f X_f [\log(NADH / NAD)_f + M] \left(\frac{S_{2f}}{K_{s2} + S_{2f}} \right) \left(\frac{S_{2f}}{K_{sa} + S_{2f}} \right) \quad (3.26)$$

where D_{2f} is the diffusion coefficient of the secondary substrate through the biofilm ($L_f^2 T^{-1}$). The overall bulk phase secondary substrate removal rate within the CSTR is similar to the equation describing the bulk phase electron donor removal rate (equation (3.22))

$$\frac{dS_{2b}}{dt} = \frac{Q}{V} (S_{2bi} - S_{2b}) - a k_{m2} (S_{2b} - S_{2s}) \quad (3.27)$$

where k_{m2} is the effective diffusion-resistance layer mass transport coefficient of the secondary substrate ($L T^{-1}$) and S_{2s} is the secondary substrate concentration at the biofilm/liquid interface ($M_2 L^{-3}$).

Many researchers have focused their work on biofilm substrate removal kinetics in the last two decades (Williamson and McCarty, 1976a; Rittmann and McCarty, 1978, 1980, 1981, Namkung *et al.*, 1983; Rittmann, 1984, 1989, Kissel *et al.*, 1984, Sáez *et al.*, 1984, Wanner and Gujer, 1984; Suidan *et al.*, 1985, 1987, Wanner and Gujer, 1986, Annachhatre and Khanna, 1987, Gantzer *et al.*, 1988; Sáez and Rittmann, 1988, Gantzer, 1989, Kim and Suidan, 1989, Skowlund, 1990). The major difficulty arises from the fact that neither the substrate flux (J) nor the substrate concentration at the biofilm/liquid interface (S_s) can be measured readily and that both will vary with changes in biofilm thickness (L_f). Thus, the solution to the system of equations must generally be obtained iteratively.

3.2 BIOLOGICAL GROWTH KINETICS

A second goal of modelling is to predict the overall amount of biomass which will accumulate in the reactor. The net growth rate of the active biomass is defined by two terms. The first term represents biomass synthesis, which is proportional to electron donor utilization. The second term represents the rate at which the biomass is oxidized, thereby providing energy for maintenance, through a phenomenon called endogenous metabolism (Rittmann and Sáez, 1994).

$$r_c = Y(r_d) - bXf \quad (3.28)$$

where r_c is the net growth rate ($M_x L^{-3} T^{-1}$), Y is the yield coefficient ($M_x M_d^{-1}$) and b is the endogenous decay coefficient (T^{-1}).

3.2.1 SUSPENDED BIOMASS GROWTH

The expression for net biomass growth in a suspended system accounting for dual substrate limitation can be derived by combining equations (3.13) and (3.28)

$$r_c = Yq_{md} Xf \left(\frac{S_{sd}}{K_{sd} + S_{sd}} \right) \left(\frac{S_{sd}}{K_{sd} + S_{sd}} \right) - bXf \quad (3.29)$$

where the biomass concentration (X) may vary with time, but the active fraction of that biomass (f) will remain constant since the entire biomass is assumed to be exposed to the same electron donor and acceptor concentrations, as well as the same environmental conditions. This equation can then be applied to a CSTR of constant volume and flow rate in the following manner

$$V \frac{fdX}{dt} = Q(X_i - X)f + V(r_c) \quad (3.30)$$

where fdX/dt is the net active biomass accumulation rate in the reactor ($M_x L^{-3} T^{-1}$) and X_i is the biomass input to the system ($M_x L^{-3}$), generally from influent and/or recycling streams. By substituting equation (3.29) into (3.30), equation (3.31) can be obtained.

$$\frac{dX}{dt} = \frac{Q}{V} (X_i - X) + (Yq_{md} \left(\frac{S_{ab}}{K_{sa} + S_{ab}} \right) \left(\frac{S_{ab}}{K_{sd} + S_{ab}} \right) - b)X \quad (3.31)$$

One might note that since the active fraction is not considered to change with time in equation (3.30), the active fraction term disappears completely in the derivation of equation (3.31)

3.2.2 BIOFILM GROWTH

The development of equations describing unsteady state biomass growth in a biofilm system is more involved than for suspended systems. The complexity can be attributed to variations in biofilm thickness due to growth and variations in the active biomass fraction, in both the time and space domains. Active biomass fraction variations are related to changes in the bacterial environment (i.e. decreases in primary substrate concentrations). In general, as the electron donor and electron acceptor become more limiting (in time and space), a greater fraction of the biomass will become inactive.

The biofilm growth is represented by a simple first order differential equation which contains a growth term, r_g ($M/L_T^3 T^{-1}$), due to substrate consumption and a loss term, r_l ($M/L_T^3 T^{-1}$), due to sloughing and endogenous decay.

$$X_f \frac{dL_f}{dt} = L_f r_g - L_f r_l \quad (3.32)$$

These individual mechanisms will be incorporated into a mass balance model and defined in greater detail in Section 3.3.2.2. It should be noted that external biomass inputs to the biofilm matrix through processes such as particle deposition are considered to be negligible

3.3 NUMERICAL APPROACH

The system of non-linear ordinary and partial differential equations presented in sections 3.1 and 3.2 can not be solved directly. As such, the solution will be obtained through a numerical approximation technique.

3.3.1 INITIAL AND BOUNDARY CONDITIONS

The numerical methods employed to solve the system of equations require initial and boundary conditions. The initial and boundary conditions for the suspended biomass and biofilm systems are presented in the following sections.

3.3.1.1 SUSPENDED BIOMASS SYSTEM

The system of equations derived for modelling the suspended biomass system assumes a CSTR regime and hence, concentrations vary only in the time domain. As such, four initial conditions are necessary to describe the overall system in its initial state at time $t = 0$.

$$\begin{aligned} S_{db} &= S_{dbo} \\ S_{ab} &= S_{abo} \\ S_{2b} &= S_{2bo} \\ X &= X_o \end{aligned}$$

where the subscript o refers to substrate and biomass concentrations at time $t = 0$. These initial values are specified directly by the user.

3.3.1.2 BIOFILM SYSTEM

The system of equations describing biofilm processes is more complex because concentrations can vary in both the time and space domains. As illustrated in Figure 3.2, the biofilm system consists of the biofilm, the effective diffusion-resistance layer and the bulk liquid within the reactor. For modelling purposes, the biofilm is discretized into a series of layers as

depicted in Figure 3.3. Initial, minimum and maximum layer thicknesses are specified by the user, and layers are split and joined as the biofilm grows and decays.

Each substrate; namely electron donor, electron acceptor and secondary substrate requires two boundary conditions and one initial condition, while the active fraction variable requires one initial condition. The boundary conditions are given for the biofilm/support interface and the biofilm/liquid interface. Diffusion and mass transport through the biofilm support is considered to be negligible when compared to diffusion and mass transport processes through the biofilm matrix. As such, the following boundary condition is assumed to apply at the biofilm/support interface.

$$\frac{dS_{df}}{dz} = \frac{dS_{af}}{dz} = \frac{dS_{2f}}{dz} = 0$$

where the subscript f refers to substrate concentrations within the biofilm, in this case at the support.

One initial condition is also required for each of the overall bulk phase reactor equation. These initial conditions are essentially the same as those specified for the suspended system except for the biomass component.

$$\begin{aligned} S_{db} &= S_{dbo} \\ S_{ab} &= S_{abo} \\ S_{2b} &= S_{2bo} \end{aligned}$$

As with the suspended biomass system, these initial values can be specified by the user.

The initial conditions within the biofilm were assumed to be uniform throughout the biofilm. The user specifies the initial biofilm thickness and the initial active fraction as follows:

$$\begin{aligned} f &= f_o \\ L_f &= L_{fo} \end{aligned}$$

Two possibilities exist for assigning the initial concentrations within the biofilm. One option would be to assign an initial value based on any data available to the user regarding actual conditions within the biofilm. However, this data is difficult to obtain and seldom exists.

Figure 3-3: Biofilm system layers.

The second option would be to use a steady state approximation similar to that described by Sáez *et al.* (1984). In this case, it is assumed that the substrate flux into the biofilm is equal to the potential substrate utilization within the biofilm. This requires an iterative procedure in which an initial concentration within the biofilm is assumed, and the potential substrate utilization and flux through the biofilm/liquid interface are calculated. If the potential substrate utilization is equal to the flux through the biofilm/liquid interface, the assumed concentration represents the initial concentration for the model run. If the potential substrate utilization is not equal to the flux through the biofilm/liquid interface, the assumed concentration is increased or decreased until the flux and potential utilization are equal. This provides an initial condition which is reasonable given the initial bulk phase concentrations and avoids prescribing an arbitrary concentration. The second option was used for the model developed in this work, the equations for which are presented in section 3.3.2.2.3.

3.3.2 NUMERICAL APPROXIMATION TECHNIQUE

The system of non-linear differential equations for the suspended biomass and biofilm systems presented in Sections 3.1 and 3.2 will be solved, simultaneously, using an explicit finite difference approach. Methods employing finite differences to solve initial and boundary value problems replace each of the derivatives in the differential equation by an appropriate difference quotient approximation such as the backward-difference or centered-difference formula. The general formulas required for the suspended biomass and biofilm systems are described in the following sections. The actual finite difference equations utilized by the model are presented in Appendix A.

3.3.2.1 SUSPENDED BIOMASS SYSTEM

First order ordinary differential equations describe CSTR substrate removal and biomass growth in a suspended biomass system. The general form of the equation can be expressed as

$$V \frac{dS_b}{dt} = Q(S_{bi} - S_b) \pm Vr_b \quad (3.33)$$

where S_b is the bulk substrate or biomass concentration and r_b is the respective consumption or generation rate of the substrate and biomass.

In the explicit finite difference method, the substrate or biomass concentration at a particular time (t) can be approximated by separating the differential variables and using the concentrations estimated at the previous time step in the following manner.

$$\frac{(S_b^{j+1} - S_b^j)}{\Delta t} = \frac{Q}{V} (S_{bi} - S_b^j) \pm r_b^j \quad (3.34)$$

where the superscript j refers to the time domain. This yields an explicit finite difference equation of the form

$$S_b^{j+1} = S_b^j + \left[\frac{Q}{V} (S_{bi} - S_b^j) \pm r_b^j \right] \Delta t \quad (3.35)$$

3.3.2.2 BIOFILM SYSTEM

As previously discussed, the system of equations required to simulate the biofilm system is much more complex than the one developed to model the suspended biomass system. General finite difference expressions are necessary to describe substrate utilization at the biofilm/liquid interface, the biofilm/support interface and the intermediate layers. Relationships were also required to simulate individual biofilm layer growth and to describe bulk phase CSTR conditions. The actual finite difference equations used by the model are presented in Appendix A.

3.3.2.2.1 INTERMEDIATE LAYER SUBSTRATE UTILIZATION

Substrate utilization within a biofilm is generally expressed in the literature as a second order partial differential equation

$$\frac{\partial S_f}{\partial t} = D_f \frac{\partial^2 S_f}{\partial z^2} - r_f \quad (3.36)$$

The following explicit finite difference expression can be derived from equation (3.36)

$$\frac{(S_f^{i,j+1} - S_f^{i,j})}{\Delta t} = \frac{D_f}{z^{i,j}} \left\{ \frac{(S_f^{i-1,j} - S_f^{i,j})}{((z^{i-1,j} + z^{i,j})/2)} - \frac{(S_f^{i,j} - S_f^{i+1,j})}{((z^{i,j} + z^{i+1,j})/2)} \right\} - r_f^{i,j} \quad (3.37)$$

where the superscript i refers to the space domain. The second order diffusion term is discretized to account for a variable layer thickness (z^i). The final form of the general differential equation for substrate utilization within the intermediate biofilm layers is therefore

$$S_f^{i,j+1} = S_f^{i,j} + \left[\frac{D_f}{z^{i,j}} \left\{ \frac{(S_f^{i-1,j} - S_f^{i,j})}{((z^{i-1,j} + z^{i,j})/2)} - \frac{(S_f^{i,j} - S_f^{i+1,j})}{((z^{i,j} + z^{i+1,j})/2)} \right\} - r_f^{i,j} \right] \Delta t \quad (3.38)$$

3.3.2.2.2 BIOFILM SUPPORT INTERFACE LAYER SUBSTRATE UTILIZATION

The substrate utilization expression for the biofilm/support layer must account for a second type (Neumann) or no flux ($dS_f/dz = 0$) boundary condition. This is accomplished by setting the diffusive flux at the biofilm/support interface to zero

$$\frac{(S_f^{nf,j+1} - S_f^{nf,j})}{\Delta t} = \frac{D_f}{z^{nf,j}} \left\{ \frac{(S_f^{nf-1,j} - S_f^{nf,j})}{((z^{nf-1,j} + z^{nf,j})/2)} - 0 \right\} - r_f^{nf,j} \quad (3.39)$$

where the superscript nf refers to the layer at the biofilm/support interface. The final general differential equation for substrate utilization within the biofilm/support interface layer is

$$S_f^{nf,j+1} = S_f^{nf,j} + \left[\frac{D_f}{z^{nf,j}} \left\{ \frac{(S_f^{nf-1,j} - S_f^{nf,j})}{((z^{nf-1,j} + z^{nf,j})/2)} \right\} - r_f^{nf,j} \right] \Delta t \quad (3.40)$$

3.3.2.2.3 BIOFILM/LIQUID INTERFACE LAYER SUBSTRATE UTILIZATION

Two alternative techniques were considered in modelling the interface between the biofilm and the liquid layers. The expression must account for diffusion across the effective diffusion-resistance layer. A fully explicit finite difference approach yields equation (3.41).

$$\frac{(S_f^{1,j+1} - S_f^{1,j})}{\Delta t} = \frac{k_m}{z^{1,j}} (S_b^j - S_f^{1,j}) - \frac{D_f}{z^{1,j}} \left\{ \frac{(S_f^{1,j} - S_f^{2,j})}{((z^{1,j} + z^{2,j}) / 2)} \right\} - r_f^{1,j} \quad (3.41)$$

where the superscripts 1 and 2 refer to the biofilm/liquid interface layer and second biofilm layer, respectively. The first term on the right hand side represents the diffusion through the effective diffusion-resistance layer and the second term represents the diffusion within the biofilm from layer 1 to layer 2. The general form of the finite difference equation for substrate utilization within the biofilm/liquid interface layer would be

$$S_f^{1,j+1} = S_f^{1,j} + \left[\frac{k_m}{z^{1,j}} (S_b^j - S_f^{1,j}) - \frac{D_f}{z^{1,j}} \left\{ \frac{(S_f^{1,j} - S_f^{2,j})}{((z^{1,j} + z^{2,j}) / 2)} \right\} - r_f^{1,j} \right] \Delta t \quad (3.42)$$

Another method of evaluating substrate concentrations within the biofilm/liquid interface layer involves an iterative technique (Sáez *et al.*, 1984). The total substrate flux (J) into the biofilm is defined as

$$J = D_f \left. \frac{dS_f}{dz} \right|_{z=0} \quad (3.43)$$

If steady state conditions are assumed to prevail, Sáez *et al.* (1984) set the total diffusive flux at the biofilm/liquid interface layer equal to the total substrate utilization requirements of the layer

$$J = \int_0^{l_f} r_f dz \quad (3.44)$$

Equation (3.44) is approximated by summing the substrate utilization in each layer of the biofilm (Sáez *et al.*, 1984) in the following manner

$$J^j = \sum_{i=1}^{n_f} r_j^{i,j} z^{i,j} \quad (3.45)$$

Under both dynamic and steady state conditions, the substrate flux into the biofilm must be equal to the substrate flux across the effective diffusion-resistance layer.

$$J^j = k_m(S_h^j - S_f^{1,j}) \quad (3.46)$$

In the iterative technique, an initial value of $S_f^{1,j}$ is assumed. equations (3.45) and (3.46) are evaluated, and, if they are not equal a new $S_f^{1,j}$ value is assumed until the two equations are equal. The main drawback in utilizing this technique is that an assumption of steady state must be made at each iteration.

3.3.2.2.4 BIOFILM LAYER GROWTH

As discussed previously, the biofilm layer growth can be represented by a first order differential equation which contains a growth term (r_g) due to substrate consumption and a loss term (r_l) due to sloughing and endogenous decay.

$$X_f \frac{dz}{dt} = z r_g - z r_l \quad (3.47)$$

This expression is similar to equation (3.32) in which L_f represents the thickness of the entire biofilm, and in this case, z represents the thickness of each discretized layer.

An expression simulating biofilm growth was derived from first principles for this model. This was accomplished by dividing the initial biofilm thickness (L_f) into a number of layers each of which can grow at a different rate depending upon the concentrations of primary substrates supplied to the layer. Each layer is composed of an active biomass portion which actively consumes the substrates, and an inactive biomass portion which does not consume substrate but does occupy space within the total biofilm matrix. The growth rate of the active fraction can be described by:

$$z_a^{t+1} = z_a^{t,j} + [z_a^{t,j} (Yq_{md} (\frac{S_{df}^{t,j}}{K_{sd} + S_{df}^{t,j}}) (\frac{S_{df}^{t,j}}{K_{sd} + S_{df}^{t,j}}) - b - sl)] \Delta t \quad (3.48)$$

where z_a is the active portion of the layer (L) and sl is the sloughing coefficient of the biofilm (T^{-1}). The sloughing coefficient represents the average rate of biomass detachment (or shearing) over the entire area of the support media. The magnitude of the sloughing coefficient is dependent upon factors which create turbulence, such as fluid velocity and aeration rate (Sáez *et al*, 1984). The unsteady state behavior of the inactive fraction can be describe by the following expression

$$z_i^{t+1} = z_i^{t,j} + [z_i^{t,j} k_i b - z_i^{t,j} sl] \Delta t \quad (3.49)$$

where z_i is the inactive portion of the layer (L) and k_i is the inert material retention coefficient (dimensionless). In the model, this coefficient represents the inorganic fraction of dead or inactive cell mass which remains within the biofilm matrix as a result of endogenous decay. It is considered to be constant throughout the biofilm, and is assumed to be proportional to the concentration of active biomass. In short, this fraction contributes to the total volume of the biofilm, but does not participate in physiological functions such as substrate utilization and cell growth. The overall unsteady state thickness of the layer can be defined as the sum of the two fractions, and hence,

$$z^{t+1} = z^{t,j} + [f^{t,j} z^{t,j} (Yq_{md} (\frac{S_{df}^{t,j}}{K_{sd} + S_{df}^{t,j}}) (\frac{S_{df}^{t,j}}{K_{sd} + S_{df}^{t,j}}) - (1 - k_i)b - sl) - (1 - f^{t,j}) z^{t,j} sl] \Delta t \quad (3.50)$$

where z is the total width of the discretized layer. The active fraction of the layer can subsequently be evaluated at each time step by using the following relationship.

$$f^{t+1} = \frac{z_a^{t+1}}{z^{t+1}} \quad (3.51)$$

4.1.2 SUSPENDED BIOMASS SYSTEM ASSUMPTIONS

Two basic assumptions are made for the suspended growth system 1) the active fraction (f) remains constant in time 2) the kinetic coefficients (Y , b , μ_{ma} , μ_{md} , μ_{m2} , q_{ma} , q_{md} , q_{m2} , K_{sa} , K_{sd} , K_{s0} and K_{s2}) are also considered to remain constant in time

4.1.3 BIOFILM SYSTEM ASSUMPTIONS

A few more system specific assumptions are required for the attached growth system due to its complexity

- 1) The biofilm is assumed to be uniform in thickness (L_f) throughout its length and width, and the biomass density (X_f) is assumed to be uniform throughout its depth
- 2) The concentration gradient of the effective diffusion-resistance layer (L) is assumed to be linear
- 3) The kinetic coefficients (Y , b , k_1 , sl , μ_{ma} , μ_{md} , μ_{m2} , q_{ma} , q_{md} , q_{m2} , K_{sa} , K_{sd} , K_{s0} , and K_{s2}) and diffusion coefficients (D_{fa} , D_{fd} , D_{f2} , k_{ma} , k_{md} , and k_{m2}) are considered to remain constant in time, and throughout the biofilm depth.
- 4) Any active suspended biomass present in the system does not contribute significantly to primary or secondary substrate utilization
- 5) Particle transport and deposition processes do not significantly affect the overall growth of the biofilm.
- 6) The diffusion coefficient of a particular substrate within the biofilm matrix is assumed to be 80% of its diffusion in water (Rittmann and McCarty, 1980)

separated in half and the characteristics of the original layer (substrate concentrations and active fraction value) are assigned to each of the two layers. In the subsequent iteration, each layer then readjusts its substrate concentrations and active fraction value based upon diffusive fluxes from adjoining layers and substrate consumption rates. The importance and effects of this layer splitting feature are discussed further in Section 5.2.2.2.

3.4.2 LAYER JOINING FEATURE

As a layer becomes infinitely thin, the model can grossly overestimate substrate concentrations within this layer and eventually cause an overflow error in the numerical model. This has the potential to occur because the layer thickness (z^u) is present in the denominator of all equations required for biofilm substrate concentration calculations.

To avoid this problem, the layer joining feature was incorporated into the numerical model. When a particular layer reaches a minimum user-specified thickness (z_m), the layer is added to the adjoining layer on the biofilm/liquid interface side. The new substrate concentrations and active fraction of the combined layer are determined based upon the weighted average of characteristics of the two layers.

$$S_m^{n+1} = \frac{(z^{i-1} S_i^{n+1} + z^i S_i^n)}{(z^{i-1} + z^i)} \quad (3.52)$$

$$f_n^{i+1} = \frac{(z_a^{i-1} + z_a^i)}{(z^{i-1} + z^i)} \quad (3.53)$$

The advantages of the layer joining feature are discussed further in Section 5.2.2.1.

Figure 3-4: Biofilm profile definition with varying layer thickness.

CHAPTER 4

MODEL SIMULATION

4.1 MODEL ASSUMPTIONS

To develop the numerical model, a series of underlying assumptions were made which are particular to either the suspended growth or attached growth systems, or can be applied to both systems. These assumptions are presented in this section.

4.1.1 GENERAL ASSUMPTIONS

- 1) Steady state or equilibrium conditions prevail within the system at time $t = 0^-$ which allows for the evaluation of certain required initial parameters at time $t = 0$ and for the system to operate under dynamic conditions at time $t = 0^+$.
- 2) Reactions are dependent solely on electron donor, electron acceptor and secondary substrate concentrations, i.e. no other nutrient limitation conditions exist.
- 3) The secondary substrate is considered to be present in trace amounts. As such, the secondary substrate does not contribute significantly to growth or the electron acceptor demand in its biodegradation. It also does not exhibit any inhibitory effects on primary substrate utilization.
- 4) Secondary substrate utilization is governed by electron donor and electron acceptor concentrations, but does not directly or indirectly affect primary substrate utilization in return.
- 5) The model assumes that the biomass does not modify metabolic pathways under anaerobic conditions. This implies that in an aerobic system, once the oxygen or electron acceptor has been depleted, the model does not simulate substrate utilization under anaerobic conditions using another electron acceptor.
- 6) The model does not account for substrate removal from the bulk phase through physical processes such as adsorption.

4.1.2 SUSPENDED BIOMASS SYSTEM ASSUMPTIONS

Two basic assumptions are made for the suspended growth system 1) the active fraction (f) remains constant in time. 2) the kinetic coefficients (Y , b , μ_{ma} , μ_{md} , μ_{m2} , q_{ma} , q_{md} , q_{m2} , K_{sa} , K_{sd} , K_{s0} and K_{s2}) are also considered to remain constant in time

4.1.3 BIOFILM SYSTEM ASSUMPTIONS

A few more system specific assumptions are required for the attached growth system due to its complexity.

- 1) The biofilm is assumed to be uniform in thickness (L_f) throughout its length and width, and the biomass density (X_f) is assumed to be uniform throughout its depth.
- 2) The concentration gradient of the effective diffusion-resistance layer (L) is assumed to be linear
- 3) The kinetic coefficients (Y , b , k_1 , sl , μ_{ma} , μ_{md} , μ_{m2} , q_{ma} , q_{md} , q_{m2} , K_{sa} , K_{sd} , K_{s0} , and K_{s2}) and diffusion coefficients (D_{fa} , D_{fd} , D_{f2} , k_{ma} , k_{md} , and k_{m2}) are considered to remain constant in time, and throughout the biofilm depth.
- 4) Any active suspended biomass present in the system does not contribute significantly to primary or secondary substrate utilization
- 5) Particle transport and deposition processes do not significantly affect the overall growth of the biofilm.
- 6) The diffusion coefficient of a particular substrate within the biofilm matrix is assumed to be 80% of its diffusion in water (Rittmann and McCarty, 1980)

4.2 SIMULATION OVERVIEW

The numerical model (COMDEG) can be utilized as a predictive tool in estimating bulk phase electron donor, electron acceptor and secondary substrate removal in both suspended and attached growth systems. It can estimate biomass growth in suspended systems and biofilm growth in attached systems. The biofilm model can also generate active fraction, electron donor concentration, electron acceptor concentration and secondary substrate concentration profiles within a biofilm.

The general features of the suspended biomass (BIOSUS) and biofilm (BIOFIL) models are presented briefly in the following sections. The FORTRAN source code for the COMDEG model can be found in Appendix B.

4.2.1 SUSPENDED BIOMASS SYSTEM

A program flow chart for the suspended biomass subroutine, BIOSUS, is presented in Figure 4.1. Eight major tasks are performed by this subroutine. 1) The BIOSUS subroutine is entered from the main program, COMDEG. 2) Input parameters are read from the input file and are written to the output file. 3) Initial parameters are specified and some preliminary calculations are performed. 4) The time step loop is entered. 5) The system parameters (substrate and biomass concentrations) for time $j+1$ are evaluated. 5) Parameters are reset for the next time step. 6) Results are written to the output file. 7) The program performs the next time step or 8) returns to the main program.

4.2.2 BIOFILM SYSTEM

As can be seen from the simplified program flow chart illustrated in Figure 4.2, the biofilm system subroutine, BIOFIL, is much more involved than its BIOSUS counterpart. As in the suspended biomass subroutine, the BIOFIL subroutine is entered from the main program. Input

Figure 4-1: BIOSUS flow chart.

2

PM-1 3 1/2" x 4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

parameters are read from the input file and are written to an output file. Initial parameters are specified and some preliminary calculations are performed. Biofilm/liquid interface layer substrate concentrations are estimated using the iterative approach (equations (3.41) and (3.42)). The time loop is entered where new bulk phase substrate concentrations are evaluated.

Next, the program establishes whether to model the biofilm as a 1 layer, 2 layer or greater than 2 layer biofilm system. For a 1 layer system, the program will determine whether or not to use the layer splitting option based upon the user specified maximum layer thickness. If the layer is split, then the layer characteristics are assigned to each of the two new layers and new active fraction, layer thickness and substrate concentration values are evaluated for each layer. If the layer is not split, new layer characteristics are simply calculated for the one layer. A similar process takes place in a two layer system, where the first and/or second layer could be split. The layer joining feature is not available in 1 and 2 layer systems because total primary substrate deprivation and/or biofilm layer decay below the user specified minimum layer thickness is not anticipated at this point within the biofilm.

In the greater than 2 layer system, conditions of the biofilm/support interface layer are first assessed. If layer joining is required, then the combined layer characteristics (substrate concentrations, active fraction value and layer thickness) are reevaluated and the program proceeds to the intermediate layers portion of the program. If layer splitting is required, then as in the 1 layer system, layer characteristics are assigned to each of the two layers and new active fraction, layer thickness and substrate concentration values are estimated for each layer. If the layer is not split, new layer characteristics are simply calculated for the layer.

After the new biofilm/support interface layer characteristics have been estimated, a biofilm layer loop is entered where the new characteristics of each intermediate layer are evaluated in a manner similar to the biofilm/support interface layer. The biofilm/liquid interface layer conditions are assessed upon completion of the biofilm layer loop and new layer characteristics (thickness,

active fraction and substrate concentrations) estimated. The layer joining feature is not necessary for the biofilm/liquid interface layer since this layer is not expected to decay due to high primary substrate concentrations at the biofilm/liquid interface relative to concentrations at greater biofilm depths.

Once all new bulk phase and biofilm characteristics have been calculated, the model is reset for the next time step. Results are then sent to the appropriate biofilm and reactor output files. The program can now either perform the next time step or return to the main program upon completion of the required number of time steps.

4.3 ITERATIVE VS. EFD APPROACH

The explicit finite difference (EFD) and iterative approaches were evaluated for their use and practicality in estimating biofilm/liquid interface layer substrate concentrations. The comparison and justification for use in the numerical model are presented in this section.

4.3.1 ITERATIVE APPROACH

As demonstrated in Section 3.3.2.2.3, the iterative approach is based on the assumption of steady state within the biofilm which signifies that no accumulation of substrates can occur within the biofilm layers (Sáez *et al.*, 1984).

$$J = D_f \left. \frac{dS_f}{dz} \right|_{z=L_f} = \int_0^{L_f} r_f dz \quad (4.1)$$

The total flux into the biofilm is equal to the potential consumption of the primary and secondary substrates throughout the biofilm, which in turn must be equal to the flux through the effective diffusion-resistance layer.

$$J = k_m (S_b - S_s) \quad (4.2)$$

The iterative approach is semi-explicit in nature because the biofilm/liquid interface layer concentrations (S_i) must be evaluated using biofilm layer growth, as well as, intermediate layer, biofilm/support interface layer and bulk phase substrate concentration values from the same time step in a summation technique.

4.3.2 EFD APPROACH

As illustrated in Section 3.3.2.2.3, the explicit finite difference approach is based on control volume and mass balance concepts. The substrate is allowed to diffuse in and out of the biofilm/liquid interface layer depending upon the concentration gradient which signifies that substrate accumulation is possible within the layer.

$$\frac{dS_i^1}{dt} = \frac{k_m}{z^1} (S_b - S_i^1) - \frac{D_f}{z^1} \frac{(S_f^1 - S_i^1)}{((z^1 + z^2)/2)} - r_i^1 \quad (4.3)$$

However, as discussed in section 3.3.1.2, the steady state approximation described by Sáez *et al.* (1984) was utilized to determine the initial concentrations within the biofilm at time $t = 0$.

4.3.3 COMPARISON AND JUSTIFICATION OF USE

The numerical model was run with each of the biofilm/liquid interface layer approximation techniques using the input parameters found in Table 4.1 (please refer to Table 6.2 for corresponding references). Electron donor and electron acceptor concentrations were computed, over a 24 hour simulation period, for the bulk phase and biofilm/liquid interface and are presented in Figures 4.3 and 4.4. As can be seen from Figure 4.3, the two methods did not yield significantly different ($>1\%$) electron donor concentrations for the biofilm/liquid interface (0.76%) or the bulk phase (0.53%). However, the results obtained from the two techniques were found to be significantly different for electron acceptor concentrations at the biofilm/liquid interface (3.5%) and in the bulk phase (1.1%).

Table 4-1: Biofilm input parameters for approximation technique comparison.

Parameter	Value
q_{md}	0.892 $\text{mg}_d/\text{mg}_s\text{-hr}$
q_{ma}	0.383 $\text{mg}_d/\text{mg}_s\text{-hr}$
K_{sd}	4.6 mg_d/L
K_{sa}	0.084 mg_d/L
$v_{s,s}$	1.42 mg_d/mg_s
b	0.01 hr^{-1}
sl	0.01 hr^{-1}
k_1	0.25
M	1.827
Y	0.5 mg_d/mg_a
X_i	$5 \times 10^6 \text{ mg}_s/\text{m}_i^3$
D_{dt}	$3.5 \times 10^{-6} \text{ m}_i^2/\text{hr}$
D_{df}	$6.2 \times 10^{-6} \text{ m}_i^2/\text{hr}$
k_{md}	0.1784 m/hr
k_{ma}	0.31 m/hr
L	0.000025 m
a	100 m_i^2/m^3
$S_{dbi} = S_{dbo}$	200 mg_d/L
$S_{abi} = S_{abo}$	6.2 mg_d/L
f_0	0.5
L_{f0}	2940 μm_i
C_s	9.6 mg_d/L
$k_1 \alpha$	60 hr^{-1}
V/Q	4 hr
z_m	40 μm_i
z_i	100 μm_i

Figure 4-3: Numerical approximation technique comparison: electron donor.

reduce the total number of biofilm layers, which in turn decreases the required computation time. As such, the layer joining feature is considered a valuable addition to the numerical model.

5.2.2.2 MAXIMUM LAYER THICKNESS

The substrate concentration within a particular biofilm layer represents an average value for the entire layer. In general, as a layer becomes larger, the model will tend to underestimate concentrations on the biofilm/liquid interface side and overestimate concentrations on biofilm/support interface side of the layer. In Figure 3-4, it was shown that for an average concentration of 10 mg/m^3 in each of the three $500 \text{ }\mu\text{m}$ layers, the biofilm profile becomes more defined as the number of layers is increased or the thickness of the individual layers is decreased.

In Figure 5-1, it can be seen that as the maximum layer thickness was increased from $100 \text{ }\mu\text{m}$ to $750 \text{ }\mu\text{m}$, the slope of the electron donor profile was substantially impacted within the first $500 \text{ }\mu\text{m}$. This can be attributed to higher electron donor consumption in the upper layers of the biofilm due to the high primary substrate availability. As a result of the rapid decrease in electron donor in the upper layers of the biofilm, a smaller maximum layer thickness is needed to more accurately define the electron donor profile.

In Figure 5-2, high bulk phase electron acceptor concentrations (9.0 mg/L - 9.2 mg/L) enabled the biomass to utilize some of the electron acceptor for respiration while allowing a portion to diffuse into the biofilm to a depth of $1000 \text{ }\mu\text{m}$. For each maximum biofilm thickness, $1000 \text{ }\mu\text{m}$ was required for the electron acceptor to become depleted. The slope of the profile was not affected by the maximum layer thickness. However, since diffusion processes played a greater role in establishing the electron acceptor profile than for the electron donor, i.e. the substrate was not depleted as quickly, the impact of increasing the maximum layer thickness on the electron acceptor profile is more pronounced than for the electron donor profile. For the larger maximum

Time step analysis, which is discussed in detail in Section 5.2.1, revealed that the results of each techniques were not significantly ($\pm 1\%$) influenced by the size of the time step. Thus, it was concluded that the difference in the results was probably caused by the assumption of steady state in the iterative approach. As such, because the numerical model was designed to model dynamic systems, the explicit finite difference approach was selected as the more desirable approach. Furthermore, since the assumption of steady state is not unreasonable for time $t = 0$ and since the explicit finite difference approach can not be utilized for approximating layer 1 conditions at time $t = 0$, the iterative approach was employed to establish initial substrate concentrations at the biofilm/liquid interface and within the biofilm.

CHAPTER 5

MODEL VERIFICATION

5.1 NUMERICAL VERIFICATION

To verify the performance of the numerical model, the suspended biomass subroutine and components of the biofilm subroutine were compared to Microsoft EXCEL spreadsheet calculations. For the suspended growth system, expected steady state substrate and biomass concentrations were determined via a spreadsheet. The program was then run until steady state conditions were reached. Both methods were found to yield identical results.

Due to the extensive nature of the biofilm system, it was impossible to obtain exact steady state solutions with the use of a spreadsheet. Instead, substrate concentrations, biofilm layer thickness and active fraction values were evaluated for five (5) consecutive time steps in a three (3) layer biofilm system using the spreadsheet. The program was then run for five time steps and the results compared. Once again these were found to be identical, which indicated that model equations had been entered correctly and that the model itself was functioning as intended.

Other components of the BIOFIL subroutine such as the layer splitting and joining features and the iterative biofilm/liquid interface layer concentrations estimation technique, were more easily verified using the Video Debugger feature of WATCOM, a software package for compiling and debugging FORTRAN programs. These portions of the program were also found to be performing as expected.

5.2 SPATIAL AND TEMPORAL DISCRETIZATION

The use of an appropriate time step and layer size is extremely important in numerical modelling. On the one hand, gross approximation errors can result from the use of too large a step, whereas valuable computing time can be wasted if the step size is too small.

5.2.1 TIME STEP

The selection of the model time step was conducted in the following manner. First the program was run with a series of different time steps (0.1 hr to 0.00001 hr). Upon completion, the model outputs were compared and the time step which minimized potential numerical simulation errors and the required computing time was selected. Next, the time step was verified by dividing and multiplying it by a factor of 2, 5 and 10. If output results were not found to fluctuate significantly (i.e. $\leq 1\%$), then the time step was judged to be adequate.

This verification process was performed for both the suspended biomass and biofilm systems whenever system conditions, process variables or kinetic parameters were altered significantly.

5.2.2 BIOFILM LAYER THICKNESS

As discussed in Section 4.4, the size and/or number of layers used to model the biofilm system is important to ensure accurate biofilm profiles, and hence, the layer splitting and layer joining options were incorporated into the numerical model. These features are discussed in the following sections.

5.2.2.1 MINIMUM LAYER THICKNESS

As a layer becomes infinitely small, the model can grossly overestimate the substrate concentrations within this layer and eventually cause an overflow error. In order to avoid this problem, a minimum layer thickness is specified by the user. When a particular layer reaches this value, it is combined with an adjoining layer in the manner described in Section 4.4.2.

The joining of small layers, in this case less than 30 μm , was not found to alter bulk phase results nor the biofilm profile significantly (i.e. $\leq 1\%$). However, the combining process does

reduce the total number of biofilm layers, which in turn decreases the required computation time. As such, the layer joining feature is considered a valuable addition to the numerical model.

5.2.2.2 MAXIMUM LAYER THICKNESS

The substrate concentration within a particular biofilm layer represents an average value for the entire layer. In general, as a layer becomes larger, the model will tend to underestimate concentrations on the biofilm/liquid interface side and overestimate concentrations on biofilm/support interface side of the layer. In Figure 3.4, it was shown that for an average concentration of 10 mg/m^3 in each of the three $500 \text{ }\mu\text{m}$ layers, the biofilm profile becomes more defined as the number of layers is increased or the thickness of the individual layers is decreased.

In Figure 5.1, it can be seen that as the maximum layer thickness was increased from $100 \text{ }\mu\text{m}$ to $750 \text{ }\mu\text{m}$, the slope of the electron donor profile was substantially impacted within the first $500 \text{ }\mu\text{m}$. This can be attributed to higher electron donor consumption in the upper layers of the biofilm due to the high primary substrate availability. As a result of the rapid decrease in electron donor in the upper layers of the biofilm, a smaller maximum layer thickness is needed to more accurately define the electron donor profile.

In Figure 5.2, high bulk phase electron acceptor concentrations ($9.0 \text{ mg/L} - 9.2 \text{ mg/L}$) enabled the biomass to utilize some of the electron acceptor for respiration while allowing a portion to diffuse into the biofilm to a depth of $1000 \text{ }\mu\text{m}$. For each maximum biofilm thickness, $1000 \text{ }\mu\text{m}$ was required for the electron acceptor to become depleted. The slope of the profile was not affected by the maximum layer thickness. However, since diffusion processes played a greater role in establishing the electron acceptor profile than for the electron donor, i.e. the substrate was not depleted as quickly, the impact of increasing the maximum layer thickness on the electron acceptor profile is more pronounced than for the electron donor profile. For the larger maximum

Figure 5-1: Electron donor biofilm profile vs. maximum layer thickness.

Figure 5-2: Electron acceptor profile vs. maximum layer thickness.

layer thicknesses, the high electron acceptor concentrations obtained were attributed to the electron donor which is allowed to advance further into the biofilm as a result of layer discretization.

As with the electron acceptor, the secondary substrate profile was greatly affected by increases in maximum layer thickness (Figure 5.3). The secondary substrate concentration profile reflected electron acceptor conditions within the biofilm. Once the electron acceptor was depleted within a particular layer, the secondary substrate was no longer consumed by the biomass and the concentration profile became dominated by diffusive processes.

Most biomass activity and substrate profile definition due to substrate consumption and diffusion occurred in the upper layers of the biofilm. A layer splitting feature was incorporated into the program to prevent layers from getting too large which could affect the accuracy of substrate concentration profiles. A maximum layer thickness of 100 μm was selected to maximize the substrate profile definition while minimizing computing time. A smaller maximum layer thickness value would require a smaller time step which would, consequently, increase the computing time.

When the user-specified maximum thickness is reached, the layer is split in half in the manner described in Section 4.4.1. The layer splitting feature improves the accuracy of the concentration profile, especially in the outer layers of the biofilm. However, the sudden reduction in the first and second layer depths tends to create an oscillatory response. This is mainly due to the steepness of the concentration profiles in the upper layers. This oscillatory effect is discussed further in Section 5.3.

5.3 DYNAMIC BIOFILM MODEL RESPONSES

When sudden changes were introduced to the biofilm model, the response of the system was to reach new equilibrium conditions. Dynamic responses in the biofilm profile characteristics and the bulk phase concentrations during start-up and layer splitting are presented in this section.

Figure 5-3: Secondary substrate biofilm profile vs. maximum layer thickness.

5.3.1 START-UP BIOFILM PROFILE

The model was started with the input parameters presented in Table 4.1 and the secondary substrate biokinetic values found in Table 5.1 (see Table 6.2 for references). The initial conditions of the biofilm model were defined such that fully penetrated biofilm conditions existed prior to start-up. Under start-up conditions, the biofilm system was automatically in a dynamic state. As the system progressed toward steady state conditions, the biofilm profile and bulk phase substrate concentrations moved toward an equilibrium.

Figures 5.4 to 5.6 illustrate the profile developments for the electron donor, electron acceptor and secondary substrates as they advance toward steady state. The electron donor and secondary substrate profiles were most sensitive to the start-up phase of the model. This can be attributed to the aeration system provided in the reactor. This factor is also apparent in the reactor itself, as can be seen from Figure 5.7, where bulk phase electron acceptor concentrations were relatively unaffected by start-up conditions.

The sagging effect present in the electron donor and secondary substrate profiles was a product of dual substrate limitation (Figures 5.4 and 5.6). At these points in the biofilm, substrate utilization was dominated by the consumption component of the equation since the oxygen supply to these layers was not limiting. At greater biofilm depths, the substrate utilization was limited by low oxygen concentrations that resulted from utilization and diffusion limitation in the outer layers. The outer layers were dominated by diffusion processes due to the strong concentration gradients provided by the bulk phase rather than oxygen limitation. The sagging effect disappeared once the system had stabilized from the initial input settings and steady state conditions were achieved.

5.3.2 BULK PHASE AND BIOFILM LAYER FLUCTUATIONS

As indicated in Section 5.2.2.2, the layer splitting feature created an oscillatory response in biofilm and bulk phase substrate concentrations. The fluctuation was especially apparent

**Table 5-1: Secondary substrate
biokinetic parameters.**

Parameter	Value
q_{m2}	0.02 mg ₂ /mg _x -hr
K_{s2}	2.0 mg ₂ /L
K_{so}	0.06 mg ₂ /L
D_{2f}	2.2×10^{-6} m ² /hr
k_{m2}	0.11 m/hr
$S_{2bi} = S_{2bo}$	1 mg ₂ /L

Figure 5-4: Electron donor biofilm profile development.

Figure 5-5: Electron acceptor biofilm profile development

Figure 5-6: Secondary substrate biofilm profile development.

Figure 5-7: Bulk phase dynamic response.

when the layer at the biofilm/liquid interface layer was split. The pronounced response in this layer was due to the high biomass activity and substrate concentrations in the outer biofilm layers. Figure 5.8 illustrates the process of layer splitting and the subsequent changes in the concentration profile.

Prior to the splitting of layer 1 (Figure 5.8a), diffusion occurs between layer 1 and its adjacent layers; the bulk phase and layer 2. When the layer is split (Figure 5.8b), diffusion occurs between layer 1a and the bulk phase, as well as between layer 1b and layer 2, but not between layer 1a and 1b because they have the same concentration. As the diffusion process proceeds, the concentration within the split layers is able to reach a new equilibrium and provide a slightly more defined profile since the layers are thinner (Figure 5.8c).

The biofilm/liquid interface layer and bulk phase model response to the layer splitting is demonstrated in Figures 5.9 to 5.11 for electron donor, electron acceptor and secondary substrates, respectively. Although the layer splitting effect was also evident in deeper biofilm layers, it was most pronounced in the biofilm/liquid interface layer which was selected for illustrative purposes. As previously discussed, when the biofilm/liquid interface layer was split, the profile became more defined yielding higher concentrations near the biofilm surface as the profile reached equilibrium. Then, as the layer thickness increased, substrate concentrations within the layer began to decrease again. Bulk phase concentrations followed the same oscillatory response (Figures 5.9 to 5.11) since bulk phase concentrations are dependent upon the substrate fluxes into the biofilm which are calculated from the biofilm/liquid layer concentrations (S_1). This would suggest that the concentration fluctuations within the biofilm and bulk phase were caused solely by the layer splitting feature. In this thesis, the average steady state concentrations were utilized in the presentation of results, calculations and further analyses.

The total biofilm thickness also exhibited an oscillatory response, although the effect was much less pronounced than for the substrate concentrations (Figure 5.12). The fluctuation was

Figure 5-8: Effect of layer splitting on biofilm concentration profile.

Figure 5-9: Biofilm/liquid interface and bulk phase electron donor fluctuations due to layer splitting.

Figure 5-10: Biofilm/liquid interface and bulk phase electron acceptor fluctuations due to layer splitting.

Figure S-11: Biofilm/liquid interface and bulk phase secondary substrate fluctuations due to layer splitting.

Figure 5.12: Total biofilm thickness fluctuations due to layer splitting.

found to follow a similar pattern as that described for the bulk phase and biofilm profile substrate concentrations. The total biofilm thickness decreased as the outer biofilm layers increased in thickness and the substrate concentrations decreased within these layers. The total thickness was found to increase again after a layer split which defined the substrate concentration profile, especially in the upper layers of the biofilm.

The steady state biofilm thickness oscillations are considered to be solely influenced by the layer splitting feature. This is not to be confused with the fluctuations in thickness which may be exhibited as the biofilm attempts to reach an equilibrium thickness based upon steady state substrate concentration profiles. The average steady state thickness was employed in the presentation of results, calculations and any further model analyses.

5.4 MODEL COMPARISON TO PREVIOUS STUDIES

The performance of the numerical model under simple conditions was examined by comparing it to published model and experimental results. A comparison with results presented in other studies will assess and verify the performance of model simulations prior to its applications to more complex systems conditions.

5.4.1 SUSPENDED BIOMASS SYSTEM

The three cases examined included primary substrate removal under single and dual substrate limitation, as well as cometabolic substrate removal under dual limitation for the suspended biomass system.

5.4.1.1 PRIMARY SINGLE SUBSTRATE LIMITATION CASE

Shamat and Maier (1980) conducted batch and continuous flow experiments to establish whether activated sludge biomass could be used to develop microbial populations capable of

completely metabolizing chlorinated organic compounds. As a second objective, they studied the kinetic parameters of these populations to assess the feasibility of chlorinated organic waste removal by activated sludge processes. Chlorinated benzoic acids (*o*-CB, *m*-CB, *p*-CB, 2,5-DCB and 3,5-DCB), as well as 2,4-dichlorophenoxyacetic acid (2,4-D) were selected as representative chlorinated compounds on the basis of their prevalence and structural variety with respect to the position and number of chlorine atoms on the aromatic molecule.

Batch experiments were carried out in 2 L glass bottles placed on rotary shakers with oxygen supplied by surface mass transfer, and oxygen was not considered to be a limiting factor. Reactors containing 50 mg/L of target substrate as the only source of carbon and energy (electron donor) were inoculated with MLSS at a concentration of approximately 0.5 mg/L.

Growth parameters calculated from the batch reactor data are presented in Table 5.2. As can be seen from the table, the actual yield coefficient (Y) was not determined for 3,5-DCB, thus, no attempt was made to simulate primary substrate removal using this compound. The kinetic parameters were then used in the numerical model and the model simulations (Figure 5.14) were compared to the experimental results (Figure 5.13) obtained by Shamat and Maier (1980).

Shamat and Maier did not consider the lag time in the kinetic parameter calculations. As such, the lag time apparent in Figure 5.13 was not simulated by the numerical model and was simply added to the simulation output as a time delay (Figure 5.14).

Continuous flow experiments were carried out in chemostat reactors with a volume of 220 ml and a constant air flow of 200 ml/min. It was assumed that oxygen was not a limiting factor in the experiment. The influent feed concentrations ranged between 50 and 200 mg/l for each test. The system was then tested at various residence times ranging from 1 to 50 days. Influent substrate concentrations of 100 mg/L and the kinetic parameters of Table 5.2 were utilized for numerical modelling purposes. The observed and model predicted values are presented in Figures 5.15 and 5.16, respectively.

Table 5-2: Growth parameters from batch reactor data (Shamat and Maier, 1980).

Compound	b (day ⁻¹)	$\mu_{\max}$ (day ⁻¹)	K_{sd} (mg _s /L)	Y (mg _s /mg _d)
2,4-Dichlorophenoxyacetate	-	2.3	5.4	0.14
<i>m</i> -Chlorobenzoate	-	0.6	2.0	0.14
<i>p</i> -Chlorobenzoate	-	1.2	1.1	0.25
<i>o</i> -Chlorobenzoate	-	1.0	2.4	0.22
2,5-Dichlorobenzoate	-	0.6	1.5	0.16
3,5-Dichlorobenzoate	-	0.05	25.3	-

Figure 5-13: Chlorinated substrate remaining in batch reactors after inoculation with MLSS (Shamat and Maier, 1980).

Figure 5-14: Chlorinated substrate remaining in batch reactors after inoculation with MLSS (COMDEG).

Figure 5-15: Effluent chlorinated substrate concentrations for different residence time values (Shamat and Maier, 1980).

Figure S-16: Effluent chlorinated substrate concentrations for different residence time values (COMDEG).

Numerical model results were found to adequately simulate both the batch and continuous flow experiments. This would suggest that the model is able to reproduce electron donor removal under single substrate limitation conditions.

5.4.1.2 PRIMARY DUAL SUBSTRATE LIMITATION CASE

Sinclair and Ryder (1975) evaluated two interacting substrate models, Monod/Monod kinetics (equation 5.1) and Monod/Contois kinetics (equation 5.2), for predicting substrate removal and biomass growth under both oxygen limiting and carbon limiting conditions.

$$\mu = \mu_m \left(\frac{S_o}{K_{so} + S_o} \right) \left(\frac{S_d}{K_{sd} + S_d} \right) \quad (5.1)$$

$$\mu = \mu_m \left(\frac{S_o}{K_{so} + S_o} \right) \left(\frac{S_d}{BX + S_d} \right) \quad (5.2)$$

Contois kinetics assume that the specific growth rate (μ) is a function of the bacterial population (X) and a Contois constant (B), whereas, Monod kinetics assume that the specific growth rate is defined by the half maximum rate concentration (K_s). However, it should be noted that since the COMDEG numerical model was based on Monod kinetics, a comparison to the Contois kinetics results was not made.

The study was undertaken in 4 L aerobic chemostats using *Candida utilis* grown on a glycolysis suppressing glycerol medium. Experiments were performed for dilution rates ranging from 0.01 hr⁻¹ to 0.5 hr⁻¹ for glycerol concentrations of 0.2 kg/m³, 1 kg/m³, 2 kg/m³ and 5 kg/m³. The kinetic parameters utilized for modelling purposes are presented in Table 5.3. The initial biomass concentration was 5 mg/L.

The experimental and model results obtained by Sinclair and Ryder are shown in Figures 5.17 to 5.20. In general, as the dilution rate decreased the biomass was better able to consume the glycerol and biomass growth was maximized. It was also found that higher influent glycerol

Table 5-3: Experimental dual substrate limitation kinetic parameter values (Sinclair and Ryder, 1975).

Kinetic Parameter	Value	
q_{md}	0.982 mg _s /mg _x -hr	
q_{ma}	0.383 mg _s /mg _x -hr	
K_{sd}	4.6 mg _s /L	
K_{sa}	0.084 mg _s /L	
b	0.01 hr ⁻¹	
$v_{a/x}$	6.29 mg _s /mg _x	
$k_L a$	S_{0bi}	k_{La}
	0.2 kg/m ³	53.0 hr ⁻¹
	1.0 kg/m ³	46.5 hr ⁻¹
	2.0 kg/m ³	62.5 hr ⁻¹
	5.0 kg/m ³	63.0 hr ⁻¹

Figure 5-17: Experimental and interacting kinetic model results for 0.2 kg/m³ glycerol concentration (Sinclair and Ryder, 1975).

Figure 5-18: Experimental and interacting kinetic model results for 1.0 kg/m³ glycerol concentration (Sinclair and Ryder, 1975).

Figure 5-19: Experimental and interacting kinetic model results for 2.0 kg/m^3 glycerol concentration (Sinclair and Ryder, 1975).

Figure 5-20: Experimental and interacting kinetic model results for 5.0 kg/m glycerol concentration (Sinclair and Ryder, 1975).

concentrations yielded higher biomass concentrations which resulted in higher oxygen utilization rates (Figures 5.17 to 5.20). Dissolved oxygen did become limiting for glycerol concentrations of 2.0 kg/m^3 and 5.0 kg/m^3 . They concluded that both the Monod and the Contois models were satisfactory in representing suspended biomass system kinetics and that the simpler model (Monod) could be used with confidence.

The kinetic parameters presented in Table 5.3 were also utilized in the COMDEG model for identical system conditions as those described for Sinclair and Ryder. As can be seen from Figures 5.21 to 5.24, the COMDEG simulation produced outputs similar to those obtained by Sinclair and Ryder's (1975) Monod kinetic model. Thus, the COMDEG numerical model was considered to accurately reproduce primary substrate removal under dual substrate conditions in suspended growth systems.

5.4.1.3 COMETABOLIC DUAL SUBSTRATE LIMITATION CASE

Bae (1992) conducted an analysis on the effects of reducing power and dual limitation on the biodegradation of halogenated secondary substrates via reductive dehalogenation, monooxygenase or dioxygenase reactions. This was achieved by applying the multiplicative model he developed to a theoretical secondary substrate removal example.

The primary substrate kinetic parameters derived from dual substrate limitation experiments that employed *Pseudomonas putida* in a chemostat were utilized in the model (Table 5.4). In Table 5.4, k is a first order parameter representing q_{m2}/K_{s2} when $K_{s2} \gg S_{2h}$. The simulated system was assigned a residence time (V/Q) of 0.25 day and a constant suspended biomass concentration (X) of 2000 mg/L, which are typical of activated sludge.

For the simulations presented here, the model was run with a constant electron acceptor concentration and a varying electron donor concentration. The consequent secondary substrate removal response for an oxygenase reaction with half maximum rate constants (K_{so}) of 0.06 mg

Figure 5-21: Numerical model results for 0.2 kg/m³ glycerol concentration (COMDEG).

Figure 5-22: Numerical model results for 1.0 kg/m³ glycerol concentration (COMDEG).

Figure 5-23: Numerical model results for 2.0 kg/m³ glycerol concentration (COMDEG).

Figure 5-24: Numerical model results for 5.0 kg/m³ glycerol concentration (COMDEG).

**Table 5-4: Dual substrate limitation model
kinetic parameter values (Bae, 1992).**

Kinetic Parameter	Value
q_{md}	2.13 $\text{mg}_d/\text{mg}_x\text{-hr}$
q_{ma}	1.29 $\text{mg}_d/\text{mg}_x\text{-hr}$
K_{sd}	0.184 mg_d/L
K_{sa}	0.019 mg_d/L
k	0.005 $\text{L}/\text{mg}_x\text{-L}$
b	0.001 hr^{-1}
$v_{a/x}$	1.42 mg_d/mg_x
Y	0.275 mg_x/mg_d
M	1.827

DO/L and 0.019 mg DO/L were then calculated. The results of Bae are shown in Figures 5.25 and 5.26 for each of the respective cases.

In general, secondary substrate removal was found to improve with increasing electron acceptor and electron donor concentrations. The impact on secondary substrate utilization was the greatest when one of the primary substrates was present in limiting concentrations while the other was present in saturating concentrations. From Figures 5.25 and 5.26 it was also inferred that lower oxygenase half maximum rate concentrations (K_{so}) yield higher secondary substrate removal when one of the primary substrates is present in limiting concentrations.

The results presented by Bae (1992) were reproduced when the kinetic parameters were utilized in the COMDEG numerical model for each oxygenase half maximum rate concentrations (Figures 5.27 and 5.28). This was to be expected because the substrate utilization portion of COMDEG is based upon the model developed by Bae. The portion of the numerical model which could differ, namely biomass growth, was not applied in this case since the biomass concentration (X) was held constant at 2000 mg/L.

From the comparison of results (Figures 5.25 to 5.28), it was concluded that the COMDEG numerical model was able to predict secondary substrate removal adequately under dual substrate limitation conditions in a suspended biomass system.

5.4.2 BIOFILM SYSTEM

Substrate utilization modelling in biofilm systems is more complex than in suspended growth systems. This is mainly due to the presence of mass transfer limitations between the bulk liquid and the biofilm and within the biofilm itself. Electron donor removal under single substrate limitation was examined for both steady state and transient system conditions. Numerical modelling studies dealing with dual substrate limitation were not available for comparison.

Figure 5-25: Removal of secondary substrate undergoing an oxygenase reaction, $K_{so} = 0.06$ mg DO/L (Bae, 1992).

Figure 5-26: Removal of secondary substrate undergoing an oxygenase reaction, $K_{so} = 0.019$ mg DO/L (Bae, 1992).

Figure 5-27: Removal of secondary substrate undergoing an oxygenase reaction, $K_{so} = 0.06$ mg DO/L (COMDEG).

Figure 5-28: Removal of secondary substrate undergoing an oxygenase reaction, $K_{so} = 0.019$ mg DO/L (COMDEG).

5.4.2.1 STEADY STATE SINGLE SUBSTRATE LIMITATION CASES

Rittmann and McCarty (1980) have presented a model developed for steady state biofilm kinetics with a single substrate. The model coupled the substrate flux into a biofilm to the total biofilm thickness which would exist at steady state for specific bulk substrate concentrations. Based on kinetic and energetic constraints, equations were provided for calculating steady state substrate flux and biofilm thickness. The equations were applied for bulk substrate concentrations greater than a minimum concentration, below which a steady state biofilm can, theoretically, not exist. However, this pseudoanalytical solution was later found to be inaccurate for certain ranges of substrate concentrations and kinetic parameters.

A new pseudoanalytical solution considered to be highly accurate for the entire range of substrate concentrations and kinetic parameters was developed by Sáez and Rittmann (1988). This method utilized simple algebraic expressions to calculate the flux of substrate into a steady state biofilm. The equations are based upon an analysis of the results from a numerical solution of differential equations.

The COMDEG numerical model was compared with both sets of pseudoanalytical solutions using the kinetic parameters listed in Table 5.5. Steady state conditions were predicted by running the model until steady state conditions were reached. It was found that COMDEG simulation results corresponded well with the two pseudoanalytical solutions (Figures 5.29 and 5.30). Total biofilm thickness predictions were generally closer to the model presented by Rittmann and McCarty (1980) (Figure 5.29), whereas, values for substrate flux into the biofilm were closer to those obtained by Sáez and Rittmann (1988) (Figure 5.30).

This comparison demonstrated that the numerical model (COMDEG) was in agreement with the steady state models developed by Rittmann and McCarty (1980) and Sáez and Rittmann

Table 5-5: Biofilm kinetic parameter values (Rittmann and McCarty, 1980; Sáez and Rittmann, 1988).

Kinetic Parameter	Value
L	0.01 cm
D_d	0.8 cm ² /day
D_{df}	0.64 cm ² /day
q_{md}	8 mg _d /mg _x -day
K_{sd}	0.01 mg _d /cm ³
b	0.1 day ⁻¹
Y	0.5 mg _x /mg _s
X_f	40 mg _x /cm ³

Figure 5-29: Steady state biofilm thickness predictions using the Rittmann and McCarty (1980), Saez and Rittmann (1988) and COMDEG models.

Figure 5-30: Steady state substrate flux into a biofilm predictions using the Rittmann and McCarty (1980), Saez and Rittmann (1988) and COMDEG models.

(1988) for bulk substrate utilization and biofilm growth predictions under single substrate limiting conditions in an attached growth system.

5.4.2.2 TRANSIENT SINGLE SUBSTRATE LIMITATION CASE

The biofilm growth component of the numerical model was verified by comparing simulations to those obtained by Skowlund (1990). In Skowlund's model the set of differential and integral equations developed by Wanner and Gujer (1984, 1986) were employed to quantify the development of substrate and active fraction profiles within the biofilm, as well as total and active biofilm growth.

Numerical simulations were performed for a transient single species/single substrate biofilm undergoing unlimited growth (i.e. negligible sloughing) using the kinetic parameters listed in Table 5.6. The COMDEG model was started with 1 layer and employed maximum (z_m) and minimum (z_l) layer thicknesses of 100 and 30 μm , respectively. It should be noted that the Skowlund model did not account for the presence of an effective diffusion-resistance layer. For the purpose of this comparison, modifications were made to the COMDEG model to eliminate the effective diffusion-resistance layer effect.

The results for total and active biofilm growth using various inert material retention coefficients are illustrated in Figures 5.31 and 5.32 for the Skowlund and COMDEG models, respectively. Although the general trend of the curves of the two models are similar, actual steady state biofilm thicknesses do differ. This difference is attributed to differences in the development of biofilm growth equations and the definition of the inert material retention coefficient.

Skowlund developed a model based upon conservation principles and the assumption that the biomass may be treated as a continuum. The flux of active biomass was expressed as the velocity at which the active biomass is displaced with respect to the biofilm/support interface and the concentration of the microorganisms. When the biofilm grows or shrinks, the total film

Table 5-6: Transient model kinetic parameter values (Skowlund, 1990).

Kinetic Parameter	Value
q_{md}	0.837 mg _d /mg _x -hr
K_{sd}	3.9 mg _d /L
b	0.0853 hr ⁻¹
Y	0.071 mg _x /mg _d
X_f	2.5 mg _x /cm _f ³
D_{df}	0.036 cm _f ² /hr
L_{fo}	5 μm
$S_{db} = S_{ds}$	0.917 mg _d /L

Figure 5-31: Total and active biofilm growth using different activation coefficients in an unlimited biofilm (Skowlund, 1990).

Figure S-32: Total and active biofilm growth using different inert material retention coefficients in an unlimited biofilm (COMDEG).

thickness changes and the biofilm/liquid interface moves at a certain velocity with respect to the support. Skowlund defined the inert material retention parameter as a biomass inactivity rate constant very similar to the endogenous decay coefficient and used it to calculate the active fraction within the biofilm as per:

$$\frac{df}{dt} = \left\{ \left[\mu_m \left(\frac{S_{df}}{K_{sd} + S_{df}} \right) - b \right] (1 - f) - K_i \right\} f - U_z \frac{df}{dz} \quad (5.3)$$

where U_z is defined as the velocity of the biofilm at the position z within the biofilm and is dependent upon the net substrate utilization by the active biomass at that point. From equation (5.3), it can be established that the inactivation coefficient represents the rate at which active biomass becomes inactive.

The biofilm growth equations derived in this study were also based upon conservation principles, but utilized a mass balance approach to describe the changes in biofilm thickness. In this approach, the biofilm is discretized into several layers and the growth of each layer is dependent upon the substrate concentrations and active fraction value within the layer. The active biomass growth for a layer is described by equation (5.4)

$$\frac{dz_a}{dt} = z_a \left(q_{md} Y \left(\frac{S_{df}}{K_{sd} + S_{df}} \right) \left(\frac{S_{af}}{K_{sa} + S_{af}} \right) - b - sl \right) \quad (5.4)$$

The inert material coefficient (k_i) was defined as the inorganic fraction of the dead cell mass remaining and, thus, occupying space, within the biofilm matrix in the COMDEG model. The inert material retention coefficient is dependent upon the active biomass thickness and the endogenous decay rate. Only a fraction of the dead cell mass remains within the biofilm matrix. It was assumed that the remaining portion of the dead cell mass will be transformed into its soluble components and leave the biofilm matrix or be consumed by the active biomass. It should be noted, however, that the consumption of cell matter was not considered to contribute significantly

to the growth of the biofilm. This parameter was employed in the calculation of the inactive biofilm fraction growth within a particular layer.

$$\frac{dz_i}{dt} = z_a k_1 b - z_i s l \quad (5.5)$$

The active and inactive biomass growth equations are both necessary in determining the total growth of each layer as per equation (5.6). The active fraction value of the layer is subsequently determined from the ratio of active biomass volume to the total biomass volume within the layer.

$$\frac{dz}{dt} = \frac{dz_a}{dt} + \frac{dz_i}{dt} = fz(Yq_{m,d})(\frac{S_{af}}{K_{sd} + S_{af}})(\frac{S_{af}}{K_{sa} + S_{af}}) - (1 - k_1)b - sl - (1 - f)zsl \quad (5.6)$$

It is interesting to note that fluctuations in biofilm thickness due to layer splitting were more pronounced at lower biofilm thicknesses (Figure 5.32). This was attributed to the smaller number of layers and the fact that the fluctuations could not be dampened as in thicker biofilms where there were a greater number of layers. It was concluded that this effect was not caused by the value of the inert material retention coefficient since this coefficient only contributes to the growth of the inactive, and hence, total biofilm volume and not to the active portion.

The profiles of biofilm activity and substrate concentration with biofilm depth for an inert material retention coefficient of 0.5 are shown in Figures 3.33 and 3.34 for the Skowlund model and Figures 3.35 and 3.36 for the COMDEG model. Once again, the results follow similar trends with slight differences in values caused by the development of biofilm growth equations and the inert material retention parameter definition.

Figure 5-33: Active fraction profile vs. biofilm depth at five different times (Skowlund, 1990).

Figure 5-34: Substrate concentration profile vs. biofilm depth at five different times (Skowlund, 1990).

Figure S-35: Active fraction profile vs. biofilm depth at five different time (COMDEG).

Figure 5-36: Substrate concentration profile vs. biofilm depth at five different times (COMDEG).

CHAPTER 6

SENSITIVITY ANALYSIS

6.1 BASE CASES

To perform the sensitivity analysis, base cases were defined for the suspended biomass and biofilm systems. No complete data set was available from the literature which could provide the information necessary for the two systems. As such, theoretical data sets were compiled from various sources for each of the systems. Where possible, kinetic and operational parameters were applied to both systems such that a comparison could be made between the suspended and attached growth systems.

6.1.1 SUSPENDED BIOMASS SYSTEM

The kinetic and operational parameters utilized in the sensitivity analysis of the suspended biomass system are presented in Table 6.1. Initial substrate concentrations were set equal to the corresponding influent substrate concentrations. Primary substrate concentrations were selected such that non-limiting conditions would prevail within the system, whereas, only a trace amount of secondary substrate was introduced. The substrate input was assumed to remain constant throughout the duration of the analysis. Influent biomass concentrations were assumed to be negligible.

The model was executed using a time step of 0.0001 hour under the base case conditions. Electron donor and electron acceptor saturation concentrations were set at 20 times their respective half maximum saturation concentrations. The resulting bulk phase substrate removal and biomass growth are presented in Figures 6.1 and 6.2, respectively.

As expected, the bulk phase electron donor and secondary substrate concentrations decreased rapidly once the model was initiated (Figure 6.1) which corresponded to the high initial

Table 6-1: Suspended biomass system base case kinetic and operational parameters.

Parameter	Reference	Value
q_{md}	Sinclair & Ryder, 1975	0.892 mg _d /mg _x -hr
q_{ma}	Sinclair & Ryder, 1975	0.383 mg _a /mg _x -hr
q_{m2}	Assumed	0.02 mg ₂ /mg _x -hr
K_{sd}	Sinclair & Ryder, 1975	4.6 mg _d /L
K_{sa}	Sinclair & Ryder, 1975	0.084 mg _a /L
K_{s2}	Assumed	2.0 mg ₂ /L
K_{so}	Bae, 1992	0.06 mg _s /L
$v_{a/x}$	Metcalf & Eddy, 1991	1.42 mg _a /mg _x
b	Sinclair & Ryder, 1975	0.01 hr ⁻¹
M	Bae, 1992	1.827
Y	Rittmann & McCarty, 1980	0.5 mg _x /mg _d
f	Assumed	0.8
$S_{dbt} = S_{dbo}$	Assumed	200 mg _d /L
$S_{abt} = S_{abo}$	Assumed	9.2 mg _a /L
$S_{2bt} = S_{2bo}$	Assumed	1 mg ₂ /L
X_i	Assumed	0 mg _x /L
X_o	Assumed	500 mg _x /L
C_s	Metcalf and Eddy, 1991	9.6 mg _a /L
$k_L a$	Sinclair & Ryder, 1975	60 hr ⁻¹
V/Q	Assumed	4 hr

Figure 6-1: Primary and secondary bulk phase concentrations under base case conditions.

Figure 6-2: Suspended biomass growth under base case conditions.

biomass concentration. This was followed by a gradual increase and leveling off in concentration as the biomass approached its steady state concentration (Figure 6.2). The electron acceptor concentration was relatively unaffected by the biomass concentration due to the high aeration mass transfer coefficient ($k_L a$) provided in the model.

6.1.2 BIOFILM SYSTEM

The base case kinetic and operational parameters utilized for the biofilm system were essentially identical to those describing the suspended biomass system. However, additional diffusion parameters were required to model the substrate flux into the biofilm. Influent substrate concentrations and initial conditions were also identical to the suspended growth system, with the exception of the initial active fraction and biofilm layer thickness values which were assumed. The base case parameters are presented in Table 6.2.

The model was executed for an initial 5 μm layer system at a time step of 0.0001 hour. The maximum and minimum layer thicknesses were set at 100 μm and 30 μm , respectively. Electron donor and electron acceptor saturation concentrations were set at 20 times their respective half maximum saturation concentrations. From Figure 6.3 it can be seen that bulk phase concentrations were found to reach steady state concentrations relatively quickly. As in the suspended biomass system, the electron acceptor concentration was relatively unaffected by start-up conditions.

Total and active biomass growth responses are presented in Figure 6.4. The initial growth was rapid and then leveled off as steady state conditions were achieved. The response itself is expressed as a biofilm volume per unit area or, since the biofilm area is assumed to remain constant, biofilm thickness.

The oscillatory effect caused by the layer splitting option is apparent in the bulk phase electron donor and secondary substrates, and to a lesser extent, in the biofilm growth curves.

Table 6-2: Biofilm system base case kinetic and operational parameters.

Parameter	Reference	Value
q_{md}	Sinclair & Ryder, 1975	0.892 $\text{mg}_d/\text{mg}_x\text{-hr}$
q_{ma}	Sinclair & Ryder, 1975	0.383 $\text{mg}_d/\text{mg}_x\text{-hr}$
q_{m2}	Assumed	0.02 $\text{mg}_2/\text{mg}_x\text{-hr}$
K_{sd}	Sinclair & Ryder, 1975	4.6 mg_d/L
K_{sa}	Sinclair & Ryder, 1975	0.084 mg_d/L
K_{s2}	Assumed	2.0 mg_2/L
K_{s0}	Bae, 1992	0.06 mg_d/L
$v_{a/x}$	Metcalf & Eddy, 1991	1.42 mg_d/mg_x
b	Sinclair & Ryder, 1975	0.01 hr^{-1}
sl	Assumed	0.01 hr^{-1}
k_1	Assumed	0.25
M	Bae, 1992	1.827
Y	Rittmann & McCarty, 1980	0.5 mg_x/mg_d
X_f	Sáez <i>et al.</i> , 1984	$5 \times 10^6 \text{ mg}_x/\text{m}^3$
D_{df}	Namkung <i>et al.</i> , 1983	$3.5 \times 10^{-6} \text{ m}^2/\text{hr}$
D_{af}	Kissel <i>et al.</i> , 1984	$6.2 \times 10^{-6} \text{ m}^2/\text{hr}$
D_{2f}	Assumed	$2.2 \times 10^{-6} \text{ m}^2/\text{hr}$
k_{md}	Namkung <i>et al.</i> , 1983	0.1784 m/hr
k_{ma}	Kissel <i>et al.</i> , 1984	0.31 m/hr
k_{m2}	Assumed	0.11 m/hr
L	Assumed	0.000025 m
a	Assumed	100 m^2/m^3
$S_{dbi} = S_{dbo}$	Assumed	200 mg_d/L
$S_{abi} = S_{abo}$	Assumed	9.2 mg_d/L
$S_{2bi} = S_{2bo}$	Assumed	1 mg_2/L
f_0	Assumed	0.5
L_{f0}	Skowlund, 1990	5 μm_f
C_s	Metcalf and Eddy, 1991	9.6 mg_d/L
$k_L a$	Sinclair & Ryder, 1975	60 hr^{-1}
V/Q	Assumed	4 hr

Figure 6-3: Biofilm bulk phase substrate concentrations under base case conditions.

Figure 6-4: Total and active biofilm growth under base case conditions.

However, the fluctuations were small enough that relatively accurate estimates of the steady state concentration could be made.

Steady state substrate concentrations and active fraction profiles at time $t = 756$ hours are shown in Figures 6.5 and 6.6, respectively. The electron acceptor was found to penetrate the biofilm to a depth of approximately 1 mm. The electron donor was depleted within the first 0.5 mm of biofilm. The secondary substrate, on the other hand, was never fully consumed. Oxygenolytic secondary substrate degradation by the biomass was found to cease when depletion of the electron acceptor occurred, at which time diffusive processes became dominant (Figure 6.5).

As was expected, the biomass active fraction was highest in the outer layers of the biofilm where primary substrate concentrations allowed for the growth of the active layer. Within the inner layers of the biofilm, however, primary substrate concentrations became depleted and the contribution of inactive cell mass to the total biofilm volume was found to increase with increasing biofilm depth (Figure 6.6).

6.2 SENSITIVITY TO INITIAL CONDITION VALUES

The first task in performing the sensitivity analysis was to evaluate the system response to initial conditions. Steady state conditions should, in theory, be independent of the history of the system which implies that initial conditions should not have any impact on the equilibrium state.

6.2.1 SUSPENDED BIOMASS SYSTEM

To test the response of the suspended biomass system, the initial biomass concentration was varied between 250 mg/L and 2000 mg/L. As can be seen from Figures 6.7 to 6.10 changes in the initial biomass concentration did not have an effect on the steady state substrate and biomass concentrations. A sagging effect could be observed at the start of the simulation for each of the substrates which was attributed to the large initial biomass concentration compared to the

Figure 6-5: Biofilm substrate concentration profiles under base case conditions.

Figure 6-6: Biofilm steady state active fraction profile under base case conditions.

Figure 6-7: Suspended biomass system: electron donor concentration response to initial biomass concentrations.

Figure 6-8: Suspended biomass system: electron acceptor concentration response to initial biomass concentrations.

Figure 6-9: Suspended biomass system: secondary substrate concentration response to initial biomass concentrations.

Figure 6-10: Suspended biomass system: biomass growth response to initial biomass concentrations.

steady state biomass concentration. This was further supported by the fact that the substrate sag increased with increasing initial biomass concentrations.

The differential equations involved in the numerical modelling of suspended biomass systems are interdependent and must be solved simultaneously, as demonstrated in Section 3. In a CSTR, the nature of the system of equations is such that the steady state conditions are generally independent of initial substrate and biomass conditions (Levenspiel, 1972; Peavy *et al.*, 1985). As the initial biomass concentration was not found to impact steady state conditions in the suspended system, it was concluded that the initial substrate concentrations of the electron donor, electron acceptor and secondary substrate would also have no bearing on the equilibrium state of the system.

6.2.2 BIOFILM SYSTEM

The response of the biofilm system to changes in initial conditions was examined by varying the initial biofilm thickness between 1 mm and 5 mm and the initial active fraction throughout the film between 0.25 and 0.75. The results for each of the bulk phase substrate concentrations and the total biofilm growth are presented in Figures 6.11 to 6.14. Although some oscillation due to layer splitting can be observed, it is clear that steady state substrate concentrations and total biofilm thickness converged to the same point in every case.

Steady state ($t = 336$ hours) profiles for each of the substrates and the active fraction profile of the biofilm are shown in Figures 6.15 to 6.18. The profiles are similar for each of the initial conditions which also implies that the system converged. The discrepancies in upper layer concentrations of primary and secondary substrates (Figures 6.15 to 6.17) were attributed to layer thickness differences, for each of the cases presented, at the time steady state was achieved ($t = 336$ hours). Thus, it was concluded that initial conditions do not impact the equilibrium state of the biofilm system.

Figure 6-11: Biofilm system: bulk phase electron donor concentration response to initial biofilm thickness and active fraction conditions.

Figure 6-12: Biofilm system: bulk phase electron acceptor concentration response to initial biofilm thickness and active fraction conditions.

Figure 6-13: Biofilm system: bulk phase secondary substrate concentration response to initial biofilm thickness and active fraction conditions.

Figure 6-14: Biofilm system: biofilm thickness response to initial biofilm thickness and active fraction conditions.

Figure 6-15: Biofilm System: electron donor profile at steady state for various initial biofilm thickness and active fraction conditions.

Figure 6-16: Biofilm system: electron acceptor profile at steady state for various initial biofilm thickness and active fraction conditions.

Figure 6-17: Biofilm system: secondary substrate profile at steady state for various initial biofilm thickness and active fraction conditions.

Figure 6-18: Biofilm system: active fraction profile at steady state for various initial biofilm thickness and active fraction conditions.

6.3 NORMALIZED SENSITIVITY ANALYSIS

A sensitivity analysis was performed to determine the response of the biofilm and suspended growth system to uncertainty in the system parameters. Since a number of system parameters were assumed, there was a need to assess the significance of variations in input values on the performance of the numerical model. To allow a direct comparison, of the changes within each system, a normalized sensitivity analysis was utilized.

The normalized sensitivity indicates the change in a state variable, such as concentration, for a change in a particular system parameter and was calculated in the following fashion (Sykes *et al.*, 1985; Sykes and Thomson, 1988).

$$C_m = \frac{d(\ln \phi)}{d(\ln \alpha_i)} = \frac{d\phi}{d\alpha_i} \frac{\alpha_i}{\phi} \quad (6.1)$$

where C_m is the normalized sensitivity coefficient for a specified system parameter, ϕ is the state variable and α_i is the system parameter. A negative coefficient indicates that an increase in the system parameter will result in a decrease in the state variable.

The normalized sensitivity coefficient is dimensionless and can, thus, be utilized to compare the sensitivity to various system parameters. The analysis will indicate the parameters which have the greatest (or least) impact on the state variable. In this case, the state variables included the substrate concentrations and suspended biomass growth in the suspended biomass system, and bulk phase substrate concentrations in the biofilm system.

Kinetic parameters were increased by 1% and the model was executed until steady state conditions were reached in each of the systems. The corresponding bulk phase concentration responses were then recorded. The results of the sensitivity analysis are discussed in the following sections.

6.4 RESULTS OF SENSITIVITY ANALYSIS

Since an infinite number of electron donor and electron acceptor combinations are possible, the numerical model sensitivity analysis was performed for one set of parameters under aerobic (oxygenase reaction) conditions. However, the model could be adapted to different electron acceptors under reductive dehalogenation conditions at which point the reductive dehalogenation reaction would be employed in the determination of secondary substrate utilization. The electron acceptor would need to be expressed in terms of dissolved oxygen equivalents (Bae, 1992).

6.4.1 SUSPENDED BIOMASS SYSTEM

The state variables considered for the sensitivity analysis of the suspended biomass system included; electron donor, electron acceptor, secondary substrate and biomass concentrations. The results of the sensitivity analysis are presented in Table 6.3.

Electron donor concentrations were most affected by changes in the apparent maximum electron donor utilization rate (-2.4123%), the yield coefficient (-2.4115%) and the half maximum electron donor concentration (1.0000%). Factors such as the active fraction (<0.0001%), apparent maximum electron acceptor utilization rate (0.0008%) and half maximum electron acceptor concentration (0.0220%) had a relatively low impact on the bulk phase electron donor concentrations.

Electron acceptor concentrations were less affected by changes in system parameters due to the non-limiting conditions existing in the reactor system. The electron acceptor (-0.0375%) and electron donor (0.0339%) apparent maximum utilization rates, as well as the yield coefficient (-0.0056), were found to have the greatest effect on bulk phase electron acceptor concentrations, whereas, active fraction (<0.0001%), electron acceptor (<0.0001%) and electron donor (0.0014%) half maximum concentrations had the least effect.

Table 6-3: Normalized sensitivity coefficients: suspended biomass system.

Parameter	Electron Donor	Electron Acceptor	Secondary Substrate	Biomass
f	<0.0001	<0.0001	<0.0001	-0.9992
Y	-2.4115	-0.0056	-0.0469	1.0835
M	--	--	-3.7931	--
b	0.0939	-0.0022	0.0017	-0.0432
q _{ms1}	-2.4123	0.0339	-0.0252	0.0864
q _{ma}	0.0008	-0.0375	-0.0007	<0.0001
K _{s1}	1.0000	0.0014	0.0108	0.0346
K _{sa}	0.0220	<0.0001	-0.0206	<0.0001
K _{s2}	--	--	0.002	--
q _{m2}	--	--	-0.3189	--
K _{s2}	--	--	0.2341	--

It was determined that the M coefficient (-3.7931%) will have the greatest impact on the assessment of secondary substrate removal followed by the apparent maximum secondary substrate utilization rate (-0.3189%) and the half maximum secondary substrate concentration (0.2341%). Once again the active fraction (<0.0001%) and apparent maximum electron acceptor utilization rate (0.0007%) were found to have little effect on the bulk phase secondary substrate concentration.

The biomass concentration was most affected by fluctuations in yield coefficient (1.0835%), active fraction (-0.9992%) and apparent maximum electron donor utilization rate (0.0864%). Variations in apparent maximum electron acceptor utilization rate (<0.0001%), half maximum electron acceptor and electron donor (0.0346%) concentrations were found to least affect the biomass generation.

In general, variations in apparent maximum substrate utilization rates and half maximum substrate concentrations were found to have the greatest impact on bulk phase substrate consumption. This would have been more obvious in the case of electron acceptor parameters, if the system had approached electron acceptor limiting as opposed to electron acceptor saturation conditions. The yield coefficient value also greatly affected electron donor, electron acceptor and biomass concentrations. The importance of a relatively accurate M coefficient was demonstrated for the removal of the secondary substrate.

6.4.2 BIOFILM SYSTEM

The state variables considered for the sensitivity analysis of the biofilm system included; bulk phase electron donor, electron acceptor and secondary substrate concentrations. Results obtained for the 1% variation in system parameters are presented in Table 6.4.

Electron donor concentrations were most affected by changes in the apparent maximum electron donor utilization rate (-0.6435%), the biomass density (-0.6248%) and the half

Table 6-4: Normalized sensitivity coefficient: biofilm system.

Parameter	Electron Donor	Electron Acceptor	Secondary Substrate
L	-0.0792	-0.0047	-0.0043
X_f	-0.6248	0.0006	-0.1798
s_l	-0.1960	-0.0015	-0.0082
k_1	0.0023	-0.0003	0.0126
Y	-0.1326	-0.0116	0.0159
M	--	--	-5.7837
b	0.0403	-0.0019	0.0395
q_{m1}	-0.6435	0.0590	0.0735
q_{m2}	-0.0206	-0.0353	-0.0325
K_{sd}	0.2150	0.0327	0.0136
K_{sa}	0.0165	0.0011	-0.1376
K_{s0}	--	--	0.0403
q_{m2}	--	--	-0.3500
K_{s2}	--	--	0.3111

maximum electron donor concentration (0.2150%). Factors such as the inactivation coefficient (0.0023%), half maximum electron acceptor concentration (0.0165%) apparent maximum electron acceptor utilization rate (-0.0206%) had a relatively low impact on the bulk phase electron donor concentrations.

Electron acceptor concentrations were, again, less affected by changes in system parameters due to the non-limiting conditions existing in the reactor system. However, parameters such as electron donor (0.0590%) and electron acceptor (-0.0353%) apparent maximum utilization rates, as well as the half maximum electron donor concentration (0.0327%), were found to have the greatest effect on bulk phase electron acceptor concentrations, whereas, the inactivation coefficient (-0.0003%), biofilm density (0.0006%) and electron acceptor (0.0011%) half maximum concentration had the least effect.

It was, once again, observed that the M coefficient (-5.7837%) will have the greatest impact on the assessment of secondary substrate removal followed by the apparent maximum secondary substrate utilization rate (-0.3500%) and the half maximum secondary substrate concentration (0.3111%). The effective diffusion-resistance layer thickness (-0.0043%), sloughing coefficient (-0.0082%) and inactivation coefficient (0.0126%) were found to have little effect on the bulk phase secondary substrate concentration.

In general, variations in apparent maximum substrate utilization rates and half maximum substrate concentrations were found to have the greatest impact on the bulk phase substrate concentrations. Once again, a system approaching electron acceptor limiting, as opposed to electron acceptor saturated, conditions would have exemplified this fact in a clearer manner. The importance of a relatively accurate M coefficient was demonstrated for the removal of the secondary substrate. The relatively low impact of fluctuations in the effective diffusion-resistance thickness would suggest that substrate removal within this system was driven by substrate

consumption rather than substrate diffusion within the biofilm. Variations in the decay coefficients were also found to have a small impact on bulk phase substrate concentrations.

6.4.3 SUSPENDED BIOMASS SYSTEM VS. BIOFILM SYSTEM

When comparing the suspended growth system to the biofilm system, it was found that, for the system conditions defined by the base cases, the results were relatively similar. The two systems demonstrated overall responses of the same order of magnitude for the electron acceptor and secondary substrates. However, electron donor responses appeared to be slightly dampened in the biofilm system. Electron acceptor bulk phase concentrations were relatively insensitive to the 1% system parameter increases due to the electron acceptor saturated conditions existing in the CSTR.

Apparent maximum substrate utilization rates and half maximum saturation concentrations were demonstrated to greatly affect all bulk phase substrate concentrations in both systems. On the other hand, the M coefficient was found to have the greatest impact on secondary substrate removal. As such, a relatively high degree of accuracy would be warranted in the experimental evaluation of these parameters.

Variations in the yield coefficient affected bulk phase substrate removal more in the suspended biomass system than in the biofilm system. When the primary substrates are not limiting, substrate removal within a suspended biomass system will be governed by the growth of the biomass. Whereas, other factors such as diffusion, biomass density and active fraction profile will ultimately play a role in substrate consumption within the biofilm, thereby, lessening the effect of the yield coefficient.

CHAPTER 7

CASE STUDIES

As discussed in Section 6, an infinite number of electron donor and electron acceptor combinations are possible. However, the numerical model case studies were performed for one set of parameters (base case) under aerobic (oxygenase reaction) conditions. It should be noted that the model could be adapted to different electron acceptors (other than oxygen) under reductive dehalogenation conditions ($K_{so} = 0$) at which point the reductive dehalogenation reaction would be employed in the determination of secondary substrate utilization (Bae, 1992).

7.1 CASE 1: SYSTEM AERATION VARIATIONS

The suspended biomass and biofilm base cases devised for illustrative purposes represented electron acceptor saturated conditions due to the presence of a highly efficient ($k_L a = 60 \text{ hr}^{-1}$) aeration system in the reactor. To further understand the response of both systems under more limiting conditions, the oxygen mass transfer coefficient ($k_L a$) was varied between 60 hr^{-1} (base case) and 0 hr^{-1} . A decrease in the oxygen mass transfer coefficient could represent single or multiple aeration unit failure within the CSTR which would ultimately affect substrate removal and biomass growth in both the suspended biomass and biofilm systems.

7.1.1 CASE 1 SUSPENDED BIOMASS SYSTEM RESULTS AND DISCUSSION

Nine different oxygen transfer coefficients were tested in the suspended biomass system. The base case described in Section 6.1.1 was also utilized as the suspended biomass base case in this section. A time step of 0.0001 hours was employed to execute the program. The simulation was run for a period of 168 hours under base case conditions, then the new $k_L a$ was introduced and the simulation was allowed to continue for another 168 hours at which time steady state

3

PM-1 3 1/2" x 4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

conditions had been reached. The resulting steady state bulk phase electron donor, electron acceptor, secondary substrate and biomass concentrations vs. $k_L a$ are presented in Figures 7.1 to 7.4, respectively.

The effluent electron donor concentration (Figure 7.1) decreased quickly with increasing aeration. On the other hand, the biomass concentration (Figure 7.4) increased rapidly with increasing $k_L a$. This would suggest that as the electron acceptor or dissolved oxygen is present in larger concentrations ($k_L a > 3 \text{ hr}^{-1}$), electron donor consumption is facilitated which encourages biomass growth. At oxygen transfer coefficient values below 3 hr^{-1} , biomass growth and electron donor utilization were comparatively lower due to bulk phase oxygen limitation ($\text{DO} < 2 \text{ mg/L}$) conditions (Figure 7.2).

The electron acceptor response of the system was relatively slower than for electron donor and biomass concentrations (Figure 7.2). Dissolved oxygen became present in saturating concentrations only for $k_L a$ values greater than 30 hr^{-1} . Below this value, the rate of electron acceptor utilization approaches the rate of mass transfer.

Higher secondary substrate removal rates were predicted for oxygen transfer coefficients larger than 0 hr^{-1} and saturating bulk phase electron donor concentrations. Once the bulk phase electron donor concentration reached subsaturating concentrations, secondary substrate removal was found to decrease with increasing oxygen mass transfer coefficient (Figure 7.3).

These results are consistent with those presented by Bae (1992) and can be explained in terms of reducing power (Table 3.1). Figure 7.5 illustrates the impact of bulk phase primary substrate concentrations on secondary substrate removal in the suspended biomass system. When electron donor concentrations are saturating ($S_{bd} > 20 K_d$), the reaction rate (q_d) and $\log(\text{NADH/NAD})$ are high which results in a high secondary substrate removal rate (Stage I). At more limiting electron donor concentrations ($S_{bd} < 20 K_d$), increases in the bulk phase electron acceptor concentrations cause a decrease in the reaction rate and $\log(\text{NADH/NAD})$ ratio which,

Figure 7-1: Suspended biomass system: steady state electron donor concentration with variations in $k_L a$.

Figure 7-2: Suspended biomass system: steady state electron acceptor concentrations with variations in $k_L a$.

Figure 7-3: Suspended biomass system: steady state secondary substrate concentration with variations in $k_L a$.

Figure 7-4: Suspended biomass system: steady state biomass concentration with variations in $k_L a$.

Figure 7-5: Suspended biomass system: secondary substrate removal as a function of primary substrate concentrations.

consequently, decrease secondary substrate removal (Stage II). The lowest point on the curve ($k_L a = 1 \text{ hr}^{-1}$) represents the best combination of electron donor and electron acceptor concentrations for optimum secondary substrate removal under these system conditions. This optimum point, can be identified for oxygenolytic cometabolic biodegradation systems.

Wastewater treatment operational conditions with respect to dissolved oxygen were represented by an oxygen transfer coefficient of 3 hr^{-1} , which yielded a bulk phase oxygen concentration between 2 mg/L and 5 mg/L. Steady state conditions for this suspended biomass system can be expected to remain fairly constant for all bulk phase variables at $k_L a$ values greater than 30 hr^{-1} . At this point, the system is considered to be saturated with electron acceptor.

7.1.2 CASE 1 BIOFILM SYSTEM RESULTS AND DISCUSSION

For the biofilm system, six different oxygen transfer coefficients were examined. The base case was the same as that used in the sensitivity analysis of the biofilm. The program was executed with a time step of 0.0001 hours, an initial biofilm thickness of 2.94 mm, and maximum and minimum layer thicknesses of 100 μm and 30 μm , respectively. The simulation was run under base case conditions for a period of 336 hours, at which time a new $k_L a$ was introduced and the simulation was allowed to continue under the new system conditions for another 336 hours, when an equilibrium state had been reached. The bulk phase steady state electron donor, electron acceptor and secondary substrate concentrations are shown in Figures 7.6 to 7.8, and the total biofilm thickness for each case can be viewed in Figure 7.9.

As in the suspended biomass system, the bulk phase electron donor concentration (Figure 7.6) and biofilm thickness (Figure 7.9) of the biofilm system were found to, respectively, decrease and increase rapidly with increasing $k_L a$ value. This can once again be attributed to the facilitation of electron donor consumption, and consequent biomass growth, at higher dissolved oxygen concentrations ($k_L a > 5 \text{ hr}^{-1}$). The bulk phase electron acceptor response was more gradual

Figure 7-6: Biofilm system: steady state bulk phase electron donor concentration with variations in $k_L a$.

Figure 7-7: Biofilm system: steady state bulk phase electron acceptor concentration with variations in $k_L a$.

Figure 7-8: Biofilm system:: steady state bulk phase secondary substrate concentration with variations in $k_L a$.

Figure 7-9: Biofilm system: steady state biofilm thickness with variations in $k_L a$.

than the electron donor and biofilm thickness responses (Figure 7.7). At lower $k_L a$ values, high biosynthesis and electron donor consumption demands, consumed much of the available dissolved oxygen. Saturating concentrations could only be maintained for oxygen mass transfer coefficients in excess of 30 hr^{-1} .

The bulk phase oxygenolytic secondary substrate responses of the biofilm system (Figure 7.8) is similar to that described for the suspended biomass system. The behavior was consistent with the results presented by Bae (1992) and can also be explained in terms of reducing power (Table 3.1). Figure 7.10 demonstrates the impact of bulk phase primary substrate concentrations on secondary substrate removal in the biofilm system. For saturating electron donor concentrations ($0 \text{ hr}^{-1} < k_L a \leq 1 \text{ hr}^{-1}$), the reaction rate (q_d) and $\log(\text{NADH}/\text{NAD})$ were relatively high and the secondary substrate removal increased with increasing electron acceptor (oxygen) concentrations which were available to react with the oxygenase enzyme (Stage I). Thus, at lower electron acceptor concentrations, the availability of oxygen limits the oxygenase reaction and decreases secondary substrate removal directly.

Lower secondary substrate removal could be expected when the electron donor was present in limiting concentrations ($S_{db} < 20 K_{sd}$) and the electron acceptor was also present in limiting concentrations (Stage II). The peak which can be observed at 1.5 hr^{-1} was caused by both a decrease in reducing power ($\log(\text{NADH}/\text{NAD})$ ratio) at limiting electron donor and electron acceptor concentrations. At limiting electron donor concentrations and saturating electron acceptor concentrations ($S_{db} > 20 K_{sd}$), the secondary substrate removal was again found to increase with increasing electron acceptor concentration, until an optimum secondary substrate removal rate was achieved (Stage III). This occurred because the bulk phase electron donor concentration maintained a relatively high reducing power factor and the change in removal was mainly affected by the electron acceptor concentration which affected the oxygenase reaction. The

Figure 7.10: Biofilm system: bulk phase secondary substrate removal as a function of primary substrate concentrations.

low points on the curve found at $k_L a = 1 \text{ hr}^{-1}$ and $k_L a \geq 5 \text{ hr}^{-1}$, represent the best combinations of electron donor and electron acceptor concentrations for optimum secondary substrate removal under the specified system conditions.

Steady state substrate concentration and active fraction profiles are presented in Figures 7.11 to 7.14. The steady state electron acceptor profile (Figure 7.12) demonstrates that for oxygen mass transfer coefficients less than 5 hr^{-1} , the dissolved oxygen penetration within the biofilm was very shallow. Greater electron acceptor penetration within the biofilm were obtained for higher mass transfer coefficients ($k_L a \geq 5 \text{ hr}^{-1}$). Electron donor and secondary substrate utilization within the biofilm were, consequently, affected.

Electron acceptor concentrations within the biofilm greatly affected the electron donor profile (Figure 7.11). At $k_L a$ values below 5 hr^{-1} , electron acceptor concentrations were too limiting to support electron donor consumption. Thus, diffusive processes became dominant which accounts for the almost fully penetrated appearance of the profile. When electron acceptor concentrations were less limiting ($k_L a \geq 5 \text{ hr}^{-1}$), the electron donor was consumed rapidly in the upper layers of the biofilm.

Under lower oxygen mass transfer conditions ($k_L a < 5 \text{ hr}^{-1}$), the low electron acceptor concentrations and negligible electron donor consumption (low reducing power) lead to low secondary substrate utilization (Figure 7.13). At higher $k_L a$ values ($k_L a \geq 5 \text{ hr}^{-1}$), secondary substrate removal occurred in the upper layers of the biofilm until electron acceptor concentrations became limiting, at which point diffusive processes became dominant. The biofilm depth at which dissolved oxygen depletion occurred can be identified by the flattening out of the secondary substrate profile.

The active fraction profile was relatively unaffected by changes in aeration efficiency (Figure 7.14). A normalized active fraction profile vs. biofilm depth, not shown here,

Figure 7-11: Biofilm system: steady state electron donor profile with variations in $k_L a$.

Figure 7-12: Biofilm system: steady state electron acceptor profile with variations in $k_L a$.

Figure 7-13: Biofilm system: steady state secondary substrate profile with variations in $k_L a$.

Figure 7-14: Biofilm system: steady state active fraction profile with variations in $k_L a$.

demonstrated that for each of the different mass transfer coefficients examined, the active fraction profiles could be superimposed upon the profile generated for base case conditions.

7.2 CASE 2: ORGANIC LOADING VARIATIONS

Biological wastewater treatment systems are often exposed to fluctuations in organic loading. The ability to predict the general behavior of the system under such fluctuations is crucial from an operational stand point. As such, the response of the suspended biomass and biofilm systems under high loading and electron donor limiting conditions were examined. This was accomplished by varying the CSTR influent electron donor concentration (S_{da}) between 2000 mg/L and 25 mg/L, where the base case concentration used for both systems was 200 mg/L.

7.2.1 CASE 2 SUSPENDED BIOMASS SYSTEM RESULTS AND DISCUSSION

Seven different influent electron donor concentrations were tested in the suspended biomass system. As in the previous case study, the base case described in Section 6.2.1 was also utilized as the suspended biomass base case in this section. The simulation was run for a period of 168 hours under base case conditions, then the new influent electron donor concentration was introduced and the simulation was allowed to continue for another 168 hours upon which time steady state conditions had been reached. The resulting bulk phase electron donor, electron acceptor, secondary substrate and biomass concentrations are presented in Figures 7.15 to 7.18, respectively.

The effluent concentration of electron donor in the suspended biomass system was found to be relatively insensitive to the wide variations in influent substrate concentrations (Figure 7.15). However, a slight increase in steady state effluent electron donor concentration could be observed with increasing S_{da} .

Figure 7-15: Suspended biomass system: steady state electron donor concentration with variations in S_{dH} .

Figure 7-16: Suspended biomass system: steady state electron acceptor concentration with variations in S_{db} .

Figure 7-17: Suspended biomass system: steady state secondary substrate concentration with variations in S_{dbi} .

Figure 7-18: Suspended biomass system: steady state biomass concentration with variations in S_{dbi} .

The steady state electron acceptor concentration was shown to decrease linearly with increasing influent electron donor concentration (Figure 7.16). In theory, this could be attributed to the increased cell maintenance requirement which would be associated with the corresponding biomass concentration response and the electron donor consumption requirements which must increase drastically for effluent concentrations to remain relatively unchanged.

As bulk phase electron donor concentrations increased the efficiency of steady state secondary substrate removal improved significantly. This is due to the increase in reducing power brought about by higher influent electron donor concentrations and the consequent increase in biomass concentration (X). From Figure 7.17, it can be concluded that at influent electron donor concentrations greater than 2000 mg/L, even higher secondary substrate utilization rates could be achieved. The optimum system conditions could and should be identified in a real suspended system.

Steady state biomass generation within the reactor could be seen to increase linearly with increasing influent electron donor concentration (Figure 7.18). As expected, this demonstrated that as long as electron donor and electron acceptor concentrations were not limiting, biomass growth will proceed unhindered by the system.

7.2.2 CASE 2 BIOFILM SYSTEM RESULTS AND DISCUSSION

For the biofilm system, six different influent electron donor concentrations were examined. As in the first case study, the base case was the same as that used in the sensitivity analysis of the biofilm. The program was executed with a time step of 0.0001 hours, an initial biofilm thickness of 2.94 mm, and maximum and minimum layer thicknesses of 100 μm and 20 μm , respectively. The simulation was run under base case conditions for a period of 336 hours, at which time a new influent electron donor concentration was introduced and the simulation was allowed to continue under the new system conditions for another 336 hours, when an equilibrium state had been

reached. The bulk phase steady state electron donor, electron acceptor and secondary substrate concentrations are shown in Figures 7.19 to 7.21, and the total biofilm thickness for each case can be viewed in Figure 7.22.

The steady state bulk phase electron donor concentration was found to be very sensitive to changes in influent electron donor concentrations (Figure 7.19), especially at concentrations greater than 500 mg/L. This would suggest that the biofilm system could not adapt to high organic loading conditions as well as the suspended biomass system.

The reasons for this phenomenon can be understood more clearly by examining the response of the total biofilm thickness (Figure 7.22). The steady state biofilm thickness increased with increasing S_{db} , until the concentration surpassed 500 mg/L. At this point, the biofilm reached a maximum thickness which would indicate that at higher influent electron donor concentrations, the electron acceptor became more limiting and could not sustain further biofilm growth. Thus, the rate of biofilm growth equaled the rate of biofilm loss by endogenous decay and sloughing. Biofilm growth is ultimately restricted by the system unlike the suspended biomass system which could sustain unlimited growth under ideal system conditions. Once the maximum thickness was attained, the biofilm was not able to compensate for increases in influent substrate concentrations. This is largely due to the assumption that the suspended biomass does not contribute to substrate consumption or biomass growth in the biofilm system. If the response of the suspended biomass present in the CSTR were incorporated into the biofilm component of the model, the system would be better able to accommodate variations in electron donor loading, as is generally observed in actual biofilm systems.

Steady state electron acceptor concentrations in the reactor were shown to initially decrease with increasing S_{db} (Figure 7.20). Once the maximum biofilm thickness was reached, however, cell maintenance and electron donor consumption requirements exerted a constant demand which allowed the bulk phase electron acceptor concentration to level-off.

Figure 7-19: Biofilm system: steady state bulk phase electron donor concentration with variations in S_{dM} .

Figure 7-20: Biofilm system: steady state bulk phase electron acceptor concentration with variations in S_{db} .

Figure 7-21: Biofilm system: steady state bulk phase secondary substrate concentration with variations in S_{db} .

Figure 7-22: Biofilm system: steady state biofilm thickness with variations in S_{dM} .

A similar steady state bulk phase response was demonstrated by the secondary substrate (Figure 7.21). In general, secondary substrate removal increased with increasing influent electron donor concentrations and increasing biomass until the maximum biofilm thickness was attained. At this point ($S_{\text{don}} > 500 \text{ mg/L}$), the rate of secondary substrate removal remained constant.

Biofilm substrate concentration and active reaction profiles in response to changes in influent electron donor concentrations are presented in Figures 7.23 to 7.26. At influent electron donor concentrations greater than 500 mg/L, dissolved oxygen concentrations within the biofilm were exhausted too quickly to support electron donor consumption beyond the upper layers (Figure 7.23 and 7.24). Thus, diffusive processes became the dominant process which accounts for the almost fully penetrated appearance of the profile (Figure 7.23). When influent electron donor concentrations were lower ($S_{\text{don}} < 500 \text{ mg/L}$), the electron donor was consumed rapidly in the upper layers of the biofilm.

The steady state electron acceptor profile (Figure 7.24) demonstrated that for influent electron donor concentrations greater than 500 mg/L, the electron acceptor penetration within the biofilm was shallow due to the high electron donor consumption in the upper layers of the biofilm. Biofilm growth, electron donor and secondary substrate utilization within the biofilm were, thus, affected. Under lower loadings ($S_{\text{don}} = 25 \text{ mg/L}$), the biosynthesis and electron donor consumption demands on the electron acceptor were lower and the corresponding steady state profile remained saturated.

The steady state secondary substrate response within the biofilm was similar to that of the electron donor (Figure 7.25). Under higher influent electron donor concentrations, the depleted electron acceptor conditions and corresponding negligible electron donor consumption lead to low secondary substrate utilization. At lower S_{don} values, secondary substrate removal was shown to occur in the upper layers of the biofilm until electron acceptor concentrations became limiting, at

Figure 7-23: Biofilm system: steady state electron donor profile with variations in S_{dbi} .

Figure 7-24: Biofilm system: steady state electron acceptor profile with variations in S_{dbi} .

Figure 7-25: Biofilm system: steady state secondary substrate profile with variation in S_{dbi} .

Figure 7-26: Biofilm system: steady state active fraction profile with variations in S_{dH} .

which point diffusive processes became dominant. The biofilm depth at which dissolved oxygen depletion occurred can be identified by the flattening out of the secondary substrate profile.

As in the previous case study, the steady state active fraction profile was relatively unaffected by changes in influent electron donor concentrations (Figure 7.26). A normalized active fraction profile vs. biofilm depth, not shown here, demonstrated that for each of the influent electron donor concentrations examined, the active fraction profiles could be superimposed upon the profile generated for base case conditions.

7.3 CASE 3: HYDRAULIC RETENTION TIME VARIATIONS

Biological wastewater treatment systems are often exposed to variations in flow rates which would affect the hydraulic residence time of the substrates in the system. The ability to predict the general behavior of the system under such fluctuations is crucial from an operations stand point. As such, the response of the suspended biomass and biofilm systems under variable flow rate conditions were examined. This was accomplished by varying the hydraulic residence time (HRT) between 1 hour and 8 hours, where the base case hydraulic residence time used for both systems was 4 hours.

7.3.1 CASE 3 SUSPENDED BIOMASS SYSTEM RESULTS AND DISCUSSION

Eight different hydraulic residence times were tested in the suspended biomass system. As in the two previous case studies, the base case described in Section 6.2.1 was utilized as the suspended biomass base case in this section. The simulation was run for a period of 168 hours under base case conditions, then the new hydraulic residence time was introduced and the simulation was allowed to continue for another 168 hours upon which time steady state conditions had been reached. The resulting bulk phase electron donor, electron acceptor, secondary substrate and biomass concentrations are presented in Figures 7.27 to 7.30, respectively.

Figure 7-27: Suspended biomass system steady state electron donor concentration with variations in HRT.

Figure 7-28: Suspended biomass system steady state electron acceptor concentration with variations in HRT.

Figure 7-29: Suspended biomass system steady state secondary substrate concentration with variations in HRT.

Figure 7-30: Suspended biomass system steady state biomass concentration with variations in HRT.

The steady state electron donor concentrations were found to decrease with increasing hydraulic residence time and leveled off at HRT values greater than 3 hours (Figure 7.27). On the other hand, biomass growth was observed to increase with increasing HRT and leveled off at HRT values greater than 3 hours (Figure 7.30). This can be attributed to the limited electron donor availability for hydraulic retention times lasting longer than 3 hours.

At hydraulic retention times approaching washout conditions ($HRT < 2.5$), the steady state electron acceptor concentrations were shown to decrease rapidly. A sagging effect, representing the point of highest electron donor consumption requirements for electron acceptor, could be observed at a hydraulic retention time of 3 hours (Figure 7.28). This was followed by a more gradual increase in steady state electron acceptor concentrations corresponding to a lower oxygen demand exerted by the system.

The steady state secondary substrate response of the system was found to be very similar to that of the electron donor. From Figure 7.29, it can be seen that the system achieved its maximum secondary substrate removal at a hydraulic retention time of approximately 3 hours. Beyond this point, electron donor concentrations would become limiting which would account for the leveling off of the secondary substrate removal curve for HRT values greater than 3 hours.

7.3.2 CASE 3 BIOFILM SYSTEM RESULTS AND DISCUSSION

For the biofilm system, six different hydraulic retention times were examined. As in the other two case studies, the base case was the same as that used in the sensitivity analysis of the biofilm. The program was executed with a time step of 0.0001 hours, an initial biofilm thickness of 2.94 mm, and maximum and minimum layer thicknesses of 100 μm and 30 μm , respectively. The simulation was run under base case conditions for a period of 336 hours, at which time a hydraulic retention time was introduced and the simulation was allowed to continue under the new system conditions for another 336 hours, when an equilibrium state had been reached. The bulk

phase steady state electron donor, electron acceptor and secondary substrate concentrations are shown in Figures 7.31 to 7.33, and the total biofilm thickness for each case can be viewed in Figure 7.34.

When compared with the suspended biomass system, the general biofilm system response of the bulk phase effluent electron donor concentration (Figure 7.31) the electron donor concentration of the biofilm system did not level off as rapidly as in the suspended biomass system. As well, washout conditions were neither expected nor observed within the biofilm system.

The reasons for this phenomenon can be understood more clearly by examining the response of the total biofilm thickness (Figure 7.34). The steady state biofilm thickness was observed to decrease with increasing hydraulic retention time. As the HRT increased, more time was available for electron donor consumption within the biofilm. Thus, as bulk phase electron donor concentrations decreased, electron donor concentrations within the biofilm became increasingly limiting until they were no longer sufficient to support the biofilm even within the upper layers and a decrease in total biofilm thickness ensued.

The steady state electron acceptor response of the biofilm system is relatively similar to that of the suspended biomass system (Figure 7.32). Bulk phase electron acceptor concentrations increased with increasing HRT.

The steady state bulk phase secondary substrate response of the biofilm system was also very similar to the suspended biomass response (Figure 7.33). In general secondary substrate removal increased with increasing hydraulic retention time, but leveled off much more slowly than in the suspended biomass system.

Biofilm substrate concentration and active fraction profiles in response to fluctuations in hydraulic retention time are shown in Figures 7.35 to 7.38. At hydraulic retention times of less than 2 hours, dissolved oxygen concentrations within the biofilm were exhausted too quickly to support electron donor consumption beyond the upper layers. As well, the replenishment of

Figure 7-31: Biofilm system steady state bulk phase electron donor concentration with variations in HRT.

Figure 7-32: Biofilm system steady state bulk phase electron acceptor concentration with variations in HRT.

Figure 7-33: Biofilm system steady state bulk phase secondary substrate concentration with variations in HRT.

Figure 7-34: Biofilm system steady state total biofilm thickness with variations in HRT.

Figure 7-35: Biofilm system steady state electron donor profile with variations in HRT.

Figure 7-36: Biofilm system steady state electron acceptor profile with variations in HRT.

Figure 7-37: Biofilm system steady state secondary substrate profile with variations in HRT.

Figure 7-38: Biofilm system steady state active fraction profile with variations in HRT.

electron donor within the CSTR was rapid compared to the biomass consumption capability which resulted in diffusive processes becoming dominant (Figure 7.35). When the hydraulic retention time was higher, ($HRT > 2$ hours), the electron donor was consumed rapidly in the upper layers of the biofilm.

The steady state electron acceptor profile (Figure 7.36) demonstrated that for hydraulic retention times of less than 2 hours, the electron acceptor penetration within the biofilm was very shallow due to the high electron donor consumption demand in the upper layers of the biofilm and the limited amount of time for diffusive processes to occur. At HRT values greater than 2 hours, the development of the dissolved oxygen profile had proceeded to greater depths within the biofilm due to the decrease in biosynthesis and electron donor consumption demand on the electron acceptor at higher hydraulic retention times.

Under lower hydraulic retention times, the depleted electron acceptor conditions and corresponding negligible electron donor consumption lead to lower secondary substrate utilization (Figure 7.37). At higher HRT values, secondary substrate removal was shown to occur in the upper layers of the biofilm until electron acceptor concentrations became limiting, at which point diffusive processes became dominant. The biofilm depth at which dissolved oxygen depletion occurred can be identified by the flattening out of the secondary substrate profile.

As in the two previous case studies, the steady state active fraction profile was relatively unaffected by changes in hydraulic retention time (Figure 7.38). A normalized active fraction profile vs. biofilm depth, not shown here, demonstrated that for each hydraulic retention time examined, the active fraction profiles could be superimposed upon the profile generated for base case conditions.

SUSPENDED BIOMASS SYSTEM VS. BIOFILM SYSTEM

The same kinetic and operational parameters were utilized for the base cases of the suspended biomass and biofilm systems. The only differences between the two systems lie in the introduction of diffusive, decay and biomass retention parameters, as well as a specific area term in the biofilm system (Tables 6.1 and 6.2). However, regardless of these additional parameters, the two systems could be considered equivalent and a direct comparison between the suspended biomass and biofilm system can be made.

Under base case conditions, steady state was generally reached much more quickly with the suspended biomass system (Figures 6.1 and 6.3). Electron acceptor concentrations remained saturated in both systems due to the high base case oxygen mass transfer coefficient. The suspended biomass system was slightly more efficient in terms of bulk phase electron donor removal. Whereas, the biofilm system could achieve higher bulk phase secondary substrate removal.

The two systems exhibited very similar responses to aeration failure represented by decreases in the oxygen mass transfer coefficient. The only difference was that the suspended biomass system developed a secondary substrate sag at which point the ideal electron donor and electron acceptor concentrations for optimum secondary substrate removal had been reached. The biofilm system only displayed a secondary substrate low for that same oxygen transfer coefficient (Figure 7.3). This removal efficiency could be obtained at other combinations of electron donor and electron acceptor concentrations (Figure 7.8).

The biofilm system was found to be very sensitive to fluctuations in organic loading in terms of effluent electron donor concentrations when compared to the suspended biomass system. This was partially attributed to the fact that the biofilm will reach a maximum total biofilm thickness beyond which the system can no longer compensate for increases in organic loading (Figure 7.22). It can also be partially explained by the fact that substrate removal by suspended

biomass was assumed to be negligible in the biofilm system. In the suspended biomass system, on the other hand, biomass growth is not limited by system or diffusive parameters which allows it to compensate (Figure 7.18).

The responses to hydraulic retention time were found to be relatively similar in both systems, except that substrate concentrations leveled off more rapidly in the suspended biomass system. The biofilm system was able to handle lower HRT values (higher flow rates) without reaching washout conditions (Figures 7.31 to 7.34). The suspended biomass system, however, remained more stable under fluctuations at higher HRT values (Figure 7.27 to 7.30). Thus, the selection of suspended biomass or biofilm system would depend upon whether a generally more stable system or the avoidance of washout conditions were desired.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 CONCLUSIONS

A computer model was developed which was able to account for dual substrate limitation and cosubstrate utilization in suspended biomass and biofilm systems. The model was capable of simulating and predicting transient variations in electron donor, electron acceptor and secondary substrate concentrations, as well as changes in active biomass within a CSTR. The computer model was also able to predict transient substrate concentration profiles and fluctuations in biofilm thickness and active biomass within the biofilm. In this work, the suspended biomass and biofilm system responses were tested under oxygenolytic condition; i.e. oxygen was utilized as the electron acceptor.

Step size analysis revealed that a time step of 0.0001 hour was adequate for simulations under base case conditions in both systems. Layer joining of small biofilm layers did not significantly affect simulation results and was useful in decreasing the number of biofilm layers which, consequently, decreased the required computing time. The layer splitting feature was found to create an oscillatory response in the determination of bulk phase substrate concentrations and, to a lesser extent, the biofilm profile substrate concentrations. However, this oscillatory effect could be minimized by decreasing the maximum layer thickness. Minimum and maximum layer thicknesses of 30 μm and 100 μm , respectively, were utilized for biofilm model simulations.

A comparison of the COMDEG numerical model with existing models revealed that the suspended biomass portion of the model could reproduce electron donor removal under single substrate conditions (Shamat and Maier, 1980), electron donor and electron acceptor removal under dual substrate conditions (Sinclair and Ryder, 1975), as well as secondary substrate removal

under dual substrate limitation (Bae, 1992). The biofilm portion of the numerical model was in agreement with steady state pseudoanalytical models developed by Rittmann and McCarty (1980) and Sáez and Rittmann (1988) for bulk phase electron donor utilization and biofilm growth predictions under single substrate limitation conditions. The model produced similar biofilm substrate and active fraction profiles, as well as total and active biofilm thickness curves, to those described by Skowlund (1990). However, biofilm thickness values differed due to the differences in the definition of inert material retention terms between the two models.

Neither of the systems was found to be sensitive to variations in initial parameter values. The normalized sensitivity analysis demonstrated that the suspended biomass electron donor, electron acceptor, secondary substrate and biomass concentrations were most affected by 1% fluctuations in apparent maximum electron donor utilization rate (-2.4123%), apparent maximum electron acceptor utilization (-0.0375%), M coefficient (-3.7931%) and yield coefficient (1.0835%), respectively. In the biofilm system, electron donor and electron acceptor concentrations were most affected by 1% fluctuations in apparent maximum electron donor concentration, (-0.6435%) and (0.0590), whereas the M coefficient had the greatest impact (-5.7837) on secondary substrate removal.

The response of the suspended biomass and biofilm systems were monitored under three case studies, namely; system aeration, organic loading and hydraulic retention time variations. The two systems exhibited decreases in electron donor and secondary substrate removal with decreases in aeration supply. The biofilm system was found to be very sensitive to fluctuations in organic loading when compared to the suspended biomass system. This was partially explained by the fact that suspended growth was considered negligible in the fixed-film system and that the biofilm reached a maximum total biofilm thickness beyond which the system could no longer compensate for increases in organic loading. The biofilm system was able to handle lower HRT values (higher

flow rates) without reaching washout conditions, whereas the suspended biomass system remained more stable under fluctuations at higher HRT values.

8.2 RECOMMENDATIONS

The numerical model assumed that the biomass did not modify metabolic pathways when transferring from aerobic to anaerobic conditions. However, this may not always be the case and physiological responses of bacterial populations under electron acceptor depletion should be incorporated in the model.

The model could also be modified to account for multi-species (e.g. aerobic, nitrifying and denitrifying bacteria) bacterial populations. Active fraction, electron donor, electron acceptor and, where required, secondary substrate removal could be predicted for each component of the population.

It was also assumed that the suspended biomass contribution to substrate removal was negligible in the biofilm system. However, in real systems, the suspended biomass can contribute a stabilizing effect to factors such as variations in organic loading. As such, the suspended biomass growth and substrate utilization should be included in the biofilm portion of the numerical model.

An oscillatory response in substrate utilization was created by the layer splitting feature. An interpolation of concentrations rather than an equal assignment of concentrations could be incorporated in the biofilm portion of the model. In doing so, the oscillatory effect could, perhaps, be reduced.

BIBLIOGRAPHY

Alexander, M.; 1981; Biodegradation of Chemicals of Environmental Concern; *Science*; 211:132-138.

Andersen, K.B. and K. Von Meyenburg; 1977; Charges of Nicotinamide Adenine Nucleotides and Adenylate Energy Charge as Regulatory Parameters of the Metabolism in *Escherichia coli*; *Journal of Biological Chemistry*; 252(12):4151-4156.

Annachatre, A.P. and P. Khanna; 1987; Unsteady-State Biofilm Kinetics; *ASCE Journal of Environmental Engineering*; 113(2): 429-433.

Atkinson, B. And I.J. Davies; 1974; The Overall Rate of Substrate Uptake (Reaction) by Microbial Films - Part I - A Biological Rate Equation; *Transactions of the Institution of Chemical Engineers*; 52:248-259.

Bader, F.G.; 1978; Analysis of Double-Substrate Limited Growth; *Biotechnology and Bioengineering*; 20:183-202.

Bae, W.; 1992; *Modeling Dual-Limitation Kinetics Incorporating Intracellular Cofactor Responses*; Ph.D. Thesis; University of Illinois at Urbana-Champaign.

Bae, W. and B.E. Rittmann; 1990; Effects of Electron Acceptor and Electron Donor on Biodegradation of CCl₄ by Biofilms; *Environmental Engineering: Proceedings of the 1990 Specialty Conference*, Arlington, VA; June 8-11; 390-397.

Bae, W. and B.E. Rittmann; 1994; Accelerating the Rate of Cometabolic Degradations Requiring an Intracellular Electron Source - Model and Biofilm Application;

Bajpai, A.C., L.R. Mustoe and D. Walker; 1990; *Advanced Engineering Mathematics*; John Wiley and Sons; 502 pp.

Barton, D.A. and J.J. McKeown; 1986; Evaluation of an Aerator Control Strategy Utilizing Time Varying Mathematical Model Simulations; *Water Science and Technology*; 18:189-201.

Belkhadir, R., B. Capdeville and H. Roques; 1988; Fundamental Descriptive Study and Modelization of Biological Film Growth. I. Fundamental Descriptive Study of Biological Film Growth; *Water Research*; 22(1): 59-69.

Borden, R.C. and P.B. Bedient; 1986; Transport of Dissolved Hydrocarbons Influenced by Oxygen-Limited Biodegradation - 1. - Theoretical Development; *Water Resources Research*; 22(13):1973-1982.

Bouwer, E.J.; 1985; Secondary Utilization of Trace Halogenated Organic Compounds in Biofilms; *Environmental Progress*; 4():43-45.

Bouwer, E.J.; 1987; Theoretical Investigation of Particle Deposition in Biofilm Systems; *Water Research*; 21(12): 1489-1498.

Bouwer, E.J. and P.L. McCarty; 1982; Removal of Trace Organic Compounds by Activated Carbon and Fixed-Film Bacteria; *Environmental Science and Technology*; 16:836-843.

Bouwer, E.J. and P.L. McCarty; 1985; Utilization Rates of Trace Halogenated Organic Compounds in Acetate-Grown Biofilms; *Biotechnology and Bioengineering*; 27:1564-1571.

Brock, T.D. and M.T. Madigan; 1988; *Biology of Microorganisms*; Prentice-Hall; 835 pp.

Burden, R.L. and J.D. Faires; 1989; *Numerical Analysis*; PSW-Kent Publishing Company; Boston; 729 pp.

Capdeville, B., R. Belkhadir and H. Roques; 1988; Fundamental Descriptive Study and Modelization of Biological Film Growth. II. A New Concept of a Biological Film Growth Modelization; *Water Research*; 22(1): 71-77.

Chang, M., T.C. Voice and C.S. Criddle; 1993; Kinetics of Competitive Inhibition and Cometabolism in the Biodegradation of Benzene, Toluene, and *p*-Xylene by Two *Pseudomonas* Isolates; *Biotechnology and Bioengineering*; 41:1057-1065.

Characklis, W.G.; 1981; Fouling Biofilm Development: a Process Analysis; *Biotechnology and Bioengineering*; 23: 1923-1960.

Choi, E. and C.E. Burkhead; 1975; Kinetics of a Fixed Mediate Activated Sludge System; *Progress in Water Technology*; 7(2):251-263.

Colwell, R.R. and G.S. Slater; 1978; Microbial Degradation of Industrial Chemicals; in *Water Pollution Microbiology*; vol. 2; R. Mitchell ed.; Wiley-Interscience; p. 111-134.

Criddle, C.S.; 1993; The Kinetics of Cometabolism; *Biotechnology and Bioengineering*; 41(11):1048-1056.

Ensign, S.A., M.R. Hyman and D.J. Arp; 1992; Cometabolic Degradation of Chlorinated Alkenes by Alkene Monooxygenase in a Propylene-Grown *Xanthobacter* Strain; *Applied and Environmental Microbiology*; 58(9):3038-3046.

Evans, W.C.; 1977; Biochemistry of the Bacterial Catabolism of Aromatic Compounds in Anaerobic Environments; *Nature*; 270(3):17-22.

Fathepure, B.Z. and T.M. Vogel; 1991; Complete Degradation of Polychlorinated Hydrocarbons by a Two-Stage Biofilm Reactor; *Applied and Environmental Microbiology*; 57(12):3418-3422.

Finlayson, B.A.; 1972; *The Method of Weighted Residuals and Variational Principles with Applications in Fluid Mechanics, Heat and Mass Transfer*; Academic Press; 412 pp.

Flora, J.R., M.T. Suidan, P. Biswas and G.D. Sayles; 1993; Modeling Substrate Transport into Biofilms: Role of Multiple Ions and pH Effects; *ASCE Journal of Environmental Engineering*; 119(5):908-930.

Freedman; D.L. and S.D. Herz; Use of Ethylene and Ethane as Primary Substrates for Aerobic Cometabolism of Vinyl Chloride; p. 623-634.

Gantzer, C.J.; 1989; Inhibitory Substrate Utilization by Steady-State Biofilms; *ASCE Journal of Environmental Engineering*; 115(2):302-319.

Gantzer, C.J., H.P. Kollig, B.E. Rittmann and D.L. Lewis; 1988; Predicting the Rate of Trace-Organic Compound Removal by Natural Biofilms; *Water Research*; 22(2):191-200.

Gaudy, A.F. and E.T. Gaudy; 1980; *Microbiology for Environmental Scientists and Engineers*; McGraw-Hill; 736pp.

Gibson, D.T.; 1971; The Microbial Oxidation of Aromatic Hydrocarbons; *Critical Reviews in Microbiology*; 1:199-223

Grady, C.P.L.; 1985; Biodegradation: Its Measurement and Microbiological Basis; *Biotechnology and Bioengineering*; 27:660-674.

Grady, C.P.L.; 1990; Biodegradation of Toxic Organics: Status and Potential; *ASCE Journal of Environmental Engineering*; 116(5):805-828.

Hamoda, M.F.; 1989; Kinetics of Aerated Submerged Fixed-Film (ASFF) Bioreactors; *Water Research*; 23(9): 1147-1154

Harder, W. and L. Dijkhuizen; 1983; Physiological Responses to Nutrient Limitation; *Annual Review of Microbiology*; 37:1-23.

Harremoës, P.; 1978; Biofilm Kinetics; in *Water Pollution Microbiology*; vol. 2; R. Mitchell ed.; Wiley Interscience; p. 71-110.

Harvey, M.; 1984; *Anaerobic Digestion of Swine Waste Using Downflow Stationary Fixed Film Digesters*; M.Sc. Thesis; University of Guelph; Guelph, Ontario; 153 pp.

Haug, E. And K. K. Choi; 1993; *Methods of Engineering Mathematics*; Prentice-Hall; 585 pp.

Hess, T.F., S.K. Schmidt, J. Silverstein and B. Howe; 1990; Supplemental Substrate Enhancement of 2,4-Dinitrophenol Mineralization by a Bacterial Consortium; *Applied and Environmental Microbiology*; 56(6):1551-1558.

Howell, J.A. and B. Atkinson; 1976; Influence of Oxygen and Substrate Concentrations on the Ideal Film Thickness and the Maximum Overall Substrate Uptake Rate in Microbial Fermenters; *Biotechnology and Bioengineering*; 18:15-35.

Hughes, J.B. and G.F. Parkin; 1992; The Effect of Mixtures of Xenobiotics and Primary Electron Donor on the Anaerobic Biotransformation of High Concentrations of Chlorinated Aliphatics; *Water Science and Technology*; 26(1-2):117-126.

Jackman, A.P. and R.L. Powell; 1991; *Hazardous Waste Treatment Technologies*; Noyes Publications; Park Ridge, New Jersey; 276 pp.

Kim, B.R. and M.T. Suidan; 1989; Approximate Algebraic Solution for a Biofilm Model With the Monod Kinetic Expression; *Water Research*; 23(12): 1491-1498.

Kim, C.J. and W.J. Maier; 1986; Acclimation and Biodegradation of Chlorinated Organic Compounds in the Presence of Alternate Substrates; *Research Journal Water Pollution Control Federation*; 58(2):157-164.

Kishimoto, T., Y. Akagi, T. Takamatsu and S. Shioya; 1991; A Fundamental Study of Wastewater Treatment by a Biofilm; *International Chemical Engineering*; 31(3): 501-507.

Kissel, J.C.; 1986; Modelling Mass Transfer in Biological Waste Water Treatment Process; *Water Science and Technology*; 18(6): 35-45.

Kissel, J.C., P.L. McCarty and R.L. Street; 1984; Numerical Simulation of Mixed-Culture Biofilm; *ASCE Journal of Environmental Engineering*; 110(2):393-411.

Klecka, G.M. and W.J. Maier; 1988; Kinetics of Microbial Growth on Mixtures of Pentachlorophenol and Chlorinated Aromatic Compounds; *Biotechnology and Bioengineering*; 31:328-335.

Knackmuss, H.; 1981; Degradation of Halogenated and Sulfonated Hydrocarbons; in *Microbial Degradation of Xenobiotic and Recalcitrant Compounds*; T. Leisinger, R. Hütter, A.M. Cook and J. Nüesch, Ed.; FEMS Symposium No. 12; Academic Press; 415 pp.

Kobayashi, H. and B.E. Rittmann; 1982; Microbial Removal of Hazardous Organic Compounds; *Environmental Science and Technology*; 16(3):170A-183A.

Kreyszig, E.; 1988; *Advanced Engineering Mathematics*; John Wiley and Sons; 1294 pp.

Lau, A.O., P.F. Strom and D. Jenkins; 1984; The competitive Growth of Floc-Forming and Filamentous Bacteria: A Model for Activated Sludge Bulking; *Journal of the Water Pollution Control Federation*; 56(1):52-61.

Lawrence, A.W. and P.L. McCarty; 1970; Unified Basis for Biological Treatment Design and Operation; *ASCE Journal of the Sanitary Engineering Division*; 96:757-778.

Levenspiel, O.; 1972; *Chemical Reaction Engineering*; John Wiley and Sons; Second Edition; 578 pp.

Logan, B.E. and J.W. Dettmer; 1990; Increased Mass Transfer to Microorganisms with Fluid Motion; *Biotechnology and Bioengineering*; 35:1135-1144.

Logan, B.E. and S.W. Hermanowicz; 1987; Application of the Penetration Theory to Oxygen Transfer to Biofilms; *Biotechnology and Bioengineering*; 29:762-766.

Lu, C.J. and S.J. Chen; 1992; The Effects of the Secondary Carbon Source on the Biodegradation of Chlorinated Phenols in Biofilm Reactors; *Water Science Technology*; 26(9-11): 2113-2116.

- Malmstead, M.J., F.J. Brockman, A.J. Valocchi and B.E. Rittmann; 1995; Modeling Biofilm Biodegradation Requiring Cosubstrates: The Quinoline Example; *Water Science and Technology*; 31(1):71-84.
- Mandelstam, J., K. McQuillen and I. Dawes; 1982; *Biochemistry of Bacterial Growth*; John Wiley and Sons; 449 pp.
- Manem, J.A. and B.E. Rittmann; 1992; Removing Trace-Level Organic Pollutants in a Biological Filter; *Journal of the American Water Works Association*; 84(4):152-157.
- van der Meer, J.R., W. Roelofsen, G. Schraa and A.J. Zehnder; 1987; Degradation of Low Concentrations of Dichlorobenzenes and 1,2,4-Trichlorobenzene by *Pseudomonas* sp. Strain P51 in Nonsterile Soil Columns; *FEMS Microbiology Ecology*; 45:333-341.
- Metcalf and Eddy, Inc; 1991; *Wastewater Engineering: Treatment, Disposal and Reuse*; Third Edition; McGraw-Hill; 1334 pp.
- Montgomery, L., N. Assaf-Anid, L. Nies, P. Anid and T.M. Vogel; 1991; Anaerobic Biodegradation of Chlorinated Organic Compounds; in *Biodegradation and Bioremediation Technologies*; G.R. Chaudhry, Ed.; Dioscordes Publ.
- Namkung, E., R.G. Stratton and B.E. Rittmann; 1983; Predicting Removal of Trace Organic Compounds by Biofilms; *Journal of the Water Pollution Control Federation*; 55(11):1366-1372.
- Neilson, A.H.; 1990; A Review: The Biodegradation of Halogenated Organic Compounds; *Journal of Applied Bacteriology*; 69:445-470.
- Nies, L. and T.M. Vogel; 1990; Effects of Organic Substrates on Dechlorination of Aroclor 1242 in Anaerobic Sediments; *Applied and Environmental Microbiology*; 56(9):2612-2617.
- Odenrantz, J.E., W. Bae, A.J. Valocchi and B.E. Rittmann; 1990; Stimulation of Biologically Active Zones (BAZ's) in Porous Media by Electron-Acceptor Injection; *Journal of Contaminant Hydrology*; 6:35-50.
- Ong, S.L.; 1990; A Comparison of Estimates of Kinetic Constants for a Suspended Growth Treatment System from Various Linear Transformations; *Journal of the Water Pollution Control Federation*; 62(7):894-900.
- Papanastasiou, A.C. and W.J. Maier; 1982; Kinetics of Biodegradation of 2,4-Dichlorophenoxyacetate in the Presence of Glucose; *Biotechnology and Bioengineering*; 24:2001-2011.
- Peavy, S., D.R. Rowe and G. Tchobanoglous; 1985; *Environmental Engineering*; McGraw-Hill, Inc.; 699 pp.
- Petrasek, A.C., I.J. Kugelman, B.M. Austern, T.A. Pressley, L.A. Winslow and R.H. Wise; 1983; Fate of Toxic Organic Compounds in Wastewater Treatment Plants; *Journal of the Water Pollution Control Federation*; 55(10):1286-1296.

- Reineke, W. and H. Knackmuss; 1979; Construction of Haloaromatic Utilizing Bacteria; *Nature*; 277:385-386.
- Reineke, W. and H. Knackmuss; 1988; Microbial Degradation of Haloaromatics; *Annual Review of Microbiology*; 42:263-287.
- Rittmann, B.E.; 1984; Choosing and Determining Loading Types for Fixed-Film Reactors; *2nd International Conference on Fixed Film Biological Processes*; pp. 565-577.
- Rittmann, B.E.; 1989; Mathematical Modelling of Fixed-Film Growth; in *Dynamic Modeling and Expert Systems in Wastewater Engineering*, G.G. Patry and D. Chapman, eds.; Lewis Publishers; Chelsea, Michigan; 395 pp.
- Rittmann, B.E. and K. Dovantsiz; 1983; Dual Limitation of Biofilm Kinetics; *Water Research*; 17(12):1727-1734.
- Rittmann, B.E., D. Jackson and S.L. Storck; 1988; Potential for Treatment of Hazardous Organic Chemicals With Biological Processes; in *Biotreatment Systems*; D. Wise ed.; vol. 3.
- Rittmann, B.E. and P.L. McCarty; 1978; Variable-Order Model of Bacterial-Film Kinetics; *ASCE Journal of Environmental Engineering*; 104:889-900.
- Rittmann, B.E. and P.L. McCarty; 1980; Model of Steady-State-Biofilm Kinetics; *Biotechnology and Bioengineering*; 22:2343-2357.
- Rittmann, B.E. and P.L. McCarty; 1981; Substrate Flux into Biofilms of any Thickness; *ASCE Journal of Environmental Engineering*; 107:831-849.
- Rittmann, B.E. and J.A. Manem; 1992; Development and Experimental Evaluation of a Steady-State, Multispecies Biofilm Model; *Biotechnology and Bioengineering*; 39:914-922.
- Rittmann, B.E. and P.B. Sáez; 1994; Modeling Biological Processes Involved in Degradation of Hazardous Organic Substrates; in *Principles and Practices in Biotreatment*, M. Levin and M. Gealt Ed.; McGraw-Hill.
- Rochkind-Dubinsky, M.L., G.S. Sayler and J.W. Blackburn; 1987; *Microbiological Decomposition of Chlorinated Aromatic Compounds*; Microbiology Series, Vol. 18; Marcel Dekker; New York, New York; p. 315.
- Sáez, P.B., J.F. Muñoz and R.A. Celedón; 1984; Biofilm Kinetics Models; *2nd International Conference on Fixed Film Biological Processes*; pp. 495-528.
- Sáez, P.B. and B.E. Rittmann; 1988; Improved Pseudoanalytical Solution for Steady-State Biofilm Kinetics; *Biotechnology and Bioengineering*; 32:379-385.
- Sáez, P.B. and B.E. Rittmann; 1990; Error Analysis of Limiting-Case Solutions to the Steady-State-Biofilm Model; *Water Research*; 24(10):1181-1185.

Sáez, P.B. and B.E. Rittmann; 1994; Biodegradation Kinetics of a Mixture Containing a Primary Substrate (Phenol) and an Inhibitory Co-Metabolite (4-Chlorophenol).

Schmidt, S.K., S. Simkins and M. Alexander; 1985; Models for the Kinetics of Biodegradation of Organic Compounds Not Supporting Growth; *Applied and Environmental Microbiology*; 50(2):323-331.

Schwarzenbach, R.P., P.M. Gschwend and D.M. Imboden; 1993; *Environmental Organic Chemistry*; Wiley; New York; 681 pp.

Shamat, N.A. and W.. Maier, 1980; Kinetics of Biodegradation of Chlorinated Organics; *Research Journal Water Pollution Control Federation*; 52(8):2158-2166.

Siegrist, H. and W. Gujer; 1985; Mass Transfer Mechanisms in a Heterotrophic Biofilm; *Water Research*; 19(11): 1369-1378.

Siegrist, H. and W. Gujer; 1987; Demonstration of Mass Transfer and pH Effects in a Nitrifying Biofilm; *Water Research*; 21(12): 1481-1487.

Sinclair, C.G. and D.N. Ryder; 1975; Models for the Continuous Culture of Microorganisms under both Oxygen and Carbon Limiting Conditions; *Biotechnology and Bioengineering*; 17:375-398.

Skowlund, C.T.; 1990; Effect of Biofilm Growth on Steady-State Biofilm Models; *Biotechnology and Bioengineering*; 35(5): 502-510.

Stover, E.L. and D.F. Kincannon; 1983; Biological Treatability of Specific Organic Compounds Found in Chemical Industry Wastewaters; *Research Journal Water Pollution Control Federation*; 55(1):97-109.

Suidan, M.T. and Y. Wang; 1985; Unified Analysis of Biofilm Kinetics; *ASCE Journal of Environmental Engineering*; 111(5):634-646.

Suidan, M.T., B.E. Rittmann and U.K. Traegner; 1987; Criteria Establishing Biofilm-Kinetic Types; *Water Research*; 21(4):491-498.

Sykes, J.F., J.L. Wilson and R.W. Andrews; 1985; Sensitivity Analysis for Steady State Groundwater Flow Using Adjoint Operators; *Water Resources Research*; 21(3):359-371.

Sykes, J.F. and N.R. Thomson; 1988; Parameter Identification and Uncertainty Analysis for Variably Saturated Flow; *Advances in Water Resources*; 11:185-191.

Sykes, R.M.; 1973; Identification of the Limiting Nutrient and Specific Growth Rate; *Journal of the Water Pollution Control Federation*; 45(5):888-895.

Tabak, H.H., S.A. Quave, C.I. Mashini and E.F. Barth; 1981; Biodegradability Studies with Organic Priority Pollutant Compounds; *Journal of the Water Pollution Control Federation*; 53(10):1503-1518.

- Tabak, H.H., S. Desai and R. Govind; 1990; Determination of Biodegradability Kinetics of RCRA Compounds Using Respirometry for Structure-Activity Relationships; *44th Purdue Industrial Waste Conference Proceedings*; 405-423.
- Tchobanoglous, G. and E.D. Schroeder; 1987; *Water Quality*; Addison-Wesley; 768 pp.
- Trulear, M.G. and W.G. Characklis; Dynamic Biofilm Processes; *Journal of the Water Pollution Control Federation*; 54(9):1288-1301.
- Vogel, T.M.; 1988; Chemical and Biological Transformations of Hazardous Wastes - Halogenated Aliphatic Compounds; *Proceedings for American Water Works Association Annual Meeting*; Orlando, Florida.
- Vogel, T.M., C.S. Criddle and P.L. McCarty; 1987; Transformations of Halogenated Compounds; *Environmental Science and Technology*; 21(8):722-736.
- Wang, B., Q. Yang, R. Liu, J. Yuang, F. Ma, J. He and G. Li; 1991; A Study of Simultaneous Organics and Nitrogen Removal by Extended Aeration Submerged Biofilm Process; *Water Science and Technology*; 24(5): 197-213.
- Wanner, O. and W. Gujer; 1984; Competition in Biofilms; *Water Science and Technology*; 17:27-44.
- Wanner, O. and W. Gujer; 1986; A Multispecies Biofilm Model; *Biotechnology and Bioengineering*; 28:314-328.
- Watanabe, Y., S. Masuda and M. Ishiguro; 1992; Simultaneous Nitrification and Denitrification in Micro-Aerobic Biofilms; *Water Science and Technology*; 26(3-4):511-522.
- Weightman, A.J. and J.H. Slater; 1979; The Problem of Xenobiotics and Recalcitrance; in *Micro-Organisms in Action: Concepts and Applications in Microbial Ecology*; J.M. Lynch and J.E. Hobbie, eds.; Blackwell Scientific Publications; Boston.
- Welty, J.R., C.E. Wicks and R.E. Wilson; 1984; *Fundamentals of Momentum, Heat and Mass Transfer*; Wiley; New York; 803 pp.
- Wiggins, B.A. and M. Alexander; 1988; Role of Chemical Concentration and Second Carbon Sources in Acclimation of Microbial Communities for Biodegradation; *Applied and Environmental Microbiology*; 54(11):2803-2807.
- Williamson, K. and P.L. McCarty; 1976a; A Model of Substrate Utilization by Bacterial Films; *Journal of the Water Pollution Control Federation*; 48(1):9-24.
- Williamson, K. and P.L. McCarty; 1976b; Verification Studies of the Biofilm Model for Bacterial Substrate Utilization; *Journal of the Water Pollution Control Federation*; 48(2):281-296.
- Young, J.C. and H.H. Tabak; 1993; Multilevel Protocol for Assessing the Fate and Effect of Toxic Organic Chemicals in Anaerobic Treatment Processes; *Water Environment Research*; 65(1):34-45.

APPENDIX A

FINITE DIFFERENCE EQUATIONS

SUSPENDED BIOMASS SYSTEM

Bulk phase electron donor concentration

$$S'_{dh}{}^{t+1} = S'_{dh} + \left[\frac{Q}{V} (S_{dhi} - S'_{dh}) - q_{md} \left(\frac{S'_{dh}}{K_{sd} + S'_{dh}} \right) \left(\frac{S'_{ab}}{K_{sa} + S'_{ab}} \right) fX' \right] \Delta t \quad (\text{A.1})$$

Bulk phase electron acceptor concentration

$$S'_{ab}{}^{t+1} = S'_{ab} + \left[\frac{Q}{V} (S_{abi} - S'_{ab}) + k_{La} (C_s - S'_{ab}) - \left\{ q_{md} \left(\frac{S'_{dh}}{K_{sd} + S'_{dh}} \right) \left(\frac{S'_{ab}}{K_{sa} + S'_{ab}} \right) + v_{a/x} b \right\} fX' \right] \Delta t \quad (\text{A.2})$$

Bulk phase secondary substrate concentration

$$S'_{2b}{}^{t+1} = S'_{2b} + \left[\frac{Q}{V} (S_{2bi} - S'_{2b}) - q_{m2} \{ \log(NADH/NAD)' + M \} \left(\frac{S'_{2b}}{K_{2d} + S'_{2b}} \right) \left(\frac{S'_{ab}}{K_{sa} + S'_{ab}} \right) fX' \right] \Delta t \quad (\text{A.3})$$

Bulk phase suspended biomass concentration

$$X'^{t+1} = X' + \left[\frac{Q}{V} (X_i - X') - \left\{ Y q_{md} \left(\frac{S'_{dh}}{K_{sd} + S'_{dh}} \right) \left(\frac{S'_{ab}}{K_{sa} + S'_{ab}} \right) - b \right\} X' \right] \Delta t \quad (\text{A.4})$$

Bulk phase electron donor or dual substrate limited log(NADH/NAD) ratio

$$\log(NADH/NAD)' = 0.273 q_{md} \left(\frac{S'_{dh}}{K_{sd} + S'_{dh}} \right) \left(\frac{S'_{ab}}{K_{sa} + S'_{ab}} \right) - 1.11 \left(\frac{S'_{ab}}{K_{sa} + S'_{ab}} \right) - 0.717 \quad (\text{A.5})$$

Bulk phase electron acceptor limited log(NADH/NAD) ratio

$$\log(NADH/NAD)' = 0.215 q_{md} \left(\frac{S'_{dh}}{K_{sd} + S'_{dh}} \right) \left(\frac{S'_{ab}}{K_{sa} + S'_{ab}} \right) - 0.717 \quad (\text{A.6})$$

BIOFILM SYSTEM - BULK PHASE

Bulk phase biofilm electron donor concentration

$$S'_{db}{}^{t+1} = S'_{db} + \left[\frac{Q}{V} (S_{dbi} - S'_{db}) - a k_{md} (S'_{db} - S'_{df}) \right] \Delta t \quad (\text{A.7})$$

Bulk phase biofilm electron acceptor concentration

$$S_{ab}^{j+1} = S_{ab}^j + \left[\frac{Q}{V} (S_{abi} - S_{ab}^j) + k_{La} (C_s - S_{ab}^j) - ak_{ma} (S_{ab}^j - S_{af}^{1,j}) \right] \Delta t \quad (\text{A.8})$$

Bulk phase biofilm secondary substrate concentration

$$S_{2b}^{j+1} = S_{2b}^j + \left[\frac{Q}{V} (S_{2bi} - S_{2b}^j) - ak_{m2} (S_{2b}^j - S_{2f}^{1,j}) \right] \Delta t \quad (\text{A.9})$$

The equations for the bulk phase biofilm log(NADH/NAD) ratios are identical to those presented for the suspended biomass system; Equations (A.5) and (A.6).

BIOFILM SYSTEM - BIOFILM LAYERS

Biofilm layer electron donor concentration - biofilm/liquid interface layer

$$S_{df}^{1,j+1} = S_{df}^{1,j} + \left[\frac{k_{md}}{z^{1,j}} (S_{ab}^j - S_{df}^{1,j}) - \frac{D_{df}}{z^{1,j}} \left\{ \frac{(S_{df}^{1,j} - S_{df}^{2,j})}{((z^{1,j} + z^{2,j})/2)} \right\} - q_{md} \left(\frac{S_{df}^{1,j}}{S_{df}^{1,j} + K_{sd}} \right) \right. \\ \left. \left(\frac{S_{df}^{1,j}}{S_{df}^{1,j} + K_{sa}} \right) f^{1,j} X_f \right] \Delta t \quad (\text{A.10})$$

Biofilm layer electron donor concentration - intermediate layers

$$S_{df}^{i,j+1} = S_{df}^{i,j} + \left[\frac{D_{df}}{z^{i,j}} \left\{ \frac{(S_{df}^{i-1,j} - S_{df}^{i,j})}{((z^{i-1,j} + z^{i,j})/2)} - \frac{(S_{df}^{i,j} - S_{df}^{i+1,j})}{((z^{i,j} + z^{i+1,j})/2)} \right\} - q_{md} \left(\frac{S_{df}^{i,j}}{S_{df}^{i,j} + K_{sd}} \right) \right. \\ \left. \left(\frac{S_{df}^{i,j}}{S_{df}^{i,j} + K_{sa}} \right) f^{i,j} X_f \right] \Delta t \quad (\text{A.11})$$

Biofilm layer electron donor concentration - biofilm/support interface layer

$$S_{df}^{j+1} = S_{df}^j + \left[\frac{D_{df}}{z^{1,j}} \left\{ \frac{(S_{df}^{i-1,j} - S_{df}^{i,j})}{((z^{i-1,j} + z^{i,j})/2)} \right\} - q_{md} \left(\frac{S_{df}^{i,j}}{S_{df}^{i,j} + K_{sd}} \right) \left(\frac{S_{df}^{i,j}}{S_{df}^{i,j} + K_{sa}} \right) f^{i,j} X_f \right] \Delta t \quad (\text{A.12})$$

Biofilm layer electron acceptor concentration - biofilm/liquid interface layer

$$S_{af}^{1,j+1} = S_{af}^{1,j} + \left[\frac{k_{ma}}{z^{1,j}} (S_{ab}^j - S_{af}^{1,j}) - \frac{D_{af}}{z^{1,j}} \left\{ \frac{(S_{af}^{1,j} - S_{af}^{2,j})}{((z^{1,j} + z^{2,j})/2)} \right\} - \{ q_{ma} \left(\frac{S_{af}^{1,j}}{S_{af}^{1,j} + K_{sd}} \right) \right. \\ \left. \left(\frac{S_{af}^{1,j}}{S_{af}^{1,j} + K_{sa}} \right) + v_{a/1} b \} f^{1,j} X_f \right] \Delta t \quad (\text{A.13})$$

Biofilm layer electron acceptor concentration - intermediate layers

$$S_{af}^{i,j+1} = S_{af}^{i,j} + \left[\frac{D_{af}}{z^{i,j}} \left\{ \frac{(S_{af}^{i-1,j} - S_{af}^{i,j})}{((z^{i-1,j} + z^{i,j})/2)} - \frac{(S_{af}^{i,j} - S_{af}^{i+1,j})}{((z^{i,j} + z^{i+1,j})/2)} \right\} - \left\{ q_{ma} \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{sd}} \right) \right. \right. \\ \left. \left. \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{sa}} \right) + v_{a/x} b \right\} f^{i,j} X_f \right] \Delta t \quad (\text{A.14})$$

Biofilm layer electron acceptor concentration - biofilm/support interface layer

$$S_{af}^{i,j+1} = S_{af}^{i,j} + \left[\frac{D_{af}}{z^{i,j}} \left\{ \frac{(S_{af}^{i-1,j} - S_{af}^{i,j})}{((z^{i-1,j} + z^{i,j})/2)} \right\} - \left\{ q_{ma} \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{sd}} \right) \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{sa}} \right) \right. \right. \\ \left. \left. + v_{a/x} b \right\} f^{i,j} X_f \right] \Delta t \quad (\text{A.15})$$

Biofilm layer secondary substrate concentration - biofilm/liquid interface layer

$$S_{2f}^{1,j+1} = S_{2f}^{1,j} + \left[\frac{k_{m2}}{z^{1,j}} (S_{2b}^j - S_{2f}^{1,j}) - \frac{D_{2f}}{z^{1,j}} \left\{ \frac{(S_{2f}^{1,j} - S_{2f}^{2,j})}{((z^{1,j} + z^{2,j})/2)} \right\} - q_{m2} \right. \\ \left. \{ \log(NADH/NAD)^{1,j} + M \} \left(\frac{S_{2f}^{1,j}}{S_{2f}^{1,j} + K_{s2}} \right) \left(\frac{S_{af}^{1,j}}{S_{af}^{1,j} + K_{so}} \right) f^{1,j} X_f \right] \Delta t \quad (\text{A.16})$$

Biofilm layer secondary substrate concentration - intermediate layers

$$S_{2f}^{i,j+1} = S_{2f}^{i,j} + \left[\frac{D_{2f}}{z^{i,j}} \left\{ \frac{(S_{2f}^{i-1,j} - S_{2f}^{i,j})}{((z^{i-1,j} + z^{i,j})/2)} - \frac{(S_{2f}^{i,j} - S_{2f}^{i+1,j})}{((z^{i,j} + z^{i+1,j})/2)} \right\} - q_{m2} \right. \\ \left. \{ \log(NADH/NAD)^{i,j} + M \} \left(\frac{S_{2f}^{i,j}}{S_{2f}^{i,j} + K_{s2}} \right) \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{so}} \right) f^{i,j} X_f \right] \Delta t \quad (\text{A.17})$$

Biofilm layer secondary substrate concentration - biofilm/support interface layer

$$S_{2f}^{i,j+1} = S_{2f}^{i,j} + \left[\frac{D_{2f}}{z^{i,j}} \left\{ \frac{(S_{2f}^{i-1,j} - S_{2f}^{i,j})}{((z^{i-1,j} + z^{i,j})/2)} \right\} - q_{m2} \{ \log(NADH/NAD)^{i,j} + M \} \right. \\ \left. \left(\frac{S_{2f}^{i,j}}{S_{2f}^{i,j} + K_{s2}} \right) \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{so}} \right) f^{i,j} X_f \right] \Delta t \quad (\text{A.18})$$

Biofilm layer growth

$$z^{i,j+1} = z^{i,j} + [f^{i,j} z^{i,j} (Yq_{md} - (1 - k_1)b - sl) - (1 - f^{i,j}) z^{i,j} sl] \Delta t \quad (\text{A.19})$$

Biofilm layer active fraction

$$f^{i,j+1} = \frac{f^{i,j} z^{i,j} + [f^{i,j} z^{i,j} (Yq_{md} - b - sl)] \Delta t}{z^{i,j} + [f^{i,j} z^{i,j} (Yq_{md} - (1 - k_1)b - sl) - (1 - f^{i,j}) z^{i,j} sl] \Delta t} \quad (\text{A.20})$$

Biofilm layer electron donor or dual substrate limited log(NADH/NAD) ratio

$$\log(NADH/NAD)^{i,j} = 0.273 q_{md} \left(\frac{S_{ab}^{i,j}}{K_{sd} + S_{ab}^{i,j}} \right) \left(\frac{S_{ab}^{i,j}}{K_{sa} + S_{ab}^{i,j}} \right) - 1.11 \left(\frac{S_{ab}^{i,j}}{K_{sa} + S_{ab}^{i,j}} \right) - 0.717 \quad (\text{A.21})$$

Biofilm layer electron acceptor limited $\log(\text{NADH}/\text{NAD})$ ratio

$$\log(\text{NADH} / \text{NAD})^{i,j} = 0.215 q_{md} \left(\frac{S_{db}^{i,j}}{K_{sd} + S_{db}^{i,j}} \right) \left(\frac{S_{ab}^{i,j}}{K_{sa} + S_{ab}^{i,j}} \right) - 0.717 \quad (\text{A.22})$$

BIOFILM SYSTEM - ITERATIVE TECHNIQUE

Bulk phase electron donor flux into biofilm

$$J_d' = k_{md} (S_{db}' - S_{df}^{1,j}) \quad (\text{A.23})$$

Bulk phase electron acceptor flux into biofilm

$$J_a' = k_{ma} (S_{ab}' - S_{af}^{1,j}) \quad (\text{A.24})$$

Bulk phase secondary substrate flux into biofilm

$$J_2' = k_{m2} (S_{2b}' - S_{2f}^{1,j}) \quad (\text{A.25})$$

Potential biofilm electron donor consumption rate

$$J_d' = q_{md} \left(\frac{S_{df}^{1,j}}{S_{df}^{1,j} + K_{sd}} \right) \left(\frac{S_{af}^{1,j}}{S_{af}^{1,j} + K_{sa}} \right) f^{1,j} X_f z^{1,j} + \sum_{i=2}^{nf} q_{mi} \left(\frac{S_{df}^{i,j}}{S_{df}^{i,j} + K_{sd}} \right) \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{sa}} \right) f^{i,j} X_f z^{i,j} \quad (\text{A.26})$$

Potential biofilm electron acceptor consumption rate

$$J_a' = \left\{ q_{ma} \left(\frac{S_{df}^{1,j}}{S_{df}^{1,j} + K_{sd}} \right) \left(\frac{S_{af}^{1,j}}{S_{af}^{1,j} + K_{sa}} \right) + v_{a,1} b \right\} f^{1,j} X_f z^{1,j} + \sum_{i=2}^{nf} \left\{ q_{mi} \left(\frac{S_{df}^{i,j}}{S_{df}^{i,j} + K_{sd}} \right) \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{sa}} \right) + v_{a,i} b \right\} f^{i,j} X_f z^{i,j} \quad (\text{A.27})$$

Potential biofilm secondary substrate consumption rate

$$J_2' = q_{m2} \{ \log(\text{NADH} / \text{NAD})^{i,j} + M \} \left(\frac{S_{2f}^{1,j}}{S_{2f}^{1,j} + K_{s2}} \right) \left(\frac{S_{af}^{1,j}}{S_{af}^{1,j} + K_{s0}} \right) f^{1,j} X_f z^{1,j} + \sum_{i=2}^{nf} q_{mi} \{ \log(\text{NADH} / \text{NAD})^{i,j} + M \} \left(\frac{S_{2f}^{i,j}}{S_{2f}^{i,j} + K_{s2}} \right) \left(\frac{S_{af}^{i,j}}{S_{af}^{i,j} + K_{s0}} \right) f^{i,j} X_f z^{i,j} \quad (\text{A.28})$$

APPENDIX B

SOURCE CODE

```
c
c Dual Substrate Cometabolic Biodegradation Code c
c Pascale Champagne-Hartley c
c April 1995 c
c Main Program c
c Variable Definition
    implicit real(a-h,k-m,q-z)
    implicit integer(i,j,n-p)
    ob=5000
    op=5000
    cl=0.999
    cu=1.001
    cd=-92
    ca=1.68
    cinc=0.02
    cdec=0.95
    cs=9.6
c -----
c File Statements
    open(7,file='injust.txt')
    open(8,file='scase.txt')
    open(9,file='fjjust.txt')
    open(10,file='fzjust.txt')
c -----
c Read Data Line 1
    nread=7
    read(nread,* )psb
c -----
c Suspended Vs. Biofilm Reactor
    if(psb.eq.1) then
        call biosus(op,cd,ca,cs,nread)
    else
        call biofil(ob,op,cl,cu,cd,ca,cinc,cdec,cs,nread)
    end if
c -----
c End of Main Program
end
```

```
c Biosub.c
c Biosus Subroutine c
c
```

```
subroutine biosus(op,cd,ca,cs,nread)
```

```

c -----
c Variable Definition
  implicit real(a-h,k-m,q-z)
  implicit integer(i,j,n-p)
  double precision sdb,sdbn,sab,sabn,s2b,s2bn,xs,xsn,lnhn
c -----
c Format Statements
1001 format(' ')
1101 format('qmd',2x,'qma',2x,'qm2',3x,'ksd',3x,'ksa',3x,'ks2',3x
1      'kso',3x,'vax',3x,'b',5x,'M',5x,'f',4x,'Y',4x,'kma',1x)
1102 format(f4.2,f5.2,f5.2,f6.3,f6.3,f6.3,f6.3,f5.2,f7.4,f6.3,f5.2
1      'f6.3,f5.2)
1105 format('mg',2x,'mg',2x,'mg',3x,'mgd',3x,'mga',3x,'mg2'
1      '3x,'mga',2x,'mga',2x,'1/h',8x,'fract',1x,'mgv',2x
1      'm/h',1x)
1106 format('mg-h',1x,'mg-h',1x,'mg-h',2x,'/l',4x,'/l',4x,'/l'
1      '4x,'/l',3x,'mgx',20x,'mga',7x)
1201 format(4x,'Q',5x,'V',4x,'db',5x,'Fa',5x,'vab',3x,'rab',4x,'Sdbi'
1      '2x,'Sabi',4x,'S2bi',6x,'Xi',3x)
1202 format(f7.1,f7.1,f6.3,f6.3,f7.1,f7.4,f7.1,f6.3,f10.6,f8.1)
1205 format(2x,'m^3/h',2x,'m^3',4x,'m',2x,'m^3/m^3',3x,'m/h',4x,'m'
1      '4x,'mgd/l',2x,'mga/l',3x,'mg2/l',3x,'mgx/l',2x)
1301 format(4x,'Time',9x,'Sdb',8x,'Sab',7x,'S2b',8x,'X',4x)
1302 format(f12.6,f12.6,f10.6,f10.6,f10.2)
1305 format(6x,'h',9x,'mgd/l',6x,'mga/l',5x,'mg2/l',5x,'mgx/l',2x)
c -----
c Data Input and Output File Setup
  write(8,*)'Suspended Biomass Reactor Output'
  read(nread,*)qmd,qma,qm2,ksd,ksa,ks2,kso,vax,b,m,f,y,kma
  write(8,1001)
  write(8,*)'Model Parameters'
  write(8,1101)
  write(8,1105)
  write(8,1106)
  write(8,1102)qmd,qma,qm2,ksd,ksa,ks2,kso,vax,b,m,f,y,kma
  read(nread,*)q,v,db,fa,vab,rab,sdbi,sabi,s2bi,xi
  write(8,1001)
  write(8,*)'Reactor Parameters'
  write(8,1201)
  write(8,1205)
  write(8,1202)q,v,db,fa,vab,rab,sdbi,sabi,s2bi,xi
  read(nread,*)sdbo,sabo,s2bo,xo
  tsr=0
  write(8,1001)
  write(8,*)'Simulation'
  write(8,1301)
  write(8,1305)
  write(8,1302)tsr,sdbo,sabo,s2bo,xo
  read(nread,*)h,tt
c -----
c Set Reactor Calculation Parameter Values
  nt=tt/h
  ntt=nt/op
  oh=1/h
  sdb=sdbo
  sab=sabo
  s2b=s2bo
  xs=xo
  poh=1

```

```

oj:=1
ts 168
c -----
c Reactor Concentrations Calculation Loop
do 200 i=1,ntt
  do 100 j=1,op
c Dual, Electron Donor or Electron Acceptor Limited Conditions
    if(sab.le.ca.and.sdb.gt.cd) then
      lnhn=0.215*(qmd*(sab/(ksa+sab))*(sdb/(ksd+sdb)))-0.717
    else
      lnhn=0.273*(qmd*(sab/(ksa+sab))*(sdb/(ksd+sdb)))-(1.11
+      *(sab/(ksa+sab)))-0.717
    end if
c New Condition Calculations Using Finite Difference Approach
    sdbn=sdb+(((q/v)*(sdbi-sdb))-(qmd*(sab/(ksa+sab))*(sdb
+      /(ksd+sdb))*f*xs))*h
    sabn=sab+(((q/v)*(sabi-sab))+(kma*(c1*c2*c3)/(vab
+      *rab))*(cs-sab))-((qma*(sa)/(ksa+sab))*(sdb
+      /(ksd+sdb))+vax*b)*f*xs))*h
    s2bn=s2b+(((q/v)*(s2bi-s2b))-(qm2*(s2b/(ks2+s2b))*(sab
+      /(kso+sab))*(lnhn+m)*f*xs))*h
    xsn=xs+(((q/v)*(xi-xs))+((v*qmd*(sab/(ksa+sab))*(sdb
+      /(ksd+sdb))-b)*f*xs))*h
    if(sdbn.lt.0) then
      sdbn=0
    end if
    if(sabn.lt.0) then
      sabn=0
    end if
    if(s2bn.lt.0) then
      s2bn=0
    end if
    if(xsn.lt.0) then
      xsn=0
    end if
c Reset Reactor Calculation Parameter Values
    sdb=sdbn
    sab=sabn
    s2b=s2bn
    xs=xsn
c Send Results to Screen
    if(poh.eq.oh) then
      tsh=(oh*h)*oj
      write(6,1001)
      write(6,1301)
      write(6,1305)
      write(6,1302)tsh,sdb,sab,s2b,xs
      oj=oj+1
      poh=0
    end if
c Increment Counter
    poh=poh+1
100  continue
    tsr=(op*h)*i
    if(tr.ge.ts) then
      kma=60
      sdbi=200
      v=4
    end if
c Send Results To Suspended Biomass File

```



```

read(nread,*)qmd,qma,qm2,ksd,ksa,ks2,kso,vax,b,m,xf,y
write(9,1003)
write(9,*)'Model Parameters'
write(9,1103)
write(9,1107)
write(9,1108)
write(9,1104)qmd,qma,qm2,ksd,ksa,ks2,kso,vax,b,m,xf,y
read(nread,*)ddf,daf,d2f,kmd,kma,km2,a,sl,kl
write(9,1003)
write(9,*)'Biofilm Parameters'
write(9,1403)
write(9,1407)
write(9,1404)ddf,daf,d2f,kmd,kma,km2,a,sl,kl
read(nread,*)q,v,db,fa,vab,rab,sdbi,sabi,s2bi
write(9,1003)
write(9,*)'Reactor Parameters'
write(9,1203)
write(9,1207)
write(9,1204)q,v,db,fa,vab,rab,sdbi,sabi,s2bi
read(nread,*)sdbo,sabo,s2bo,fo,lfo
tbr=0
write(9,1003)
write(9,*)'Simulation'
write(9,1303)
write(9,1307)
read(nread,*)h,tt,nf
lfao=(lfo/nf)*fo
write(9,1304)tbr,sdbo,sabo,s2bo,lfo,lfao

```

c

c Set Reactor Calculation Parameter Values

```

nt=tt/h
ntt=nt/op
oh=1/(h*10)
zo=lfo/nf
zao=(lfo/nf)*fo
zio=(lfo/nf)*(1-fo)
zl=0.000100
zm=0.00003
sdb=sdbo
sab=sabo
s2b=s2bo
tb=336

```

c

c Biofilm/Liquid Donor and Acceptor Concentration Iteration at Time

c t=0 Using Summation Technique

```

sdsn=sdb*cdec
sasn=sab*cdec
s2sn=s2b*cdec
900 fdo=nf*zo*(qmd*((sdsn/(ksd+sdsn))*(sasn/(ksa+sasn)))*xf*fo)
    lfao=nf*zo*((qma*((sdsn/(ksd+sdsn))*(sasn/(ksa+sasn)))+vax*b)*xf
    + *fo)
    sdbl=(fdo/kmd)+sdsn
    sabl=(lfao/kma)+sasn
    sdbd=1-((sdbl-sdb)/sdb)
    sabd=1-((sabl-sab)/sab)
    if(sdbd.lt.cl.and.sabd.lt.cl) then
        sdsn=cdec*sdsn
        sasn=cdec*sasn
        go to 900
    end if

```

```

      if(sdbd.lt.cl.and.sabd.ge.cl.and.sabd.le.cu) then
        sdsn=cdec*sdsn
        go to 900
      end if
      if(sdbd.ge.cl.and.sdbd.le.cu.and.sabd.lt.cl) then
        sasn=cdec*sasn
        go to 900
      end if
      if(sdbd.gt.cu.and.sabd.gt.cu) then
        sdsn=sdsn+cinc*sdsn
        sasn=sasn+cinc*sasn
        go to 900
      end if
      if(sdbd.gt.cu.and.sabd.lt.cl) then
        sdsn=sdsn+cinc*sdsn
        sasn=cdec*sasn
        go to 900
      end if
      if(sdbd.lt.cl.and.sabd.gt.cu) then
        sdsn=cdec*sdsn
        sasn=sasn+cinc*sasn
        go to 900
      end if
      if(sdbd.ge.cl.and.sdbd.le.cu.and.sabd.gt.cu) then
        sasn=sasn+cinc*sasn
        go to 900
      end if
      if(sdbd.gt.cu.and.sabd.ge.cl.and.sabd.le.cu) then
        sdsn=sdsn+cinc*sdsn
        go to 900
      end if
c -----
c Biofilm/Liquid Secondary Substrate Concentration Iteration at time
c t=0 Using Summation Technique
c
      if(sasn.le.ca.and.sdsn.gt.cd) then
        lnhn=0.215*(qmd*(sasn/(ksa+sasn))*(sdsn/(ksd+sdsn)))-0.717
      else
        lnhn=0.273*(qmd*(sasn/(ksa+sasn))*(sdsn/(ksd+sdsn)))-(1.11
+      *(sasn/(ksa+sasn)))-0.717
      end if
950 fl2o=nl*zo*(qm2*(lnhn+m)*((s2sn/(ks2+s2sn))*(sasn/(kso+sasn)))
+      *xf*fo)
      s2bl=(fl2o/km2)+s2sn
      s2bd=1-((s2bl-s2b)/s2b)
      if(s2bd.lt.cl) then
        s2sn=cdec*s2sn
        go to 950
      end if
      if(s2bd.gt.cu) then
        s2sn=s2sn+cinc*s2sn
        go to 950
      end if
c -----
c Send Results To Biofilm Layer File
      write(10,*)'Biofilm Concentration Profile at Time t=0'
      write(10,1503)
      write(10,1507)
      nff=0
      sdsnf=sdsn*1000
      sasnf=sasn*1000

```

```
s2snf=s2sn*1000
write(10,1504)nff,zo,fo,sdsnf,sasnf,s2snf,zao
```

```
c
c Set Biofilm Boundary Parameter Values
```

```
do 410 iii=1,nf
  fl(iii)=fo
  zl(iii)=zo
  za(iii)=zao
  zi(iii)=zio
  sdf(iii)=sdsn
  saf(iii)=sasn
  s2f(iii)=s2sn
```

```
410 continue
```

```
c
c Starting Time Loop
```

```
read(nread,*)ppp
pob=1
poh=1
oi=1
oj=1
lf=0
lfa=0
lfs=0
lfas=0
lfss=0
lfass=0
lft=0
lfat=0
lflt=0
lfatt=0
zd=0
zad=0
do 400 i=1,ntt
  do 300 j=1,op
```

```
c
c New Reactor Concentration Calculations
```

```
sdbn=sdb+((q/v)*(sdbi-sdb)-(a*kmd*(sdb-sdf(1))))*h
sabn=sab+((q/v)*(sabi-sab)+(((fa*db)/(vab
+      *rab))*(cs-sab))-(a*kma*(sab-saf(1))))*h
s2bn=s2b+((q/v)*(s2bi-s2b)-(a*km2*(s2b-s2f(1))))*h
if(sdbn.lt.0) then
  sdbn=0
end if
if(sabn.lt.0) then
  sabn=0
end if
if(s2bn.lt.0) then
  s2bn=0
end if
lf=0
lfa=0
lfs=0
lfas=0
lfss=0
lfass=0
lft=0
lfat=0
lflt=0
lfatt=0
zd=0
```

```

      zad=0
c -----
c   Subroutine for Condition Where Nf=1 Layer
      if(nf.eq.1) then
c   New Profile Conditions for Layer 1 - Biofilm/Liquid Donor and
c   Acceptor Concentration Iteration at time t=tbl
c   Separating Layers If They Will Increase by 100% Due to Growth
      zan(1)=za(1)+(za(1)*(y*qmd*(sdf(1)/(ksd+sdf(1))))
+      *(saf(1)/(ksa+saf(1))-b-sl))*h
      zin(1)=zi(1)+(za(1)*k1*b-zi(1)*sl)*h
      zn(1)=zan(1)+zin(1)
      if(zn(1).ge.zl) then
        z(2)=z(1)/2
        z(1)=z(1)/2
        za(2)=za(1)/2
        za(1)=za(1)/2
        zi(2)=zi(1)/2
        zi(1)=zi(1)/2
        f(2)=f(1)
        sdf(2)=sdf(1)
        saf(2)=saf(1)
        s2f(2)=s2f(1)
        nf=nf+1
c   New Profile Conditions for Layer 2 Using Finite Difference -
      sdfn(2)=sdf(2)+(((ddf/z(2))*((sdf(1)-sdf(2))/(z(1)
+      +z(2)/2)))-(qmd*(sdf(2)/(ksd+sdf(2)))*(saf(2)
+      /(ksa+saf(2)))*f(2)*xf))*h
      safn(2)=saf(2)+(((daf/z(2))*((saf(1)-saf(2))/(z(1)
+      +z(2)/2)))-((qma*(sdf(2)/(ksd+sdf(2)))*(saf(2)
+      /(ksa+saf(2)))+vax*b)*f(2)*xf))*h
      if(sdfn(2).lt.0) then
        sdfn(2)=0
      end if
      if(safn(2).lt.0) then
        safn(2)=0
      end if
c   Dual. Electron Donor or Electron Acceptor Limited Conditions
      if(saf(2).le.ca.and.sdf(2).gt.cd) then
        lnhn=0.215*(qmd*(saf(2)/(ksa+saf(2)))*(sdf(2)
+      /(ksd+sdf(2))))-0.717
      else
        lnhn=0.273*(qmd*(saf(2)/(ksa+saf(2)))*(sdf(2)
+      /(ksd+sdf(2))))-(1.11*(saf(2)/(ksa
+      +saf(2))))-0.717
      end if
      s2fn(2)=s2f(2)+(((d2f/z(2))*((s2f(1)-s2f(2))/(z(1)
+      +z(2)/2)))-(qm2*(lnhn+m)*(s2f(2)/(ks2+s2f(2)))
+      *(saf(2)/(kso+saf(2)))*f(2)*xf))*h
      if(s2fn(2).lt.0) then
        s2fn(2)=0
      end if
c   New Active Fraction and Layer 2 Thickness Calculations
      zan(2)=za(2)+(za(2)*(y*qmd*(sdf(2)/(ksd+sdf(2)))
+      *(saf(2)/(ksa+saf(2))-b-sl))*h
      zin(2)=zi(2)+(za(2)*k1*b-zi(2)*sl)*h
      zn(2)=zan(2)+zin(2)
      fn(2)=zan(2)/zn(2)
      if(zn(2).lt.0) then
        zn(2)=0
      end if

```



```

      if fn(2).lt.0) then
        fn(2)=0
      end if
      lftt=zn(2)
      lfatt=zan(2)
c New Active Fraction and Layer 1 Thickness Calculations
      zan(1)=za(1)+(za(1)*(y*qmd*(sdf(1)/(ksd+sdf(1))))
+      *(saf(1)/(ksa+saf(1)))-b-sl))*h
      zin(1)=zi(1)+(za(1)*k1*b-zi(1)*sl))*h
      zn(1)=zan(1)+zin(1)
      fn(1)=zan(1)/zn(1)
      if zn(1).lt.0) then
        zn(1)=0
      end if
      if fn(1).lt.0) then
        fn(1)=0
      end if
      lft=lftt+zn(1)
      lfat=lfatt+zan(1)
c New Profile Conditions of Layer 1
      if(ppp.eq.1) then
c Fully Explicit Method
        sdfn(1)=sdf(1)+(((kmd/z(1))*(sdb-sdf(1)))-((ddf
+          /z(1))*((sdf(1)-sdf(2))/((z(1)+z(2))
+          /2)))-(qmd*(sdf(1)/(ksd+sdf(1)))*(saf(1)
+          /(ksa+saf(1)))*xf*f(1)))*h
        safn(1)=saf(1)+(((kma/z(1))*(sab-saf(1)))-((daf
+          /z(1))*((saf(1)-saf(2))/((z(1)+z(2))
+          /2)))-(qma*(sdf(1)/(ksd+sdf(1))
+          *(saf(1)/(ksa+saf(1)))+vax*b)*xf*f(1))
+          *h
        if sdfn(1).lt.0) then
          sdfn(1)=0
        end if
        if safn(1).lt.0) then
          safn(1)=0
        end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
        if(saf(1).le.ca.and.sdf(1).gt.cd) then
          lnhn=0.215*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+          /(ksd+sdf(1)))-0.717
        else
          lnhn=0.273*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+          /(ksd+sdf(1)))-1.11*(saf(1)/(ksa
+          +saf(1)))-0.717
        end if
        s2fn(1)=s2f(1)+(((km2/z(1))*(s2b-s2f(1)))-((d2f
+          /z(1))*((s2f(1)-s2f(2))/((z(1)+z(2))
+          /2)))-(qm2*(lnhn+m)*(s2f(1)/(ks2
+          +s2f(1)))*(saf(1)/(kso+saf(1)))*xf
+          *f(1)))*h
        if s2fn(1).lt.0) then
          s2fn(1)=0
        end if
      else
c Summation Technique
        sdfn(1)=cdec*sdbn
        safn(1)=cdec*sabn
        s2fn(1)=cdec*s2bn
        fpd=0

```

```

fpa=0
fp2=0
ftd=0
fta=0
ft2=0
fjd(2)=zn(2)*qmd*((sdfn(2)/(ksd+sdfn(2)))
+      *(safn(2)/(ksa+safn(2))))*xf*fn(2)
fja(2)=zn(2)*(qma*((sdfn(2)/(ksd+sdfn(2)))
+      *(safn(2)/(ksa+safn(2))))+vax*b)*xf*fn(2)
fpd=fjd(2)
fpa=fja(2)
826   fsd=zn(1)*qmd*((sdfn(1)/(ksd+sdfn(1)))*(safn(1)
+      /(ksa+safn(1))))*xf*fn(1)
fsa=zn(1)*(qma*((sdfn(1)/(ksd+sdfn(1)))
+      *(safn(1)/(ksa+safn(1))))+vax*b)*xf*fn(1)
ftd=fpd+fsd
fta=fpa+fsa
sdbnl=sdfn(1)+(ftd/kmd)
sabnl=safn(1)+(fta/kma)
sdbnd=1-((sdbnl-sdbn)/sdbn)
sabnd=1-((sabnl-sabn)/sabn)
if(sdbnd.lt.cl.and.sabnd.lt.cl) then
    sdfn(1)=cdec*sdfn(1)
    safn(1)=cdec*safn(1)
    go to 826
end if
if(sdbnd.lt.cl.and.sabnd.ge.cl.and.sabnd.le.cu)
1   then
    sdfn(1)=cdec*sdfn(1)
    go to 826
end if
if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.lt.cl)
1   then
    safn(1)=cdec*safn(1)
    go to 826
end if
if(sdbnd.gt.cu.and.sabnd.gt.cu) then
    sdfn(1)=sdfn(1)+cinc*sdfn(1)
    safn(1)=safn(1)+cinc*safn(1)
    go to 826
end if
if(sdbnd.gt.cu.and.sabnd.lt.cl) then
    sdfn(1)=sdfn(1)+cinc*sdfn(1)
    safn(1)=cdec*safn(1)
    go to 826
end if
if(sdbnd.lt.cl.and.sabnd.gt.cu) then
    sdfn(1)=cdec*sdfn(1)
    safn(1)=safn(1)+cinc*safn(1)
    go to 826
end if
if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.gt.cu)
1   then
    safn(1)=safn(1)+cinc*safn(1)
    go to 826
end if
if(sdbnd.gt.cu.and.sabnd.ge.cl.and.sabnd.le.cu)
1   then
    sdfn(1)=sdfn(1)+cinc*sdfn(1)
    go to 826

```

```

        end if
        if(sdfn(1).lt.0) then
            sdfn(1)=0
        end if
        if(safn(1).lt.0) then
            safn(1)=0
        end if
c   Biofilm/Liquid Secondary Substrate Concentration Iteration at time
c   t=tbl Using Summation
        if(safn(2).le.ca.and.sdfn(2).gt.cd) then
            lnhn2(2)=0.215*(qmd*(safn(2)/(ksa+
+             safn(2)))*(sdfn(2)/(ksd+sdfn(2))))
+             -0.717
        else
            lnhn2(2)=0.273*(qmd*(safn(2)/(ksa+
+             safn(2)))*(sdfn(2)/(ksd+sdfn(2))))
+             -(1.11*(safn(2)/(ksa+safn(2))))
+             -0.717
        end if
        fj2(2)=zn(2)*qm2*xf*((s2fn(2)/(ks2
+             +s2fn(2)))*(safn(2)/(kso+safn(2)))
+             *fn(2)*(lnhn2(2)+m))
        fp2=fj2(2)
851    if(safn(1).le.ca.and.sdfn(1).gt.cd) then
            lnhn2(1)=0.215*(qmd*(safn(1)/(ksa+safn(1)))
+             *(sdfn(1)/(ksd+sdfn(1))))-0.717
        else
            lnhn2(1)=0.273*(qmd*(safn(1)/(ksa+safn(1)))
+             *(sdfn(1)/(ksd+sdfn(1))))-(1.11*(safn(1)
+             /(ksa+safn(1))))-0.717
        end if
        fs2=zn(1)*qm2*xf*((s2fn(1)/(ks2+s2fn(1)))
+             *(safn(1)/(kso+safn(1)))*fn(1)*(lnhn2(1)
+             +m))
        ft2=fp2+fs2
        s2bni=s2fn(1)+(ft2/km2)
        s2bnd=1-((s2bni-s2bn)/s2bn)
        if(s2bnd.lt.cl) then
            s2fn(1)=cdec*s2fn(1)
            go to 851
        end if
        if(s2bnd.gt.cu) then
            s2fn(1)=s2fn(1)+cinc*s2fn(1)
            go to 851
        end if
        if(s2fn(1).lt.0) then
            s2fn(1)=0
        end if
    end if
else
c   New Active Fraction and Layer 1 Thickness Calculations
        zan(1)=za(1)+(za(1)*(y*qmd*(sdf(1)/(ksd+sdf(1)))
+             *(saf(1)/(ksa+saf(1)))-b-sl))*h
        zin(1)=zi(1)+(za(1)*k1*b-zi(1)*sl)*h
        zn(1)=zan(1)+zin(1)
        fn(1)=zan(1)/zn(1)
        if(zn(1).lt.0) then
            zn(1)=0
        end if
        if(fn(1).lt.0) then

```

```

        fn(1)=0
    end if
    lft=zn(1)
    lfut=zan(1)
c New Profile Conditions of Layer 1
    if(ppp.eq.1) then
c Fully Explicit Method
        sdfn(1)=sdf(1)+(((kmd/z(1))*(sdb-sdf(1)))-((ddf
+           /z(1))*((sdf(1)-sdf(2))/(z(1)+z(2))
+           /2)))-(qmd*(sdf(1)/(ksd+sdf(1)))*(saf(1)
+           /(ksa+saf(1)))*xf*f(1)))*h
        safn(1)=saf(1)+(((kma/z(1))*(sab-saf(1)))-((daf
+           /z(1))*((saf(1)-saf(2))/(z(1)+z(2))
+           /2)))-(qma*(sdf(1)/(ksd+sdf(1))
+           *(saf(1)/(ksa+saf(1)))+vax*b)*xf*f(1))
+           *h
        if(sdfn(1).lt.0) then
            sdfn(1)=0
        end if
        if(safn(1).lt.0) then
            safn(1)=0
        end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
        if(saf(1).le.ca.and.sdf(1).gt.cd) then
            lnhn=0.215*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+           /(ksd+sdf(1)))-0.717
        else
            lnhn=0.273*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+           /(ksd+sdf(1)))-(1.11*(saf(1)/(ksa
+           +saf(1)))-0.717
        end if
        s2fn(1)=s2f(1)+(((km2/z(1))*(s2b-s2f(1)))-((d2f
+           /z(1))*((s2f(1)-s2f(2))/(z(1)+z(2))
+           /2)))-(qm2*(lnhn+m)*(s2f(1)/(ks2
+           +s2f(1)))*(saf(1)/(kso+saf(1)))*xf
+           *f(1)))*h
        if(s2fn(1).lt.0) then
            s2fn(1)=0
        end if
    else
c Summation Technique - Layer 1
        sdfn(1)=cdec*sdbn
        safn(1)=cdec*sabn
        s2fn(1)=cdec*s2bn
801        fsd=zn(1)*qmd*((sdfn(1)/(ksd+sdfn(1)))*(safn(1)
+           /(ksa+safn(1)))*xf*fn(1)
        fsa=zn(1)*(qma*((sdfn(1)/(ksd+sdfn(1))
+           *(safn(1)/(ksa+safn(1)))+vax*b)*xf*fn(1)
        ftd=fsd
        fta=fsa
        sdbnl=sdfn(1)+(ftd/kmd)
        sabnl=safn(1)+(fta/kma)
        sdbnd=1-(sdbnl-sdbn)/sdbn
        sabnd=1-(sabnl-sabn)/sabn
        if(sdbnd.lt.cl.and.sabnd.lt.cl) then
            sdfn(1)=cdec*sdfn(1)
            safn(1)=cdec*safn(1)
            go to 801
        end if
        if(sdbnd.lt.cl.and.sabnd.ge.cl.and.sabnd.le.cu)

```

```

1         then
          sdfn(1)=cdec*sdfn(1)
          go to 801
        end if
        if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.lt.cl)
1         then
          safn(1)=cdec*safn(1)
          go to 801
        end if
        if(sdbnd.gt.cu.and.sabnd.gt.cu) then
          sdfn(1)=sdfn(1)+cinc*sdfn(1)
          safn(1)=safn(1)+cinc*safn(1)
          go to 801
        end if
        if(sdbnd.gt.cu.and.sabnd.lt.cl) then
          sdfn(1)=sdfn(1)+cinc*sdfn(1)
          safn(1)=cdec*safn(1)
          go to 801
        end if
        if(sdbnd.lt.cl.and.sabnd.gt.cu) then
          sdfn(1)=cdec*sdfn(1)
          safn(1)=safn(1)+cinc*safn(1)
          go to 801
        end if
        if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.gt.cu)
1         then
          safn(1)=safn(1)+cinc*safn(1)
          go to 801
        end if
        if(sdbnd.gt.cu.and.sabnd.ge.cl.and.sabnd.le.cu)
1         then
          sdfn(1)=sdfn(1)+cinc*sdfn(1)
          go to 801
        end if
        if(sdfn(1).lt.0) then
          sdfn(1)=0
        end if
        if(safn(1).lt.0) then
          safn(1)=0
        end if
c  Biofilm/Liquid Secondary Substrate Concentration Iteration at time
c  t=tbl Using Summation
876      if(safn(1).le.ca.and.sdfn(1).gt.cd) then
          lnhn2(1)=0.215*(qmd*(safn(1)/(ksa+safn(1)))
+          *(sdfn(1)/(ksd+sdfn(1))))-0.717
        else
          lnhn2(1)=0.273*(qmd*(safn(1)/(ksa+safn(1)))
+          *(sdfn(1)/(ksd+sdfn(1))))-(1.11*(safn(1)
+          /(ksa+safn(1))))-0.717
        end if
        fs2=zn(1)*qm2*xf*((s2fn(1)/(ks2+s2fn(1)))
+          *(safn(1)/(kso+safn(1)))*fn(1)*(lnhn2(1)
+          +m))
        ft2=fs2
        s2bnl=s2fn(1)+(ft2/km2)
        s2bnd=1-((s2bnl-s2bn)/s2bn)
        if(s2bnd.lt.cl) then
          s2fn(1)=cdec*s2fn(1)
          go to 876
        end if

```

```

      if(s2bnd.gt.cu) then
        s2fn(1)=s2fn(1)+cinc*s2fn(1)
        go to 876
      end if
      if(s2fn(1).lt.0) then
        s2fn(1)=0
      end if
    end if
  end if
  go to 333
end if

c -----
c Subroutine for Condition Where Nf=2 Layers
  if(nf.eq.2) then
c New Profile Conditions for Layer Nf
c Separating Layers If They Will Increase by 100% Due to Growth
    zan(nf)=za(nf)+(za(nf)*(y*qmd*(sdf(nf)/(ksd+sdf(nf))))
+      *(saf(nf)/(ksa+saf(nf)))-b-sl))*h
    zin(nf)=zi(nf)+(za(nf)*k1*b-zi(nf)*sl)*h
    zn(nf)=zan(nf)+zin(nf)
    if(zn(nf).ge.zl) then
      z(nf+1)=z(nf)/2
      z(nf)=z(nf)/2
      za(nf+1)=za(nf)/2
      za(nf)=za(nf)/2
      zi(nf+1)=zi(nf)/2
      zi(nf)=zi(nf)/2
      f(nf+1)=f(nf)
      sdf(nf+1)=sdf(nf)
      saf(nf+1)=saf(nf)
      s2f(nf+1)=s2f(nf)
c New Profile Conditions for Layer Nf Using Finite Difference -
      sdf(nf)=sdf(nf)+(((ddf/z(nf))*(((sdf(nf-1)-sdf(nf))
+      /((z(nf-1)+z(nf))/2))-((sdf(nf)-sdf(nf+1))
+      /((z(nf)+z(nf+1))/2))))-(qmd*(sdf(nf)/(ksd
+      +sdf(nf)))*(saf(nf)/(ksa+saf(nf)))*f(nf)*xf))*h
      saf(nf)=saf(nf)+(((daf/z(nf))*(((saf(nf-1)-saf(nf))
+      /((z(nf-1)+z(nf))/2))-((saf(nf)-saf(nf+1))
+      /((z(nf)+z(nf+1))/2))))-(qma*(sdf(nf)/(ksd
+      +sdf(nf)))*(saf(nf)/(ksa+saf(nf)))+vax*b)*f(nf)
+      *xf))*h
      if(sdf(nf).lt.0) then
        sdf(nf)=0
      end if
      if(saf(nf).lt.0) then
        saf(nf)=0
      end if
c Dual. Electron Donor or Electron Acceptor Limited Conditions
      if(saf(nf).le.ca.and.sdf(nf).gt.cd) then
        lnhn=0.215*(qmd*(saf(nf)/(ksa+saf(nf)))*(sdf(nf)
+      /(ksd+sdf(nf)))-0.717
      else
        lnhn=0.273*(qmd*(saf(nf)/(ksa+saf(nf)))*(sdf(nf)
+      /(ksd+sdf(nf)))-(1.11*(saf(nf)/(ksa
+      +saf(nf)))-0.717
      end if
      s2fn(nf)=s2f(nf)+(((d2f/z(nf))*(((s2f(nf-1)-s2f(nf))
+      /((z(nf-1)+z(nf))/2))-((s2f(nf)-s2f(nf+1))
+      /((z(nf)+z(nf+1))/2))))-(qm2*(lnhn+m)*(s2f(nf)
+      /(ks2+s2f(nf)))*(saf(nf)/(kso+saf(nf)))*f(nf)

```

```

+      *xf))*h
      if(s2fn(nf).lt.0) then
        s2fn(nf)=0
      end if
c New Active Fraction and Layer Nf Thickness Calculations
      zan(nf)=za(nf)+(za(nf)*(y*qmd*(sdf(nf)/(ksd
+      +sdf(nf)))*(saf(nf)/(ksa+saf(nf))-b-sl))*h
      zin(nf)=zi(nf)+(za(nf)*k1*b-zi(nf)*sl)*h
      zn(nf)=zan(nf)+zin(nf)
      fn(nf)=zan(nf)/zn(nf)
      if(zn(nf).lt.0) then
        zn(nf)=0
      end if
      if(fn(nf).lt.0) then
        fn(nf)=0
      end if
      lfs=zn(nf)
      lfass=zan(nf)
c New Profile Conditions for Layer Nf+1 Using Modified Finite Difference
      sdfn(nf+1)=sdf(nf+1)+(((ddf/z(nf+1))*((sdf(nf)
+      -sdf(nf+1))/(z(nf)+z(nf+1)/2)))-(qmd
+      *(sdf(nf+1)/(ksd+sdf(nf+1)))*(saf(nf+1)/(ksa
+      +saf(nf+1)))*f(nf+1)*xf))*h
      safn(nf+1)=saf(nf+1)+(((daf/z(nf+1))*((saf(nf)
+      -saf(nf+1))/(z(nf)+z(nf+1)/2)))-(qma
+      *(sdf(nf+1)/(ksd+sdf(nf+1)))*(saf(nf+1)/(ksa
+      +saf(nf+1)))+vax*b)*f(nf+1)*xf))*h
      if(sdfn(nf+1).lt.0) then
        sdfn(nf+1)=0
      end if
      if(safn(nf+1).lt.0) then
        safn(nf+1)=0
      end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
      if(saf(nf+1).le.ca.and.sdf(nf+1).gt.cd) then
        lnhn=0.215*(qmd*(saf(nf+1)/(ksa+saf(nf+1)))
+      *(sdf(nf+1)/(ksd+sdf(nf+1)))-0.717
      else
        lnhn=0.273*(qmd*(saf(nf+1)/(ksa+saf(nf+1)))
+      *(sdf(nf+1)/(ksd+sdf(nf+1)))-1.11
+      *(saf(nf+1)/(ksa+saf(nf+1)))-0.717
      end if
      s2fn(nf+1)=s2f(nf+1)+(((d2f/z(nf+1))*((s2f(nf)
+      -s2f(nf+1))/(z(nf)+z(nf+1)/2)))-(qm2*(lnhn
+      +m)*(s2f(nf+1)/(ks2+s2f(nf+1)))*(saf(nf+1)
+      /(kso+saf(nf+1)))*f(nf+1)*xf))*h
      if(s2fn(nf+1).lt.0) then
        s2fn(nf+1)=0
      end if
c New Active Fraction and Layer Nf+1 Thickness Calculations
      zan(nf+1)=za(nf+1)+(za(nf+1)*(y*qmd*(sdf(nf+1)/(ksd
+      +sdf(nf+1)))*(saf(nf+1)/(ksa+saf(nf+1))-b-sl))*h
+      *h
      zin(nf+1)=zi(nf+1)+(za(nf+1)*k1*b-zi(nf+1)*sl)*h
      zn(nf+1)=zan(nf+1)+zin(nf+1)
      fn(nf+1)=zan(nf+1)/zn(nf+1)
      if(zn(nf+1).lt.0) then
        zn(nf+1)=0
      end if
      if(fn(nf+1).lt.0) then

```

```

        tn(nf+1)=0
    end if
    lfs=lfs+zn(nf+1)
    lfas=lfas+zan(nf+1)
    nf=nf+1
else
c New Profile Conditions for Layer Nf Using Modified Finite Difference -
    sdfn(nf)=sdfn(nf)+(((ddf/z(nf))*((sdf(nf-1)-sdf(nf))
+      /((z(nf-1)+z(nf))/2)))-(qmd*(sdf(nf)/(ksd
+      +sdf(nf)))*(saf(nf)/(ksa+saf(nf)))*f(nf)*xf))*h
    safn(nf)=safn(nf)+(((daf/z(nf))*((saf(nf-1)-saf(nf))
+      /((z(nf-1)+z(nf))/2)))-(qma*(sdf(nf)/(ksd
+      +sdf(nf)))*(saf(nf)/(ksa+saf(nf)))+vax*b)*f(nf)
+      *xf))*h
    if sdfn(nf).lt.0 then
        sdfn(nf)=0
    end if
    if safn(nf).lt.0 then
        safn(nf)=0
    end if
c Dual. Electron Donor or Electron Acceptor Limited Conditions
    if (saf(nf).le.ca.and.sdf(nf).gt.cd) then
        lnhn=0.215*(qmd*(saf(nf)/(ksa+saf(nf)))*(sdf(nf)
+      /(ksd+sdf(nf)))-0.717
    else
        lnhn=0.273*(qmd*(saf(nf)/(ksa+saf(nf)))*(sdf(nf)
+      /(ksd+sdf(nf)))-(1.11*(saf(nf)/(ksa
+      +saf(nf)))-0.717
    end if
    s2fn(nf)=s2fn(nf)+(((d2f/z(nf))*((s2f(nf-1)-s2f(nf))
+      /((z(nf-1)+z(nf))/2)))-(qm2*(lnhn+m)*(s2f(nf)
+      /(ks2+s2f(nf)))*(saf(nf)/(kso+saf(nf)))*f(nf)
+      *xf))*h
    if s2fn(nf).lt.0 then
        s2fn(nf)=0
    end if
c New Active Fraction and Layer Nf Thickness Calculations
    zan(nf)=zan(nf)+(za(nf)*(y*qmd*(sdf(nf)/(ksd
+      +sdf(nf)))*(saf(nf)/(ksa+saf(nf)))-b-sl))*h
    zin(nf)=zi(nf)+(za(nf)*k1*b-zi(nf)*sl)*h
    zn(nf)=zan(nf)+zin(nf)
    tn(nf)=zan(nf)/zn(nf)
    if zn(nf).lt.0 then
        zn(nf)=0
    end if
    if tn(nf).lt.0 then
        tn(nf)=0
    end if
    lfs=zn(nf)
    lfas=zan(nf)
end if
c New Profile Conditions for Layer 1 - Biofilm/Liquid Donor and
c Acceptor Concentration Iteration at time t=tbl
c Separating Layers If They Will Increase by 100% Due to Growth
    zan(1)=za(1)+(za(1)*(y*qmd*(sdf(1)/(ksd+sdf(1))
+      *(saf(1)/(ksa+saf(1)))-b-sl))*h
    zin(1)=zi(1)+(za(1)*k1*b-zi(1)*sl)*h
    zn(1)=zan(1)+zin(1)
    if zn(1).ge.zl then
        do 652 jz=nf,2,-1

```



```

      z(jz+1)=z(jz)
      zn(jz+1)=zn(jz)
      za(jz+1)=za(jz)
      zan(jz+1)=zan(jz)
      zi(jz+1)=zi(jz)
      zin(jz+1)=zin(jz)
      fl(jz+1)=fl(jz)
      fn(jz+1)=fn(jz)
      sdf(jz+1)=sdf(jz)
      sdn(jz+1)=sdn(jz)
      saf(jz+1)=saf(jz)
      safn(jz+1)=safn(jz)
      s2f(jz+1)=s2f(jz)
      s2fn(jz+1)=s2fn(jz)
652    continue
      z(2)=z(1)/2
      zn(2)=zn(1)/2
      za(2)=za(1)/2
      zan(2)=zan(1)/2
      zi(2)=zi(1)/2
      zin(2)=zin(1)/2
      fl(2)=fl(1)
      sdn(2)=sdn(1)
      saf(2)=saf(1)
      s2f(2)=s2f(1)
      nf=nf+1
c New Profile Conditions for Layer 2 Using Finite Difference -
      sdn(2)=sdn(2)+(((ddf/z(2))*(((sdf(1)-sdf(2))/(z(1)
+      +z(2))/2))-((sdf(2)-sdf(3))/(z(2)+z(3))/2))))
+      -(qmd*(sdf(2)/(ksd+sdf(2)))*(saf(2)/(ksa
+      +saf(2)))*f(2)*xh)*h
      safn(2)=safn(2)+(((daf/z(2))*(((saf(1)-saf(2))/(z(1)
+      +z(2))/2))-((saf(2)-saf(3))/(z(2)+z(3))/2))))
+      -(qma*(sdf(2)/(ksd+sdf(2)))*(saf(2)/(ksa
+      +saf(2)))+vax*b)*f(2)*xf)*h
      if(sdn(2).lt.0) then
        sdn(2)=0
      end if
      if(safn(2).lt.0) then
        safn(2)=0
      end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
      if(saf(2).le.ca.and.sdf(2).gt.cd) then
        lnfn=0.215*(qmd*(saf(2)/(ksa+saf(2)))*(sdf(2)
+      /(ksd+sdf(2)))-0.717
      else
        lnfn=0.273*(qmd*(saf(2)/(ksa+saf(2)))*(sdf(2)
+      /(ksd+sdf(2)))-1.11*(saf(2)/(ksa
+      +saf(2)))-0.717
      end if
      s2fn(2)=s2fn(2)+(((d2f/z(2))*(((s2f(1)-s2f(2))/(z(1)
+      +z(2))/2))-((s2f(2)-s2f(3))/(z(2)+z(3))/2))))
+      -(qm2*(lnfn+m)*(s2f(2)/(ks2+s2f(2)))*(saf(2)
+      /(kso+saf(2)))*f(2)*xh)*h
      if(s2fn(2).lt.0) then
        s2fn(2)=0
      end if
c New Active Fraction and Layer 2 Thickness Calculations
      zan(2)=zan(2)+(za(2)*(y*qmd*(sdf(2)/(ksd+sdf(2))
+      *(saf(2)/(ksa+saf(2)))-b-sl))*h

```

```

zin(2)=zi(2)+(za(2)*k1*b-zi(2)*sl)*h
zn(2)=zan(2)+zin(2)
fn(2)=zan(2)/zn(2)
if(zn(2).lt.0) then
    zn(2)=0
end if
if(fn(2).lt.0) then
    fn(2)=0
end if
lft=lfs+zn(2)
lfat=lfas+zan(2)
c New Active Fraction and Layer 1 Thickness Calculations
zan(1)=za(1)+(za(1)*(y*qmd*(sdf(1)/(ksd+sdf(1))))
+      *(saf(1)/(ksa+saf(1)))-b-sl))*h
zin(1)=zi(1)+(za(1)*k1*b-zi(1)*sl)*h
zn(1)=zan(1)+zin(1)
fn(1)=zan(1)/zn(1)
if(zn(1).lt.0) then
    zn(1)=0
end if
if(fn(1).lt.0) then
    fn(1)=0
end if
lft=lft+zn(1)
lfat=lfat+zan(1)
c New Profile Conditions of Layer 1
if(ppp.eq.1) then
c Fully Explicit Method
    sdfn(1)=sdf(1)+(((kmd/z(1))*(sdb-sdf(1)))-((ddf
+      /z(1))*((sdf(1)-sdf(2))/(z(1)+z(2))
+      /2)))-(qmd*(sdf(1)/(ksd+sdf(1)))*(saf(1)
+      /(ksa+saf(1)))*xf*f(1))*h
    safn(1)=saf(1)+(((kma/z(1))*(sab-saf(1)))-((daf
+      /z(1))*((saf(1)-saf(2))/(z(1)+z(2))
+      /2)))-(qma*(sdf(1)/(ksd+sdf(1))
+      *(saf(1)/(ksa+saf(1)))+vax*b)*xf*f(1))
+      *h
    if(sdfn(1).lt.0) then
        sdfn(1)=0
    end if
    if(safn(1).lt.0) then
        safn(1)=0
    end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
    if(saf(1).le.ca.and.sdf(1).gt.cd) then
        lnhn=0.215*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+      /(ksd+sdf(1))))-0.717
    else
        lnhn=0.273*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+      /(ksd+sdf(1))))-(1.11*(saf(1)/(ksa
+      +saf(1))))-0.717
    end if
    s2fn(1)=s2f(1)+(((km2/z(1))*(s2b-s2f(1)))-((d2f
+      /z(1))*((s2f(1)-s2f(2))/(z(1)+z(2))
+      /2)))-(qm2*(lnhn+m)*(s2f(1)/(ks2
+      +s2f(1)))*(saf(1)/(ksa+saf(1)))*xf
+      *f(1))*h
    if(s2fn(1).lt.0) then
        s2fn(1)=0
    end if

```

```

else
c Summation Technique
sdfn(1)=cdec*sdbn
safn(1)=cdec*sabn
s2fn(1)=cdec*s2bn
fpd=0
fpa=0
fp2=0
ftd=0
fta=0
ft2=0
do 627 jj=2,nf
    fjd(jj)=zn(jj)*qmd*((sdfn(jj)/(ksd+sdfn(jj)))
+      *(safn(jj)/(ksa+safn(jj))))*xf*fn(jj)
    fja(jj)=zn(jj)*(qma*((sdfn(jj)/(ksd+sdfn(jj)))
+      *(safn(jj)/(ksa+safn(jj))))+vax*b)*xf
+      *fn(jj)
    fpd=fpd+fjd(jj)
    fpa=fpa+fja(jj)
627 continue
827 ffsd=zn(1)*qmd*((sdfn(1)/(ksd+sdfn(1)))*(safn(1)
+      /(ksa+safn(1))))*xf*fn(1)
    ffsa=zn(1)*(qma*((sdfn(1)/(ksd+sdfn(1)))
+      *(safn(1)/(ksa+safn(1))))+vax*b)*xf*fn(1)
    ftd=fpd+ffsd
    fta=fpa+ffsa
    sdbnl=sdfn(1)+(ftd/kmd)
    sabnl=safn(1)+(fta/kma)
    sdbnd=1-((sdbnl-sdbn)/sdbn)
    sabnd=1-((sabnl-sabn)/sabn)
    if(sdbnd.lt.cl.and.sabnd.lt.cl) then
        sdfn(1)=cdec*sdfn(1)
        safn(1)=cdec*safn(1)
        go to 827
    end if
    if(sdbnd.lt.cl.and.sabnd.ge.cl.and.sabnd.le.cu)
1      then
        sdfn(1)=cdec*sdfn(1)
        go to 827
    end if
    if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.lt.cl)
1      then
        safn(1)=cdec*safn(1)
        go to 827
    end if
    if(sdbnd.gt.cu.and.sabnd.gt.cu) then
        sdfn(1)=sdfn(1)+cinc*sdfn(1)
        safn(1)=safn(1)+cinc*safn(1)
        go to 827
    end if
    if(sdbnd.gt.cu.and.sabnd.lt.cl) then
        sdfn(1)=sdfn(1)+cinc*sdfn(1)
        safn(1)=cdec*safn(1)
        go to 827
    end if
    if(sdbnd.lt.cl.and.sabnd.gt.cu) then
        sdfn(1)=cdec*sdfn(1)
        safn(1)=safn(1)+cinc*safn(1)
        go to 827
    end if

```

```

      if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.gt.cu)
1      then
        safn(1)=safn(1)+cinc*safn(1)
        go to 827
      end if
      if(sdbnd.gt.cu.and.sabnd.ge.cl.and.sabnd.le.cu)
1      then
        sdfn(1)=sdfn(1)+cinc*sdfn(1)
        go to 827
      end if
      if(sdfn(1).lt.0) then
        sdfn(1)=0
      end if
      if(safn(1).lt.0) then
        safn(1)=0
      end if
c Biofilm/Liquid Secondary Substrate Concentration Iteration at time
c t=tbl Using Summation
do 727 j=2,nf
  if(safn(j).le.ca.and.sdfn(j).gt.cd) then
    lnhn2(j)=0.215*(qmd*(safn(j)/(ksa+
+ safn(j)))*(sdfn(j)/(ksd+sdfn(j))))
+ -0.717
  else
    lnhn2(j)=0.273*(qmd*(safn(j)/(ksa+
+ safn(j)))*(sdfn(j)/(ksd+sdfn(j))))
+ -(1.11*(safn(j)/(ksa+safn(j))))
+ -0.717
  end if
  fj2(j)=zn(j)*qm2*xf*((s2fn(j)/(ks2
+ s2fn(j)))*(safn(j)/(kso+safn(j)))
+ *fn(j)*(lnhn2(j)+m))
  fp2=fp2+fj2(j)
727 continue
852 if(safn(1).le.ca.and.sdfn(1).gt.cd) then
  lnhn2(1)=0.215*(qmd*(safn(1)/(ksa+safn(1)))
+ *(sdfn(1)/(ksd+sdfn(1))))-0.717
  else
    lnhn2(1)=0.273*(qmd*(safn(1)/(ksa+safn(1)))
+ *(sdfn(1)/(ksd+sdfn(1))))-(1.11*(safn(1)
+ /(ksa+safn(1))))-0.717
  end if
  fs2=zn(1)*qm2*xf*((s2fn(1)/(ks2+s2fn(1)))
+ *(safn(1)/(kso+safn(1))) *fn(1)*(lnhn2(1)
+ +m))
  ft2=fp2+fs2
  s2bnl=s2fn(1)+(ft2/kn2)
  s2bnd=1-((s2bnl-s2bn)/s2bn)
  if(s2bnd.lt.cl) then
    s2fn(1)=cdec*s2fn(1)
    go to 852
  end if
  if(s2bnd.gt.cu) then
    s2fn(1)=s2fn(1)+cinc*s2fn(1)
    go to 852
  end if
  if(s2fn(1).lt.0) then
    s2fn(1)=0
  end if
end if
end if

```

```

else
c New Active Fraction and Layer 1 Thickness Calculations
  zan(1)=za(1)+(za(1)*(y*qmd*(sdf(1)/(ksd+sdf(1))))
+   *(saf(1)/(ksa+saf(1)))-b-sl))*h
  zin(1)=zi(1)+(za(1)*k1*b-zi(1)*sl)*h
  zn(1)=zan(1)+zin(1)
  fn(1)=zan(1)/zn(1)
  if zn(1).lt.0 then
    zn(1)=0
  end if
  if fn(1).lt.0 then
    fn(1)=0
  end if
  lft=lfs+zn(1)
  lfat=lfas+zan(1)
c New Profile Conditions of Layer 1
  if ppp.eq.1 then
c Fully Explicit Method
    sdfn(1)=sdf(1)+(((kmd/z(1))*(sdb-sdf(1)))-((ddf
+   /z(1))*((sdf(1)-sdf(2))/((z(1)+z(2))
+   /2)))-(qmd*(sdf(1)/(ksd+sdf(1)))*(saf(1)
+   /(ksa+saf(1)))*xf(1)))*h
    safn(1)=saf(1)+(((kma/z(1))*(sab-saf(1)))-((daf
+   /z(1))*((saf(1)-saf(2))/((z(1)+z(2))
+   /2)))-(qma*(sdf(1)/(ksd+sdf(1))
+   *(saf(1)/(ksa+saf(1)))+vax*b)*xf(1))
+   *h
    if sdfn(1).lt.0 then
      sdfn(1)=0
    end if
    if safn(1).lt.0 then
      safn(1)=0
    end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
    if (saf(1).le.ca.and.sdf(1).gt.cd) then
      lnhn=0.215*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+   /(ksd+sdf(1)))-0.717
    else
      lnhn=0.273*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+   /(ksd+sdf(1)))-1.11*(saf(1)/(ksa
+   +saf(1)))-0.717
    end if
    s2fn(1)=s2f(1)+(((km2/z(1))*(s2b-s2f(1)))-((d2f
+   /z(1))*((s2f(1)-s2f(2))/((z(1)+z(2))
+   /2)))-(qm2*(lnhn+m)*(s2f(1)/(ks2
+   +s2f(1)))*(saf(1)/(kso+saf(1)))*xf
+   *f(1)))*h
    if s2fn(1).lt.0 then
      s2fn(1)=0
    end if
else
c Summation Technique - Layer 1
  sdfn(1)=cdec*sdbn
  safn(1)=cdec*sabn
  s2fn(1)=cdec*s2bn
  fpd=0
  fpa=0
  fp2=0
  fld=0
  fla=0

```

```

      fl2=0
      do 677 jj=2,nf
        fjd(jj)=zn(jj)*qmd*((sdfn(jj)/(ksd+sdfn(jj)))
+         *(safn(jj)/(ksa+safn(jj))))*xf*fn(jj)
        fja(jj)=zn(jj)*(qma*((sdfn(jj)/(ksd+sdfn(jj)))
+         *(safn(jj)/(ksa+safn(jj))))+vax*b)*xf
+         *fn(jj)
        fjd=fjd+fjd(jj)
        fja=fja+fja(jj)
677      continue
802      fsd=zn(1)*qmd*((sdfn(1)/(ksd+sdfn(1)))*(safn(1)
+         /(ksa+safn(1))))*xf*fn(1)
      fsa=zn(1)*(qma*((sdfn(1)/(ksd+sdfn(1)))
+         *(safn(1)/(ksa+safn(1))))+vax*b)*xf*fn(1)
      fld=fjd+fsd
      fta=fja+fsa
      sdbnl=sdfn(1)+(fld/kmd)
      sabnl=safn(1)+(fta/kma)
      sdbnd=1-((sdbnl-sdbn)/sdbn)
      sabnd=1-((sabnl-sabn)/sabn)
      if(sdbnd.lt.cl.and.sabnd.lt.cl) then
        sdfn(1)=cdec*sdfn(1)
        safn(1)=cdec*safn(1)
        go to 802
      end if
      if(sdbnd.lt.cl.and.sabnd.ge.cl.and.sabnd.le.cu)
1        then
          sdfn(1)=cdec*sdfn(1)
          go to 802
        end if
      if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.lt.cl)
1        then
          safn(1)=cdec*safn(1)
          go to 802
        end if
      if(sdbnd.gt.cu.and.sabnd.gt.cu) then
        sdfn(1)=sdfn(1)+cinc*sdfn(1)
        safn(1)=safn(1)+cinc*safn(1)
        go to 802
      end if
      if(sdbnd.gt.cu.and.sabnd.lt.cl) then
        sdfn(1)=sdfn(1)+cinc*sdfn(1)
        safn(1)=cdec*safn(1)
        go to 802
      end if
      if(sdbnd.lt.cl.and.sabnd.gt.cu) then
        sdfn(1)=cdec*sdfn(1)
        safn(1)=safn(1)+cinc*safn(1)
        go to 802
      end if
      if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.gt.cu)
1        then
          safn(1)=safn(1)+cinc*safn(1)
          go to 802
        end if
      if(sdbnd.gt.cu.and.sabnd.ge.cl.and.sabnd.le.cu)
1        then
          sdfn(1)=sdfn(1)+cinc*sdfn(1)
          go to 802
        end if

```

```

      if(sdfn(1).lt.0) then
        sdfn(1)=0
      end if
      if(safn(1).lt.0) then
        safn(1)=0
      end if
c   Biofilm/Liquid Secondary Substrate Concentration Iteration at time
c   t=tbl Using Summation
      do 777 jji=2,nf
        if(safn(jji).le.ca.and.sdfn(jji).gt.cd) then
          lnhn2(jji)=0.215*(qmd*(safn(jji)/(ksa+
+          safn(jji)))*(sdfn(jji)/(ksd+
+          sdfn(jji)))-0.717
        else
          lnhn2(jji)=0.273*(qmd*(safn(jji)/(ksa+
+          safn(jji)))*(sdfn(jji)/(ksd+sdfn(jji)))
+          -(1.11*(safn(jji)/(ksa+safn(jji))))
+          -0.717
        end if
        fj2(jji)=zn(jji)*qm2*xf*((s2fn(jji)/(ks2
+          +s2fn(jji)))*(safn(jji)/(kso+safn(jji)))
+          *fn(jji)*(lnhn2(jji)+m))
        fp2=fp2+fj2(jji)
777      continue
877      if(safn(1).le.ca.and.sdfn(1).gt.cd) then
        lnhn2(1)=0.215*(qmd*(safn(1)/(ksa+safn(1)))
+          *(sdfn(1)/(ksd+sdfn(1)))-0.717
      else
        lnhn2(1)=0.273*(qmd*(safn(1)/(ksa+safn(1)))
+          *(sdfn(1)/(ksd+sdfn(1)))-1.11*(safn(1)
+          /(ksa+safn(1)))-0.717
      end if
      fs2=zn(1)*qm2*xf*((s2fn(1)/(ks2+s2fn(1)))
+          *(safn(1)/(kso+safn(1)))*fn(1)*(lnhn2(1)
+          +m))
      ft2=fp2+fs2
      s2bnl=s2fn(1)+(ft2/km2)
      s2bnd=1-((s2bnl-s2bn)/s2bn)
      if(s2bnd.lt.cl) then
        s2fn(1)=cdec*s2fn(1)
        go to 877
      end if
      if(s2bnd.gt.cu) then
        s2fn(1)=s2fn(1)+cinc*s2fn(1)
        go to 877
      end if
      if(s2fn(1).lt.0) then
        s2fn(1)=0
      end if
    end if
  end if
  end if
  go to 333
end if

```

c Subroutine for Condition Where Nf>2 Layers

c New Profile Conditions for Layer Nf

c Separating or Joining Layers Due to Growth or Decay

```

      zan(nf)=za(nf)+(za(nf)*(y*qmd*(sdf(nf)/(ksd+sdf(nf)))
+          *(saf(nf)/(ksa+saf(nf)))-b-sl))*h

```

$$z(n) = z(n) + (z(n) * k1 * b - z(n) * sl) * h$$

$$zn(n) = zan(n) + zin(n)$$

c -----
 c Joining Layers Nf & Nf-1
 if zn(nf).le.zn then
 $f(nf-1) = (za(nf) + za(nf-1)) / (z(nf) + z(nf-1))$
 $sd(f(nf-1)) = ((z(nf) * sd(f(nf))) + (z(nf-1) * sd(f(nf-1)))) / (z(nf) + z(nf-1))$
 $sa(f(nf-1)) = ((z(nf) * sa(f(nf))) + (z(nf-1) * sa(f(nf-1)))) / (z(nf) + z(nf-1))$
 $s2(f(nf-1)) = ((z(nf) * s2(f(nf))) + (z(nf-1) * s2(f(nf-1)))) / (z(nf) + z(nf-1))$
 $za(nf-1) = za(nf) + za(nf-1)$
 $zi(nf-1) = zi(nf) + zi(nf-1)$
 $z(nf-1) = z(nf) + z(nf-1)$
 $f(nf) = 0$
 $fn(nf) = 0$
 $sd(f(nf)) = 0$
 $sdfn(nf) = 0$
 $sa(f(nf)) = 0$
 $safn(nf) = 0$
 $s2(f(nf)) = 0$
 $s2fn(nf) = 0$
 $za(nf) = 0$
 $zan(nf) = 0$
 $zi(nf) = 0$
 $zin(nf) = 0$
 $z(nf) = 0$
 $zn(nf) = 0$

c New Profile Conditions for Layer Nf-1 Using Modified Finite Difference

$sdfn(nf-1) = sdfn(nf-1) + (((ddf(z(nf-1))) * ((sdfn(nf-2) - sdfn(nf-1)) / ((z(nf-2) + z(nf-1)) / 2))) - qmd * (sdfn(nf-1) / (ksd + sdfn(nf-1))) * (sa(f(nf-1)) / (ksa + sa(f(nf-1))) * f(nf-1) * x)) * h$
 $safn(nf-1) = safn(nf-1) + (((daf(z(nf-1))) * ((safn(nf-2) - safn(nf-1)) / ((z(nf-2) + z(nf-1)) / 2))) - (qma * (sdfn(nf-1) / (ksd + sdfn(nf-1))) * (sa(f(nf-1)) / (ksa + sa(f(nf-1))) + vax * b) * f(nf-1) * x)) * h$
 if sdfn(nf-1).lt.0 then
 $sdfn(nf-1) = 0$
 end if
 if safn(nf-1).lt.0 then
 $safn(nf-1) = 0$
 end if

c Dual, Electron Donor or Electron Acceptor Limited Conditions

if sa(f(nf-1)).le.ca.and.sdfn(nf-1).gt.cd then
 $lnhn = 0.215 * (qmd * (sa(f(nf-1)) / (ksa + sa(f(nf-1)))) * (sdfn(nf-1) / (ksd + sdfn(nf-1)))) - 0.717$
 else
 $lnhn = 0.273 * (qmd * (sa(f(nf-1)) / (ksa + sa(f(nf-1)))) * (sdfn(nf-1) / (ksd + sdfn(nf-1)))) - 1.11$
 $lnhn = 0.273 * (qmd * (sa(f(nf-1)) / (ksa + sa(f(nf-1)))) * (sdfn(nf-1) / (ksd + sdfn(nf-1)))) - 0.717$
 end if
 $s2fn(nf-1) = s2fn(nf-1) + (((d2f(z(nf-1))) * ((s2fn(nf-2) - s2fn(nf-1)) / ((z(nf-2) + z(nf-1)) / 2))) - qm2 * (lnhn + m) * (s2fn(nf-1) / (ks2 + s2fn(nf-1))) * (sa(f(nf-1)) / (kso + sa(f(nf-1))) * f(nf-1) * x)) * h$
 if s2fn(nf-1).lt.0 then
 $s2fn(nf-1) = 0$
 end if

c New Active Fraction and Layer Nf-1 Thickness Calculations

```

zan(nf-1)=za(nf-1)+(za(nf-1)*(y*qmd*(sdf(nf-1))/(ksd
+
+sdff(nf-1)))*(saf(nf-1)/(ksa+saf(nf-1)))-b-sl))
+
+h
zin(nf-1)=zi(nf-1)+(za(nf-1)*k1*b-zi(nf-1)*sl)*h
zn(nf-1)=zan(nf-1)+zin(nf-1)
fn(nf-1)=zan(nf-1)/zn(nf-1)
if zn(nf-1).lt.0 then
  zn(nf-1)=0
end if
if fn(nf-1).lt.0 then
  fn(nf-1)=0
end if
lfs=zn(nf-1)
lfas=zan(nf-1)
nf=nf-1
go to 444
end if

```

c Separating Layer Nf

```

if zn(nf).ge.zl then
  z(nf+1)=z(nf)/2
  z(nf)=z(nf)/2
  za(nf+1)=za(nf)/2
  za(nf)=za(nf)/2
  zi(nf+1)=zi(nf)/2
  zi(nf)=zi(nf)/2
  f(nf+1)=f(nf)
  sdf(nf+1)=sdf(nf)
  saf(nf+1)=saf(nf)
  s2f(nf+1)=s2f(nf)

```

c New Profile Conditions for Layer Nf+1 Using Modified Finite Difference

```

sdfn(nf+1)=sdf(nf+1)+(((d2f/z(nf+1))*((sdf(nf)
+
-sdf(nf+1))/((z(nf)+z(nf+1))/2)))-qmd
+
*(sdf(nf+1)/(ksd+sdf(nf+1)))*(saf(nf+1)/(ksa
+
+saf(nf+1)))*f(nf+1)*xf)*h
safn(nf+1)=saf(nf+1)+(((daf/z(nf+1))*((saf(nf)
+
-saf(nf+1))/((z(nf)+z(nf+1))/2)))-qma
+
*(sdf(nf+1)/(ksd+sdf(nf+1)))*(saf(nf+1)/(ksa
+
+saf(nf+1))+vax*b)*f(nf+1)*xf)*h
if sdfn(nf+1).lt.0 then
  sdfn(nf+1)=0
end if
if safn(nf+1).lt.0 then
  safn(nf+1)=0
end if

```

c Dual, Electron Donor or Electron Acceptor Limited Conditions

```

if saf(nf+1).le.ca.and.sdf(nf+1).gt.cd then
  lnfn=0.215*(qmd*(saf(nf+1)/(ksa+saf(nf+1)))
+
*(sdf(nf+1)/(ksd+sdf(nf+1)))-0.717
else
  lnfn=0.273*(qmd*(saf(nf+1)/(ksa+saf(nf+1)))
+
*(sdf(nf+1)/(ksd+sdf(nf+1)))-1.11
+
*(saf(nf+1)/(ksa+saf(nf+1)))-0.717
end if
s2fn(nf+1)=s2f(nf+1)+(((d2f/z(nf+1))*((s2f(nf)
+
-s2f(nf+1))/((z(nf)+z(nf+1))/2)))-qm2*(lnfn
+
+m)*(s2f(nf+1)/(ks2+s2f(nf+1)))*(saf(nf+1)
+
/(kso+saf(nf+1)))*f(nf+1)*xf)*h
if s2fn(nf+1).lt.0 then

```

```

      s2fn(nf+1)=0
    end if
c New Active Fraction and Layer Nf+1 Thickness Calculations
      zan(nf+1)=za(nf+1)+(za(nf+1)*(y*qmd*(sdf(nf+1)/(ksd
+      +sdf(nf+1)))*(saf(nf+1)/(ksa+saf(nf+1))-b-sl))
+      *h
      zin(nf+1)=zi(nf+1)+(za(nf+1)*k1*b-zi(nf+1)*sl)*h
      zn(nf+1)=zan(nf+1)+zin(nf+1)
      fn(nf+1)=zan(nf+1)/zn(nf+1)
      if(zn(nf+1).lt.0) then
        zn(nf+1)=0
      end if
      if(fn(nf+1).lt.0) then
        fn(nf+1)=0
      end if
      lfs=zn(nf+1)
      lfas=zan(nf+1)
      nf=nf+1
    else
c -----
c No Joining or Separating Required
c New Profile Conditions for Layer Nf Using Modified Finite Difference -
      sdfn(nf)=sdf(nf)+(((dd/z(nf))*(sdf(nf-1)-sdf(nf))
+      /((z(nf-1)+z(nf))/2)))-(qmd*(sdf(nf)/(ksd
+      +sdf(nf)))*(saf(nf)/(ksa+saf(nf)))*f(nf)*x))*h
      safn(nf)=saf(nf)+(((daf/z(nf))*(saf(nf-1)-saf(nf))
+      /((z(nf-1)+z(nf))/2)))-((qma*(sdf(nf)/(ksd
+      +sdf(nf)))*(saf(nf)/(ksa+saf(nf)))+vax*b)*f(nf)
+      *x))*h
      if(sdfn(nf).lt.0) then
        sdfn(nf)=0
      end if
      if(safn(nf).lt.0) then
        safn(nf)=0
      end if
c Dual. Electron Donor or Electron Acceptor Limited Conditions
      if(saf(nf).le.ca.and.sdf(nf).gt.cd) then
        lnhn=0.215*(qmd*(saf(nf)/(ksa+saf(nf)))*(sdf(nf)
+      /(ksd+sdf(nf)))-0.717
      else
        lnhn=0.273*(qmd*(saf(nf)/(ksa+saf(nf)))*(sdf(nf)
+      /(ksd+sdf(nf)))-(1.11*(saf(nf)/(ksa
+      +saf(nf)))-0.717
      end if
      s2fn(nf)=s2f(nf)+(((d2/z(nf))*(s2f(nf-1)-s2f(nf))
+      /((z(nf-1)+z(nf))/2)))-(qn12*(lnhn+m)*(s2f(nf)
+      /(ks2+s2f(nf)))*(saf(nf)/(kso+saf(nf)))*f(nf)
+      *x))*h
      if(s2fn(nf).lt.0) then
        s2fn(nf)=0
      end if
c New Active Fraction and Layer Nf Thickness Calculations
      zan(nf)=za(nf)+(za(nf)*(y*qmd*(sdf(nf)/(ksd+sdf(nf))
+      *(saf(nf)/(ksa+saf(nf)))-b-sl))*h
      zin(nf)=zi(nf)+(za(nf)*k1*b-zi(nf)*sl)*h
      zn(nf)=zan(nf)+zin(nf)
      fn(nf)=zan(nf)/zn(nf)
      if(zn(nf).lt.0) then
        zn(nf)=0
      end if

```

```

        if fn(nf).lt.0) then
            fn(nf)=0
        end if
        if s=zn(nf)
            if as=-zan(nf)
                end if
c -----
c New Profile Conditions for Layers Nf-1 to 2
444      do 500 ii=nf-1,2,-1
c -----
c Separating or Joining Layers Due to Growth or Decay
      zan(ii)=za(ii)+(za(ii)*(y*qmd*(sdf(ii)/(ksd+sdf(ii)))
+          *(saf(ii)/(ksa+saf(ii))-b-sl))*h
      zin(ii)=zi(ii)+(za(ii)*k1*b-zi(ii)*sl)*h
      zn(ii)=zan(ii)+zin(ii)
c -----
c Joining Layers ii & ii-1
      if (zn(ii).le.zn) then
          fl(ii-1)=(za(ii)+za(ii-1))/(z(ii)+z(ii-1))
          sdf(ii-1)=((z(ii)*sdf(ii)+(z(ii-1)*sdf(ii-1)))
+              /(z(ii)+z(ii-1)))
          saf(ii-1)=((z(ii)*saf(ii)+(z(ii-1)*saf(ii-1)))
+              /(z(ii)+z(ii-1)))
          s2f(ii-1)=((z(ii)*s2f(ii)+(z(ii-1)*s2f(ii-1)))
+              /(z(ii)+z(ii-1)))
          za(ii-1)=za(ii)+za(ii-1)
          zi(ii-1)=zi(ii)+zi(ii-1)
          z(ii-1)=z(ii)+z(ii-1)
          do 515 iij=ii+1,nf
              fl(iij-1)=fl(iij)
              fn(iij-1)=fn(iij)
              sdf(iij-1)=sdf(iij)
              sdfn(iij-1)=sdfn(iij)
              saf(iij-1)=saf(iij)
              safn(iij-1)=safn(iij)
              s2f(iij-1)=s2f(iij)
              s2fn(iij-1)=s2fn(iij)
              za(iij-1)=za(iij)
              zan(iij-1)=zan(iij)
              zi(iij-1)=zi(iij)
              zin(iij-1)=zin(iij)
              z(iij-1)=z(iij)
              zn(iij-1)=zn(iij)
515      continue
      fl(nt)=0
      fn(nt)=0
      sdf(nt)=0
      sdfn(nt)=0
      saf(nt)=0
      safn(nt)=0
      s2f(nt)=0
      s2fn(nt)=0
      za(nt)=0
      zan(nt)=0
      zi(nt)=0
      zin(nt)=0
      z(nt)=0
      zn(nt)=0
      nt=nt-1
      go to 500

```

end if

c

c Separating Layer ii

if(zn(ii).ge.zl) then

do 550 iz=zf,ii+1,-1

z(iz+1)=z(iz)

zn(iz+1)=zn(iz)

za(iz+1)=za(iz)

zan(iz+1)=zan(iz)

zi(iz+1)=zi(iz)

zin(iz+1)=zin(iz)

f(iz+1)=f(iz)

fn(iz+1)=fn(iz)

sdf(iz+1)=sdf(iz)

sdfn(iz+1)=sdfn(iz)

saf(iz+1)=saf(iz)

safn(iz+1)=safn(iz)

s2f(iz+1)=s2f(iz)

s2fn(iz+1)=s2fn(iz)

550

continue

z(ii+1)=z(ii)/2

z(ii)=z(ii)/2

za(ii+1)=za(ii)/2

za(ii)=za(ii)/2

zi(ii+1)=zi(ii)/2

zi(ii)=zi(ii)/2

f(ii+1)=f(ii)

sdf(ii+1)=sdf(ii)

saf(ii+1)=saf(ii)

s2f(ii+1)=s2f(ii)

sdfn(ii+1)=sdfn(ii)+(((ddf/z(ii+1))*((sdf(ii)

+ -sdf(ii+1))/((z(ii)+z(ii+1))/2))-((sdf(ii+1)

+ -sdf(ii+2))/((z(ii+1)+z(ii+2))/2))))-(qmd

+*(sdf(ii+1)/(ksd+sdf(ii+1)))*(saf(ii+1)/(ksa

+saf(ii+1)))*f(ii+1)*xf)*h

safn(ii+1)=safn(ii)+(((daf/z(ii+1))*((saf(ii)

+ -saf(ii+1))/((z(ii)+z(ii+1))/2))-((saf(ii+1)

+ -saf(ii+2))/((z(ii+1)+z(ii+2))/2))))-(qma

+*(sdf(ii+1)/(ksd+sdf(ii+1)))*(saf(ii+1)/(ksa

+saf(ii+1))+vax*b)*f(ii+1)*xf)*h

if(sdfn(ii+1).lt.0) then

sdfn(ii+1)=0

end if

if(safn(ii+1).lt.0) then

safn(ii+1)=0

end if

c Dual, Electron Donor or Electron Acceptor Limited Conditions

if(saf(ii+1).le.ca.and.sdf(ii+1).gt.cd) then

lnhn=0.215*(qmd*(saf(ii+1)/(ksa+saf(ii+1)))

+ *(sdf(ii+1)/(ksd+sdf(ii+1))))-0.717

else

lnhn=0.273*(qmd*(saf(ii+1)/(ksa+saf(ii+1)))

+ *(sdf(ii+1)/(ksd+sdf(ii+1))))-(1.11*(saf(ii+1)

+ /(ksa+saf(ii+1))))-0.717

end if

s2fn(ii+1)=s2f(ii+1)+(((d2f/z(ii+1))*((s2f(ii)

+ -s2f(ii+1))/((z(ii)+z(ii+1))/2))-((s2f(ii+1)

+ -s2f(ii+2))/((z(ii+1)+z(ii+2))/2))))-(qm2

+*(lnhn+m)*(s2f(ii+1)/(ks2+s2f(ii+1)))*(saf(ii+1)

+ /(kso+saf(ii+1)))*f(ii+1)*xf)*h

```

    if s2fn(ii+1).lt.0) then
      s2fn(ii+1)=0
    end if
c New Active Fraction and Layer ii Thickness Calculations
    zan(ii+1)=za(ii+1)+(za(ii+1)*(y*qmd*(sdf(ii+1)/(ksd
+      +sdf(ii+1)))*(saf(ii+1)/(ksa+saf(ii+1)))-b-sl))
+      *h
    zin(ii+1)=zi(ii+1)+(za(ii+1)*k1*b-zi(ii+1)*sl)*h
    zn(ii+1)=zan(ii+1)+zin(ii+1)
    fn(ii+1)=zan(ii+1)/zn(ii+1)
    if zn(ii+1).lt.0) then
      zn(ii+1)=0
    end if
    if fn(ii+1).lt.0) then
      fn(ii+1)=0
    end if
    zd=zn(ii+1)
    zad=zan(ii+1)
    s2fn(ii)=sdf(ii)+(((ddf/z(ii))*(((sdf(ii-1)-sdf(ii))
+      /((z(ii-1)+z(ii))/2))-((sdf(ii)-sdf(ii+1))
+      /((z(ii)+z(ii+1))/2))))-(qmd*(sdf(ii)/(ksd
+      +sdf(ii)))*(saf(ii)/(ksa+saf(ii)))*f(ii)*xf)*h
    safn(ii)=saf(ii)+(((daf/z(ii))*(((saf(ii-1)-saf(ii))
+      /((z(ii-1)+z(ii))/2))-((saf(ii)-saf(ii+1))
+      /((z(ii)+z(ii+1))/2))))-(qma*(sdf(ii)/(ksd
+      +sdf(ii)))*(saf(ii)/(ksa+saf(ii)))+vax*b)*f(ii)
+      *xf)*h
    if sdfn(ii).lt.0) then
      sdfn(ii)=0
    end if
    if safn(ii).lt.0) then
      safn(ii)=0
    end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
    if (saf(ii).le.ca.and.sdf(ii).gt.cd) then
      lnhn=0.215*(qmd*(saf(ii)/(ksa+saf(ii)))*(sdf(ii)
+      /(ksd+sdf(ii))))-0.717
    else
      lnhn=0.273*(qmd*(saf(ii)/(ksa+saf(ii)))*(sdf(ii)
+      /(ksd+sdf(ii))))-(1.11*(saf(ii)/(ksa+saf(ii))))
+      -0.717
    end if
    s2fn(ii)=s2f(ii)+(((d2f/z(ii))*(((s2f(ii-1)-s2f(ii))
+      /((z(ii-1)+z(ii))/2))-((s2f(ii)-s2f(ii+1))
+      /((z(ii)+z(ii+1))/2))))-(qm2*(lnhn+m)*s2f(ii)
+      /(ks2+s2f(ii)))*(saf(ii)/(kso+saf(ii)))*f(ii)
+      *xf)*h
    if s2fn(ii).lt.0) then
      s2fn(ii)=0
    end if
c New Active Fraction and Layer ii Thickness Calculations
    zan(ii)=za(ii)+(za(ii)*(y*qmd*(sdf(ii)/(ksd
+      +sdf(ii)))*(saf(ii)/(ksa+saf(ii)))-b-sl))*h
    zin(ii)=zi(ii)+(za(ii)*k1*b-zi(ii)*sl)*h
    zn(ii)=zan(ii)+zin(ii)
    fn(ii)=zan(ii)/zn(ii)
    if zn(ii).lt.0) then
      zn(ii)=0
    end if
    if fn(ii).lt.0) then

```

```

        fn(ii)=0
    end if
    lf=lf+zn(ii)+zdl
    lfa=lfa+zan(ii)+zad
    nf=nf+1
else
c New Profile Conditions for Layers Nf-1 to 2 Using Finite Difference
    sdfn(ii)=sdf(ii)+(((ddf/z(ii))*(((sdf(ii-1)-sdf(ii))
+      /((z(ii-1)+z(ii))/2)))-(sdf(ii)-sdf(ii+1))
+      /((z(ii)+z(ii+1))/2))))-(qmd*(sdf(ii)/(ksd
+      +sdf(ii)))*(saf(ii)/(ksa+saf(ii)))*f(ii)*xf))*h
    safn(ii)=saf(ii)+(((daf/z(ii))*(((saf(ii-1)-saf(ii))
+      /((z(ii-1)+z(ii))/2)))-(saf(ii)-saf(ii+1))
+      /((z(ii)+z(ii+1))/2))))-(qma*(sdf(ii)/(ksd
+      +saf(ii)))*(saf(ii)/(ksa+saf(ii)))+vax*b)*f(ii)
+      *xf))*h
    if sdfn(ii).lt.0 then
        sdfn(ii)=0
    end if
    if safn(ii).lt.0 then
        safn(ii)=0
    end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
    if (saf(ii).le.ca.and.sdf(ii).gt.cd) then
        lnhn=0.215*(qmd*(saf(ii)/(ksa+saf(ii)))*(sdf(ii)
+      /((ksd+sdf(ii))))-0.717
    else
        lnhn=0.273*(qmd*(saf(ii)/(ksa+saf(ii)))*(sdf(ii)
+      /((ksd+sdf(ii))))-(1.11*(saf(ii)/(ksa+saf(ii))))
+      -0.717
    end if
    s2fn(ii)=s2f(ii)+(((ddf/z(ii))*(((s2f(ii-1)-s2f(ii))
+      /((z(ii-1)+z(ii))/2)))-(s2f(ii)-s2f(ii+1))
+      /((z(ii)+z(ii+1))/2))))-(qm2*(lnhn+m)*(s2f(ii)
+      /((ks2+s2f(ii)))*(saf(ii)/(kso+saf(ii)))*f(ii)
+      *xf))*h
    if s2fn(ii).lt.0 then
        s2fn(ii)=0
    end if
c New Active Fraction and Layer ii Thickness Calculations
    zan(ii)=za(ii)+(za(ii)*(y*qmd*(sdf(ii)/(ksd
+      +sdf(ii)))*(saf(ii)/(ksa+saf(ii))-b-sl))*h
    zin(ii)=zi(ii)+(za(ii)*k1*b-zi(ii)*sl)*h
    zn(ii)=zan(ii)+zin(ii)
    fn(ii)=zan(ii)/zn(ii)
    if zn(ii).lt.0 then
        zn(ii)=0
    end if
    if fn(ii).lt.0 then
        fn(ii)=0
    end if
    lf=lf+zn(ii)
    lfa=lfa+zan(ii)
end if
500 continue
c
c New Profile Conditions for Layer 1 - Biofilm/Liquid Donor and
c Acceptor Concentration Iteration at time t=tbl
c Separating Layers Due to Growth
    zan(1)=za(1)+(za(1)*(y*qmd*(sdf(1)/(ksd+sdf(1))))

```

4

OF/DE

4

PM-1 3 1/2" x 4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT

PRECISIONSM RESOLUTION TARGETS

```

      * (saf(1) * (ksa + saf(1))) - b - s1)) * h
zin(1) = zi(1) + (za(1) * k1 * b - zin(1) * s1) * h
zn(1) = zan(1) + zin(1)
if (zn(1) ge .z) then
  do 650 jz = nf, 2, -1
    z(jz+1) = z(jz)
    zn(jz+1) = zn(jz)
    za(jz+1) = za(jz)
    zan(jz+1) = zan(jz)
    zi(jz+1) = zi(jz)
    zin(jz+1) = zin(jz)
    fl(jz+1) = fl(jz)
    fn(jz+1) = fn(jz)
    sdf(jz+1) = sdf(jz)
    sdfn(jz+1) = sdfn(jz)
    saf(jz+1) = saf(jz)
    safn(jz+1) = safn(jz)
    s2f(jz+1) = s2f(jz)
    s2fn(jz+1) = s2fn(jz)
650  continue
  z(2) = z(1) / 2
  z1(1) = z(1) / 2
  za(2) = za(1) / 2
  za1(1) = za(1) / 2
  zi(2) = zi(1) / 2
  zi1(1) = zi(1) / 2
  fl(2) = fl(1)
  sdf(2) = sdf(1)
  saf(2) = saf(1)
  s2f(2) = s2f(1)
  nf = nf - 1
c New Profile Conditions for Layer 2 Using Finite Difference -
  sdfn(2) = sdf(2) + (((ddf/z(2)) * (((sdf(1) - sdf(2)) / ((z(1)
+ z(2)) / 2)) - (sdf(2) - sdf(3)) / ((z(2) + z(3)) / 2))))))
+ (qmd * (sdf(2) / (ksd + sdf(2))) * (saf(2) / (ksa
+ saf(2))) * fl(2) * xf)) * h
  safn(2) = saf(2) + (((daf/z(2)) * (((saf(1) - saf(2)) / ((z(1)
+ z(2)) / 2)) - (saf(2) - saf(3)) / ((z(2) + z(3)) / 2))))))
+ (qma * (sdf(2) / (ksd + sdf(2))) * (saf(2) / (ksa
+ saf(2))) + vax * b) * fl(2) * xf)) * h
  if sdfn(2).lt.0 then
    sdfn(2) = 0
  end if
  if safn(2).lt.0 then
    safn(2) = 0
  end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
  if (saf(2).le.ca.and.sdf(2).gt.cd) then
    lnhn = 0.215 * (qmd * (saf(2) / (ksa + saf(2))) * (sdf(2)
+ (ksd + sdf(2)))) - 0.717
  else
    lnhn = 0.273 * (qmd * (saf(2) / (ksa + saf(2))) * (sdf(2) / (ksd
+ sdf(2)))) - 1.11 * (saf(2) / (ksa + saf(2))) - 0.717
  end if
  s2fn(2) = s2f(2) + (((d2f/z(2)) * (((s2f(1) - s2f(2)) / ((z(1)
+ z(2)) / 2)) - (s2f(2) - s2f(3)) / ((z(2) + z(3)) / 2))))))
+ (qm2 * (lnhn + m) * (s2f(2) / (ks2 + s2f(2))) * (saf(2)
+ (kso + saf(2))) * fl(2) * xf)) * h
  if s2fn(2).lt.0 then
    s2fn(2) = 0

```



```

end if
c New Active Fraction and Layer 2 Thickness Calculations
zan(2)=zaf(2)+(zaf(2)*(y*qmd*(sdf(2)+(ksd+sdf(2))))
+
*(saf(2)/(ksa+saf(2)))-b-si))*h
zin(2)=zi(2)+(zaf(2)*k1*b-zi(2)*si)*h
zn(2)=zan(2)+zin(2)
fn(2)=zan(2)/zn(2)
if(zn(2) lt 0) then
  zn(2)=0
end if
if(fn(2) lt 0) then
  fn(2)=0
end if
lft=lft+1fs+zn(2)
lfat=lfat+lfas+zan(2)
c New Active Fraction and Layer 1 Thickness Calculations
zan(1)=zaf(1)+(zaf(1)*(y*qmd*(sdf(1)+(ksd+sdf(1))))
+
*(saf(1)/(ksa+saf(1)))-b-si))*h
zin(1)=zi(1)+(zaf(1)*k1*b-zi(1)*si)*h
zn(1)=zan(1)+zin(1)
fn(1)=zan(1)/zn(1)
if(zn(1) lt 0) then
  zn(1)=0
end if
if(fn(1) lt 0) then
  fn(1)=0
end if
lft=lft+zn(1)
lfat=lfat+zan(1)
c New Profile Conditions of Layer 1
if(ppp eq 1) then
c Fully Explicit Method
sdfn(1)=sdf(1)+(((kmd/z(1))*(sdb-sdf(1)))-((ddf
+
/z(1))*((sdf(1)-sdf(2))/(z(1)+z(2))
+
/2)))-qmd*(sdf(1)/(ksd+sdf(1)))*(saf(1)
+
/(ksa+saf(1)))*xf(1))*h
safn(1)=saf(1)+(((kma/z(1))*(sab-saf(1)))-((daf
+
/z(1))*((saf(1)-saf(2))/(z(1)+z(2))
+
/2)))-((qma*(sdf(1)/(ksd+sdf(1))
+
*(saf(1)/(ksa+saf(1)))+vax*b)*xf(1)))
+
*h
if(sdfn(1) lt 0) then
  sdfn(1)=0
end if
if(safn(1) lt 0) then
  safn(1)=0
end if
c Dual, Electron Donor or Electron Acceptor Limited Conditions
if(saf(1) le ca and sdf(1) gt cd) then
  lnhn=0.215*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+
/(ksd+sdf(1))))-0.717
else
  lnhn=0.273*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+
/(ksd+sdf(1))))-1.11*(saf(1)/(ksa
+
+saf(1)))-0.717
end if
s2fn(1)=s2f(1)+(((km2/z(1))*(s2b-s2f(1)))-((d2f
+
/z(1))*((s2f(1)-s2f(2))/(z(1)+z(2))
+
/2)))-qm2*(lnhn+m)*(s2f(1)/(ks2
+
+s2f(1)))*(saf(1)/(ksa+saf(1)))*xf

```

```

      *f1)))*h
      if s2fn(1) lt 0 then
        s2fn(1)=0
      end if
    else
c Summation Technique
      sdfn(1)=cdec*sdbn
      safn(1)=cdec*sabn
      s2fn(1)=cdec*s2bn
      fpd=0
      fpa=0
      fp2=0
      fld=0
      fla=0
      fl2=0
      do 625 jj=2,nf
        fjd(jj)=zn(jj)*qmd*((sdfn(jj)/(ksd+sdfn(jj)))
+         *(safn(jj)/(ksa+safn(jj))))*xi*fn(jj)
        fja(jj)=zn(jj)*(qma*((sdfn(jj)/(ksd+sdfn(jj)))
+         *(safn(jj)/(ksa+safn(jj))))+vax*b)*xi*fn(jj)
        fpd=fpd+fjd(jj)
        fpa=fpa+fja(jj)
625      continue
825      fsd=zn(1)*qmd*((sdfn(1)/(ksd+sdfn(1)))*(safn(1)
+         /(ksa+safn(1))))*xi*fn(1)
        fsa=zn(1)*(qma*((sdfn(1)/(ksd+sdfn(1))
+         *(safn(1)/(ksa+safn(1))))+vax*b)*xi*fn(1)
        fld=fpd+fsd
        fla=fpa+fsa
        sdbnl=sdfn(1)+(fld/kmd)
        sabnl=safn(1)+(fla/kma)
        sdbnd=1-((sdbnl-sdbn)/sdbn)
        sabnd=1-((sabnl-sabn)/sabn)
        if sdbnd.lt.cl.and.sabnd.lt.cl) then
          sdfn(1)=cdec*sdfn(1)
          safn(1)=cdec*safn(1)
          go to 825
        end if
        if sdbnd.lt.cl.and.sabnd.ge.cl.and.sabnd.le.cu) then
          sdfn(1)=cdec*sdfn(1)
          go to 825
        end if
        if sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.lt.cl) then
          safn(1)=cdec*safn(1)
          go to 825
        end if
        if sdbnd.gt.cu.and.sabnd.gt.cu) then
          sdfn(1)=sdfn(1)+cinc*sdfn(1)
          safn(1)=safn(1)+cinc*safn(1)
          go to 825
        end if
        if sdbnd.gt.cu.and.sabnd.lt.cl) then
          sdfn(1)=sdfn(1)+cinc*sdfn(1)
          safn(1)=cdec*safn(1)
          go to 825
        end if
        if sdbnd.lt.cl.and.sabnd.gt.cu) then
          sdfn(1)=cdec*sdfn(1)
          safn(1)=safn(1)+cinc*safn(1)
          go to 825

```

```

end if
if(sdbnd.ge.cl and sdbnd.le.eu and sabnd.gt.eu) then
  safn(1)=safn(1)+cinc*safn(1)
  go to 825
end if
if(sdbnd.gt.eu and sabnd.ge.cl and sabnd.le.eu) then
  safn(1)=sdbnd(1)+cinc*sdbnd(1)
  go to 825
end if
if(sdbnd(1).lt.0) then
  sdbnd(1)=0
end if
if(safn(1).lt.0) then
  safn(1)=0
end if
c. Biofilm Liquid Secondary Substrate Concentration Iteration at time
c. t thl Using Summation
do 725 j=2,nf
  if(safn(j).le.ca and sdbnd(j).gt.cd) then
    lnhn2(j)=0.215*(qmd*(safn(j)/(ksa+
      safn(j)))*(sdbnd(j)/(ksd+sdbnd(j)))
      -0.717
  else
    lnhn2(j)=0.273*(qmd*(safn(j)/(ksa+
      safn(j)))*(sdbnd(j)/(ksd+sdbnd(j)))
      -(1.11*(safn(j)/(ksa+safn(j)))
      -0.717
  end if
  f2(j)=znc(j)*qm2*x1*((s2fn(j)/(ks2
    +s2fn(j)))*(safn(j)/(kso+safn(j)))
    *fn(j)*(lnhn2(j)+m))
  fp2=fp2+f2-ωL
725 continue
850 if(safn(1).le.ca and sdbnd(1).gt.cd) then
  lnhn2(1)=0.215*(qmd*(safn(1)/(ksa+safn(1)))
    *(sdbnd(1)/(ksd+sdbnd(1)))-0.717
else
  lnhn2(1)=0.273*(qmd*(safn(1)/(ksa+safn(1)))
    *(sdbnd(1)/(ksd+sdbnd(1)))-(1.11*(safn(1)
    /(ksa+safn(1)))-0.717
end if
f2=znc(1)*qm2*x1*((s2fn(1)/(ks2+s2fn(1)))
  *(safn(1)/(kso+safn(1)))*fn(1)*(lnhn2(1)
  +m))
ft2=fp2+f2
s2bnd=s2fn(1)+(ft2/km2)
s2bnd=1-((s2bnd-s2bn)/s2bn)
if(s2bnd.lt.cl) then
  s2fn(1)=cdec*s2fn(1)
  go to 850
end if
if(s2bnd.gt.eu) then
  s2fn(1)=s2fn(1)+cinc*s2fn(1)
  go to 850
end if
if(s2fn(1).lt.0) then
  s2fn(1)=0
end if
end if
else

```

c New Active Fraction and Layer 1 Thickness Calculations

```

zant(1)=zai(1)+(zai(1)*(y*qmd*(sdf(1)/(ksd+sdf(1))
+
*(saf(1)/(ksa+saf(1)))-b-sl))*h
zinc(1)=zi(1)+(zai(1)*k1*b-zic(1)*sl)*h
znc(1)=zant(1)+zinc(1)
fn(1)=zant(1)-znc(1)
if(zn(1).lt.0) then
  zn(1)=0
end if
if(fn(1).lt.0) then
  fn(1)=0
end if
lft=lfi+lfz+znc(1)
lfat=lfz+lfz+znc(1)

```

c New Profile Conditions of Layer 1

```

if(ppp eq.1) then

```

c Fully Explicit Method

```

sdfn(1)=sdf(1)+(((kmd/z(1))*(sdb-sdf(1)))-((ddf
+
z(1))*((sdf(1)-sdf(2))/(z(1)+z(2))
+
2)))-qmd*(sdf(1)/(ksd+sdf(1)))*(saf(1)
+
(ksa+saf(1))*xf*f(1))*h
safn(1)=saf(1)+(((kma/z(1))*(sab-saf(1)))-((daf
+
z(1))*((saf(1)-saf(2))/(z(1)+z(2))
+
2)))-qma*(sdf(1)/(ksd+sdf(1))
+
*(saf(1)/(ksa+saf(1))-vax*b)*xf*f(1))
+
*h
if(sdfn(1).lt.0) then
  sdfn(1)=0
end if
if(safn(1).lt.0) then
  safn(1)=0
end if

```

c Dual, Electron Donor or Electron Acceptor Limited Conditions

```

if(saf(1).le.ca.and.sdf(1).gt.cd) then
  lnhn=0.215*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+
/(ksd+sdf(1))))-0.717
else
  lnhn=0.273*(qmd*(saf(1)/(ksa+saf(1)))*(sdf(1)
+
/(ksd+sdf(1))))-(1.11*(saf(1)/(ksa
+
saf(1))))-0.717
end if
s2fn(1)=s2f(1)+(((km2/z(1))*(s2b-s2f(1)))-((d2f
+
z(1))*((s2f(1)-s2f(2))/(z(1)+z(2))
+
2)))-qm2*(lnhn+m)*(s2f(1)/(ks2
+
s2f(1)))*(saf(1)/(ksa+saf(1)))*xf
+
*f(1))*h
if(s2fn(1).lt.0) then
  s2fn(1)=0
end if
else

```

c Summation Technique - Layer 1

```

sdfn(1)=cdec*sdbn
safn(1)=cdec*sabn
s2fn(1)=cdec*s2bn
fpd=0
fpa=0
fp2=0
ftd=0
fta=0
ft2=0

```

```

do 675 j=2,nf
  fjd(j)=zn(j)*qmd*((sdfn(j)/(ksd+sdfn(j)))
    * (safn(j)/(ksa+safn(j)))*xf*fn(j)
  fja(j)=zn(j)*qma*((sdfn(j)/(ksd+sdfn(j)))
    * (safn(j)/(ksa+safn(j)))+vax*b)*xf
    *fn(j)
  fpd=fpd+fjd(j)
  fpa=fpa+fja(j)
675  continue
800  fsd=zn(1)*qmd*((sdfn(1)/(ksd+sdfn(1)))*(safn(1)
    *(ksa+safn(1)))*xf*fn(1)
  fsa=zn(1)*qma*((sdfn(1)/(ksd+sdfn(1))
    * (safn(1)/(ksa+safn(1)))+vax*b)*xf*fn(1)
  ftd=fpd+fsd
  fta=fpa+fsa
  sdbnd=sdfn(1)+(ftd/kmd)
  sabnd=safn(1)+(fta/kma)
  sdbnd=1-((sdbnd-sdbn)/sdbn)
  sabnd=1-((sabnd-sabn)/sabn)
  if(sdbnd.lt.cl.and.sabnd.lt.cl) then
    sdfn(1)=cdec*sdfn(1)
    safn(1)=cdec*safn(1)
    go to 800
  end if
  if(sdbnd.lt.cl.and.sabnd.ge.cl.and.sabnd.le.cu) then
    sdfn(1)=cdec*sdfn(1)
    go to 800
  end if
  if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.lt.cl) then
    safn(1)=cdec*safn(1)
    go to 800
  end if
  if(sdbnd.gt.cu.and.sabnd.gt.cu) then
    sdfn(1)=sdfn(1)+cinc*sdfn(1)
    safn(1)=safn(1)+cinc*safn(1)
    go to 800
  end if
  if(sdbnd.gt.cu.and.sabnd.lt.cl) then
    sdfn(1)=sdfn(1)+cinc*sdfn(1)
    safn(1)=cdec*safn(1)
    go to 800
  end if
  if(sdbnd.lt.cl.and.sabnd.gt.cu) then
    sdfn(1)=cdec*sdfn(1)
    safn(1)=safn(1)+cinc*safn(1)
    go to 800
  end if
  if(sdbnd.ge.cl.and.sdbnd.le.cu.and.sabnd.gt.cu) then
    safn(1)=safn(1)+cinc*safn(1)
    go to 800
  end if
  if(sdbnd.gt.cu.and.sabnd.ge.cl.and.sabnd.le.cu) then
    sdfn(1)=sdfn(1)+cinc*sdfn(1)
    go to 800
  end if
  if(sdfn(1).lt.0) then
    sdfn(1)=0
  end if
  if(safn(1).lt.0) then
    safn(1)=0

```

```

end if
c Biofilm Liquid Secondary Substrate Concentration Iteration at time
c t=tbl Using Summation
do 775 j=2,nt
  if(safn(j) le ca.and sdfn(j) gt.cd) then
    lnhn2(j)=0.215*(qmd*(safn(j)/(ksa+
    +safn(j)))*(sdfn(j)/(ksd+
    +sdfn(j)))-0.717
  else
    lnhn2(j)=0.273*(qmd*(safn(j)/(ksa+
    +safn(j)))*(sdfn(j)/(ksd+sdfn(j)))-
    +(1.11*(safn(j)/(ksa+safn(j)))-
    -0.717
  end if
  f12(j)=zn(j)*qm2*xf*((s2fn(j)/(ks2
    +s2fn(j)))*(safn(j)/(ksa+safn(j))
    +fn(j)*(lnhn2(j)+m))
  fp2=fp2+f12(j)
775 continue
875 if(safn(1) le ca.and sdfn(1) gt.cd) then
  lnhn2(1)=0.215*(qmd*(safn(1)/(ksa+safn(1))
    *(sdfn(1)/(ksd+sdfn(1)))-0.717
  else
    lnhn2(1)=0.273*(qmd*(safn(1)/(ksa+safn(1))
    *(sdfn(1)/(ksd+sdfn(1)))+(1.11*(safn(1)
    /(ksa+safn(1)))-0.717
  end if
  fs2=zn(1)*qm2*xf*((s2fn(1)/(ks2+s2fn(1))
    *(safn(1)/(ksa+safn(1)))+fn(1)*(lnhn2(1)
    +m))
  ft2=fp2+fs2
  s2bnd=s2fn(1)+(ft2/km2)
  s2bnd=1-(s2bnd-s2bn)/s2bn
  if(s2bnd.lt.cl) then
    s2fn(1)=cdec*s2fn(1)
    go to 875
  end if
  if(s2bnd.gt.cu) then
    s2fn(1)=s2fn(1)+cinc*s2fn(1)
    go to 875
  end if
  if(s2fn(1).lt.0) then
    s2fn(1)=0
  end if
end if
end if
end if
c -----
c Reset Biofilm Layer Parameters for Next Time Step
333 do 350 pfi=1,nf
  z(pfi)=zn(pfi)
  za(pfi)=zan(pfi)
  zi(pfi)=zin(pfi)
  f(pfi)=fn(pfi)
  sdf(pfi)=sdfn(pfi)
  sdfi(pfi)=sdfn(pfi)*1000
  saf(pfi)=safn(pfi)
  safi(pfi)=safn(pfi)*1000
  s2f(pfi)=s2fn(pfi)
  s2fi(pfi)=s2fn(pfi)*1000
350 continue

```


END

1 8-0 6-9 7

FIN