To the Graduate Council:

I am submitting herewith a dissertation written by John William Davis entitled "Biologically Mediated Removal of Dissolved Organic Carbon from Coal Slurry Wastewater." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Microbiology.

Gary S. Sayler, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

The Graduate School

BIOLOGICALLY MEDIATED REMOVAL OF DISSOLVED ORGANIC CARBON FROM COAL SLURRY WASTEWATER

A Dissertation

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Degree

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John William Davis

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ABSTRACT

The biologically mediated removal of dissolved organic carbon (DOC) from pipeline slurry wastewaters was examined. BOD_{20} values for laboratory slurry wastewaters ranged from 52 to 216 mg $liter^{-1}$ indicating biological oxidation of the organic compounds. Second order kinetic rate constants (k_2) developed for DOC removal ranged from 1.39×10^{-4} to 2.30×10^{-4} liter day^{-1} (mg of sludge)$^{-1}$ providing evidence that biological treatment was an affective mechanism for reducing the pollution potential of the slurry wastewaters.

After biological treatment a residual DOC (a mean of 33 mg of carbon $liter^{-1}$) remained and ultrafiltration studies indicated this residual carbon level was of high (>1000 MW) molecular weight. The activated sludge preferentially removed the lower (<1000 MW) molecular weight compounds with the higher molecular weight DOC being more resistant to biological attack. However, extended acclimation (greater than one month) enabled the activated sludge to remove the higher molecular weight components from the slurry wastewaters.

Chlorination of the slurry wastewaters reduced the efficiency of DOC removal. The k_2 values for the chlorinated wastewaters were 60% lower than for the unchlorinated wastewaters. Furthermore, acclimation of activated sludge to chlorinated wastewaters reduced or eliminated the ability of the microorganisms to remove DOC from the unchlorinated wastewaters.

The results of this investigation suggest that a fixed film community of microorganisms was capable of the biotransformation of the wastewater DOC. Increases in the removal and mineralization of model

fulvic compounds occurred during the growth and development of the biofilm suggesting an increase in the heterotrophic potential of the microorganisms as the biofilm matures.

TABLE OF CONTENTS

SECTION		PAGE
I.	INTRODUCTION	1
	A. Coal Slurry Wastewater Characteristics	1
	B. Inorganic Constituents in Slurry Pipeline Waters	2
	C. Organic Constituents of Coal Slurry Wastewaters	4
II.	LITERATURE REVIEW	11
	A. Microbial Role in Biodegradation	11
	B. Microbial Communities and Wastewater Treatment	15
	1. Activated Sludge	17
	2. Bacterial Attachment and Biofilm Communities	23
	C. Methods for Estimating Biodegradation of Organic Chemicals	30
	1. Non-specific Methods	31
	2. Specific Methods	33
	D. Kinetics of Xenobiotic Biodegradation	35
	1. Determination of Kinetic Constants from BOD Data	36
	2. Kinetic Models	37
III.	GOALS AND OBJECTIVES	40
IV.	MATERIAL AND METHODS	41
	A. Preparation of Coal Slurry Wastewaters	41
	B. Wastewater Chlorination	43
	C. Activated Sludge	43
	D. Biofilm Growth on Coal Slurry Water	45
	E. Biofilm Sampling	49
	F. Biochemical Oxygen Demand (BOD) and Oxygen Uptake	49
	G. Dissolved Organic Carbon (DOC) Determinations	52
	H. Carbon Dioxide Determinations	52
	I. Determination of Kinetic Constants	53
	J. Molecular Weight Determinations Using Ultrafiltration	54
	K. Biomass Estimations and Microbial Enumeration	55
	L. Mineralization Assays	59
	M. Electron Microscopy	60
V.	RESULTS	62
	A. Wyodak Coal Slurry Wastewater: Biochemical Oxygen Demand	62
	B. DOC Removal from Wyodak Slurry Waters	67
	C. Molecular Weight Fractionation	74

SECTION PAGE

D. Oxygen Uptake 79
E. Semi-Continuous Reactors. 80
F. DOC Removal as a Function of the Biomass Concentration. 84
G. Biodegradation of Chlorinated Slurry Wastewaters. . . 87
1. Molecular Weight Fraction of Chlorinated Wastewater 87
2. DOC Removal from Chlorinated Slurry Waters . . . 88
3. Molecular Weight Distribution of Chlorinated Slurry Waters After Biological Treatment . . . 93
H. Biofilm Growth and Development on Black Mesa Centrate Slurry Water 95
1. Biofilm Growth. 97
2. Biomass Estimations 97
3. Biofilm Mineralization Capacity 104
4. Biofilm Removal of Wastewater DOC 113

VI. DISCUSSION. 117
A. Respirometer Studies and DOC Removal. 117
B. Chlorination Studies 125
C. Biofilm Growth and Development. 130

VII. SUMMARY. 133

VIII. CONCLUSIONS 137

LIST OF REFERENCES 141

VITA 158

LIST OF TABLES

TABLE		PAGE
1.	Inorganic Contaminants in Black Mesa and Simulated Pipeline Transport Waters.	5
2.	Degradation Products Present in Slurry Wastewater Samples.	8
3.	DOC Concentrations and BOD Constants for Wyodak Slurry Wastewaters	63
4.	DOC Removed During Respirometer Studies.	68
5.	First and Second Order Rate Constants for Removal of DOC from Slurry Wastewaters	70
6.	DOC Respired and Accumulated into Biomass During Respirometer Studies.	72
7.	Molecular Weight Distribution of DOC from Wyodak Slurry Water: Percentage Removal from Each Molecular Weight Fraction	76
8.	Oxygen Uptake by the Activated Sludge Using Different Molecular Weight Fractions of Wyodak Slurry Wastewater	81
9.	Residual DOC in Wyodak Slurry Wastewater After Treatment with Activated Sludge	86
10.	Kinetic Constants for DOC Removal from Chlorinated and Unchlorinated Slurry Wastewater by Activated Sludge	90
11.	Molecular Weight Fractionation of the DOC Present in Slurry Wastewater Before and After Biological Treatment	94
12.	Analysis of Variance (ANOVA) of the Colonization of the Cellulose Strips--AODC.	98
13.	Analysis of Variance (ANOVA) of the Colonization of the Cellulose Strips--ATP	99
14.	Incorporation of ^{14}C-Para-Hydroxybenzoic Acid into the Biofilm.	112
15.	Molecular Weight Characterization of Black Mesa Centrate After Contact with a Microbial Biofilm	114
16.	Influent/Effluent DOC Levels in a Continuous Flow System in the Presence of a Biofilm Community	115

LIST OF FIGURES

FIGURE		PAGE
1.	Exploded view of the biofilm continuous flow reactor . . .	46
2.	Assembled biofilm continuous flow reactor.	47
3.	Top and side view of the stainless steel support for the cellulose acetate strips	48
4.	Experimental and theoretical oxygen demand exerted during respirometry studies	65
5.	Molecular weight profile of Wyodak slurry wastewater before and after biological treatment.	78
6.	Rate of DOC removal by an activated sludge population in a semi-continuous (fill and draw) reactor.	83
7.	Removal of DOC from Wyodak slurry wastewater as a function of the activated sludge concentration	85
8.	Increase in the <1000 MW DOC as a result of chlorination. .	89
9.	Comparison of the DOC removed from chlorinated and unchlorinated slurry wastewater by activated sludge. . .	92
10.	Molecular weight profile of chlorinated slurry wastewater before and after biological treatment	96
11.	Electron micrograph of the biofilm community--sample A . .	101
12.	Electron micrograph of the biofilm community--sample B . .	102
13.	Acridine orange direct counts of the microorganisms in the biofilm community	103
14.	ATP determinations for the biofilm community.	105
15.	Rate of ^{14}C-glucose removal from slurry wastewaters by biofilm community	107
16.	Rate of removal of ^{14}C-para-hydroxybenzoic acid from slurry wastewater by a biofilm community	108
17.	Rate of mineralization of ^{14}C-para-hydroxybenzoic acid in slurry wastewater by a biofilm community. . . .	111

I. INTRODUCTION

Coal Slurry Wastewater Characteristics

Coal use in the United States has fluctuated widely over the last 80 years. At the beginning of this century coal supplied over 90% of the total energy used in the U.S. (114). By the end of 1972 coal only supplied 17% of U.S.'s total energy. However, coal is amply abundant in this country with proven reserves of over 280 billion tons. The coal most easily mined, surface coal, is located primarily in the western U.S. and the location of the underground coal is equally split between eastern and western U.S. (127).

As the world's oil supply decreases, the U.S. will increasingly rely on coal, its most abundant fossil fuel, to meet the country's energy demands. Coal transport from producing mines to industrial users traditionally has been by truck, rail, or a combination of the two. An alternative to these transport modes is pipeline transport as a coal-water slurry (87).

Slurry transportation of coal to power plants, shipping facilities, and coal conversion industries is an economically viable alternative for bulk coal transportation (96). While several pipeline transport processes are possible, aqueous slurries have proven to be reliable and efficient in the only functional large scale slurry pipeline in the U.S., operated by the Black Mesa Pipeline Company. The Black Mesa system connects the Mohave Generating Station in Laughlin, Nevada, to its coal supply near Kayenta, Arizona. Another pipeline,

the Cadiz Ohio Consolidated Coal Company pipeline, once operated between 1957 and 1963 but is no longer used (9).

Using anticipated data of slurry transport of 175 million tons of coal per annum, approximately 120 million gallons of water per day will be required (10, 67). Such water requirements represent special challenges to management of local and regional water reserves at the pipeline origin, especially in water limited western states (127). Use of the water at the pipeline terminus will be determined by the quality of the water and specific water needs of that locale.

Wastewater derived from the coal slurry process known as "blackwater" results during the coal/water separation. The Mohave Power Generating Station uses the "blackwater" as cooling water after chemical flocculation to remove coal fines. The overflow resulting from chemical treatment is the predominant wastewater emanating from the transport process. Since over ten additional pipelines are in the planning stage (9) there have been numerous attempts to identify the chemical alterations of the wastewater because of the slurry process (55, 82, 132, 149, 153). These investigators have centered upon both the inorganic and organic contaminants which have leached from the coal into the transport waters during the slurry process.

Inorganics Constituents in Slurry Pipeline Waters

Inorganic characteristics of slurry transport wastewaters reflect the chemical constituents of the transport water as well as the coal type. Interactions among inorganic chemical species during transport can affect the redox potential, pH, solubilization, and precipitation

of both the inorganic and organic chemical constituents of the wastewater (127).

Physical-chemical characteristics of the coal slurry wastewater are primarily dependent upon the coal type (82, 127). The pH of slurry waters ranged from a low of 2.0 for a bituminous coal (Illinois #6) (152) to a high of 9.0 for Black Mesa pipeline slurry waters (8). The level of total dissolved solids (TDS) in slurry waters varies substantially with the coal source. TDS levels as low as 903 mg liter^{-1} were reported for simulated pipeline slurries using Kerr-McGhee coal (149) while Moore (133) reported levels as high as 6230 mg liter^{-1} TDS for slurries prepared with Illinois coals. Although TDS are often amorphous in nature, one study (133) reported that over 80% of the TDS could be attributed to calcium sulfate.

The concentration of sulfates in coal slurry waters also vary widely and may be attributed to the coal type as well as the extent of pyrite oxidation in the coal (127). Values ranging from 171-3151 mg liter^{-1} sulfates are reported for simulated pipeline slurries. However, sulfate concentrations in western coals are generally low. Examination of slurries originating from the Black Mesa pipeline revealed concentrations of only 132 and 186 mg liter^{-1} for sulfates (8, 160).

The dissolution of metals in coal slurry waters has been the subject of several investigations (8, 82, 132, 144, 148, 149). These studies indicate that a wide range of variability exists with respect to coal type and the solubilization of metal species. Thus, the capacity to predict metal concentrations in slurry waters is limited by

the inherent variability associated with the coal in question (127). The following table (Table 1) lists the range of metal concentrations present in Black Mesa and simulated slurry pipeline preparations.

Organic Constituents of Coal Slurry Wastewaters

Organic material present in slurry waters has been measured in a variety of ways. Total organic carbon (TOC) (82, 97), dissolved organic carbon (DOC) (97), biochemical oxygen demand (BOD) (148, 160), and chemical oxygen demand (COD) (145, 149) have been used as techniques for assaying relative amounts of organic constituents present in slurry waters. DOC measures carbon that may be associated with organic matter in solution, but may also include easily oxidizable colloidal or microparticulate organic matter. Biochemical and chemical oxygen demand measurements relate to the quantity of oxygen required by either biochemical processes, or chemical oxidation to convert the organic matter to carbon dioxide (175). Invariably, BOD is less than COD for organic compounds in solutions, since biochemical oxidation is less complete than chemical oxidation (161).

It is readily apparent that the levels of organic material vary widely (8, 82, 132, 145, 148, 149). As with the inorganic constituents, the coal type is the most significant parameter affecting the level of organic material present in the slurry water (127). Even within a given coal type the chemical structure of the coal is not homogeneous. The heterogeneity in structure can be attributed to factors such as the original parent material available as well as the variable environmental factors occurring during the coal formation

Table 1. Inorganic Contaminants in Black Mesa and Simulated Pipeline Transport Waters

Element	Black Mesa[a] Slurry Water (mg liter^{-1})	Other Slurries (mg liter^{-1})	Reference
Al	-	1.63-8.62	132
Ag	0.005	0.002-0.005	149
As	<0.001	0.14-0.028	144
Ba	-	0.932	149
Ca	22.8	90-1660	8, 82, 132
Cd	0.014	0.005-0.014	8, 149
Co	-	0.028	149
Cr	-	0.001-0.6	8, 144, 149
Cu	0.011	0.003-0.13	8, 82, 144
Fe	0.53	0.002-2.35	8, 82
Hg	0.001	0.001-0.008	8, 144
K	-	8-29	82, 132
Li	-	0.07-0.34	82
Mn	-	0.002-8.70	82, 132, 144, 149
Mg	-	25-856	8, 82, 144
Ni	0.015	0.01-0.8	8, 132, 144
Pb	-	0.02-0.37	132, 144, 149
Zn	0.004	0.003-0.207	82, 144, 149

[a]All values reported for Black Mesa were from Reference #8.

process. Plummer and Jordan (149) reported BOD_5 (5 day BOD) values of 7-11 mg liter^{-1} for wastewaters generated from Jacob Ranch coals. The same authors reported BOD_5 levels of 70-165 mg liter^{-1} from bench scale slurries of Kerr-McGhee coals (148). Moore (132), using Kansas coals, obtained BOD_5 levels of 60 mg liter^{-1} for simulated pipeline slurries. Illinois coals tested under similar conditions were found to generate wastewaters with BOD_5 in the 10-20 mg liter^{-1} range. When Moore investigated western coals (Kansas and Wyoming coals) he found higher values in the range of 140-250 mg liter^{-1} (132). Acclimation of the microorganisms was necessary prior to significant oxygen utilization. After acclimation, minimal lag phases are observed in the BOD curves. Moore concluded that organic components of coal slurry waters were readily amenable to biologically mediated removal (132).

When COD measurements were employed to assess organic components in slurry wastewaters, wide discrepancies were noted between different coal types. Anderson et al. (8) reported values for Wasatch coals of less than 10 mg liter^{-1}. In contrast, Godwin and Manahan (82) reported COD levels as high as 2,000 mg liter^{-1} for wastewaters generated from North Dakota lignite slurries. Moore (132) reported intermediate COD levels for Kansas and Illinois slurry waters ranging from 23-92 mg liter^{-1}.

Two other variables, besides coal type, which affect the level of organic material in slurry waters are coal-water ratios (82, 97) and the length of coal storage (149). The affect of coal-water ratios on the concentration of organic material was examined for both

sub-bituminous and lignite coals. Coal-water ratios for slurries ranged from 1:100 to 1:1 and in all coals tested the organic contamination increased with increasing coal-water ratios (82, 97). Similar results are seen with coal storage age (149) in that the levels of organic material in the slurry water increase with the increase in coal storage age.

Although the BOD and COD determinations may accurately assess the level of organic carbon present in slurry waters, these methods fail to provide any information concerning the chemical structure of the organic contaminants. Few studies are available identifying the nature of the DOC contained within slurry waters. Davis and coworkers (55) examined the DOC material in Wyodak and Black Mesa slurry waters and classified over 50% of the DOC as fulvic-like material. Reid (152) performed additional studies to ascertain the chemical structure of both the fulvic and non-fulvic fractions from the same slurry wastewaters. Because of the complex nature of this DOC material, the fractionated DOC was first oxidized by potassium permganate and then methylated with diazomethane to facilitate detection by gas chromatography/mass spectrometry. The results of these degradation/derivitization experiments are listed in Table 2. The compounds are listed in order of increasing elution time.

The organic constituents in the Wyodak non-fulvic fraction are primarily aliphatic in nature while the Wyodak fulvic fraction contains both aromatic and aliphatic compounds. The degradation products contained in this fulvic fraction can be classified into five separate groups:

Table 2.[a] Degradation Products Present in Slurry Wastewater Samples[b]

Acid Esters	Scan #
Wyodak Fulvic Acid	
- Oxalic acid dimethyl ester	32
- Malonic acid dimethyl ester	49
- Succinic acid dimethyl ester	102
- Benzoic acid methyl ester	139
- Glutaric acid dimethyl ester	164
- Adipic acid dimethyl ester	178
- Pimelic acid dimethyl ester	307
- p-Methoxybenzoic acid methyl ester	352
- Suberic acid dimethyl ester	375
- Tricarboxylic acid trimethyl ester	390
- 1,2-Benzenedicarboxylic acid dimethyl ester	438
- 1,4-Benzenedicarboxylic acid dimethyl ester	450
- Methylbenzenedicarboxylic acid dimethyl ester	489
- Dimethyl furandicarboxylic dimethyl ester	507
- Phthalate ester (contaminant)	517
- Furantricarboxylic acid trimethyl ester	603
- Methoxybenzenedicarboxylic acid dimethyl ester (isomer)	611
- Methoxybenzenedicarboxylic acid dimethyl ester (isomer)	621
- 1,2,4-Benzenetricarboxylic acid trimethyl ester	683
- 1,2,3-Benzenetricarboxylic acid trimethyl ester	700
- Metoxybenzenetricarboxylic acid trimethyl ester (isomer	834
- Benzenetetracarboxylic acid tetramethyl ester (isomer))	890
Wyodak Non-Fulvic Acid	
- Oxalic acid dimethyl ester	19
- Malonic acid dimethyl ester	52
- Succinic acid dimethyl ester	106
- Glutaric acid dimethyl ester	171
- Tricarboxylic acid trimethyl ester	405
- Phthalate esters (contaminants)	525-530
- 1,2,3-Benzenetricarboxylic acid trimethyl ester	725
Black Mesa Fulvic Acid	
- Oxalic acid dimethyl ester	23
- Phthalate ester (contaminant)	542
Black Mesa Non-Fulvic Acid	
- Oxalic acid dimethyl ester	25
- Phthalate ester (contaminant)	546

[a]Table taken from reference 152 (Reid, M. C. 1984. Characterization of organic contaminants present in coal slurry wastewaters. Ph.D. Dissertation, University of Tennessee, Knoxville, TN).

[b]Compounds determined as methyl esters via derivatization.

Group	% of Total
benzenecarboxylic acid esters	30
methoxybenzenecarboxylic acid esters	20
furan carboxylic acid esters	10
aliphatic dibasic acid esters	35
aliphatic tribasic acid esters	5

Reid (152) hypothesized that the parent polymers of the fulvic acids from Wyodak slurry waters were "combinations of aryl units linked by aliphatic units."

The DOC in the Black Mesa slurry water yielded completely different results. Few compounds were observed in the Black Mesa slurry wastewater but those identified indicated the DOC was primarily aliphatic in nature. However, Reid (152) concluded that a large percentage of the Black Mesa DOC was oxidized to CO_2 and would explain the paucity of degradation products.

The previous discussion indicates that, depending upon the coal type, the pipeline slurry process will generate significant levels of inorganic and organic contaminants in the resulting wastewater. Potentially, this wastewater is available for other industrial or agricultural uses depending upon its chemical constituents and amenability to physical/chemical and biological treatment. Since up to ten additional slurry transport pipelines have been proposed or are in the design stage (10), the potential for reuse of this wastewater may control the likelihood of completion of these projects. Hence it is imperative to identify the contaminants which leach into the transport

water during the slurry process and to develop processes to improve the quality of the resulting wastewater for potential reuse.

The overall goal of this project was to improve the reuse potential of the slurry water by examining the ability of mixed microbial communities to reduce the organic load of slurry transport waters.

II. LITERATURE REVIEW

Microbial Role in Biodegradation

The continuous cycling of carbon is necessary for the perpetuation of life as we know it. Carbon dioxide is fixed into organic compounds, and these compounds are transformed by other organisms and eventually degraded and liberated as carbon dioxide. The central role of microorganisms in the decomposition of organic carbon is well established (52, 66) and the catabolic reactions of microorganisms are primarily responsible for the turnover of carbon in the environment (52).

Anthropogenic sources of organic carbon contribute significantly to the environmental load of organic carbon. The estimated annual production of synthetic chemicals is 300 million tons (78). Over 1000 new chemicals are brought to market annually and 150 chemicals are produced in excess of 50,000 tons per annum. Many of these chemicals eventually end up being released into the environment and can pose significant health threats (80).

The removal of these xenobiotics from the environment is also mediated by microorganisms. A working definition of xenobiotics is ". . . compounds that are released in any compartment of the environment by action of man and thereby occur in a concentration in this or another compartment of the environment that is higher than natural" (110). Some abiotic transformation of xenobiotics may occur (43) but this conversion rarely leads to appreciable change in the chemical structure of the parent compound (5). Since only

microorganisms utilize these xenobiotics for carbon and energy, the transformation of the parent compound can result in complete mineralization to CO_2 or production of microbial biomass. However, transformation by microbial communities is also the result of cometabolic processes (5, 93). Cometabolism is defined as "any oxidation of substances without the utilization of the energy derived from the oxidation to support growth" (93). During the cometabolic process the microbial population fails to increase in numbers or biomass and there is an accumulation of products structurally similar to the parent compound (5).

Although anaerobic catabolism of organic compounds plays an important role in the cycling of carbon (66), the role of diatomic oxygen is well established (13, 52, 79). Numerous organic compounds remain relatively unsusceptible to biodegradation until oxidation initiates the catabolic process. For example, alkanes are relatively inert until the terminal carbon is oxidized. Catabolism of fatty acids proceeds via beta-oxidation (52) and saturated rings are inert until hydroxyl groups are inserted. One of the best studied aerobic catabolic processes is the biodegradation of aromatic compounds (79, 80). This process is enzymatically mediated by dioxygenases which function primarily in the cleavage of the aromatic ring with the simultaneous incorporation of the oxygen molecule into the organic structure. This reaction appears to be mediated only by procaryotic organisms (80).

The availability of more than one carbon source has long been known to exert a regulatory effect on the catabolic response of microorganisms (131). Since microorganisms in natural environments are

constantly exposed to a wide diversity of organic substrates as potential carbon and energy sources, one would also expect that a heterogeneous substrate mixture would influence the catabolic response of the microbial communities (177).

Microbial populations display one of two types of responses when more than one carbon and energy source is available: sequential or simultaneous substrate utilization (89, 177). Sequential substrate utilization is often accompanied by diauxic growth even in mixed cultures. Sequential substrate utilization could result from the regulation of enzyme synthesis (176) or preferential transport of one substrate over another (89).

Concurrent catabolism of organic compounds by mixed microbial communities has also been reported (138, 177). Activated sludge communities are known for the simultaneous removal of glucose and benzoic acid, or glucose and 2,4-dichlorophenoxyacetic acid. Slater and Lovatt (170) described a sludge population which was capable of catabolizing 20 different isomeric forms of linear alkyl benzene sulfonates. Often this simultaneous substrate utilization can support growth rates of microbial communities which are greater than the sum of the growth rates of the population on the individual substrates (177).

Simultaneous utilization of more than one substrate may be particularly important at low substrate concentrations (89). Although E. coli typically exhibits sequential substrate utilization in the presence of glucose and lactose under batch culture conditions; simultaneous substrate utilization is seen when continuous culture

conditions are employed under carbon limited growth (122, 167). Similar results were observed with Pseudomonas oxalaticus when grown under carbon limited conditions in the presence of oxalate and acetate. However, simultaneous substrate utilization occurred only at growth rates below 0.15 hour^{-1}. When the dilution rate was raised above this value, there was an increasing percentage of oxalate in the effluent (59).

Boethling and Alexander (21) and Subba-Rao et al. (178) have proposed that the substrate concentration is the most significant parameter governing the potential for mineralization by microorganisms. Boethling and Alexander (21) reported the rate of mineralization of xenobiotics is directly proportional to the substrate concentration and there exists a threshhold concentration below which no biodegradation occurs. Although natural environments such as lake water or sewage may contain microbial communities which are capable of mineralizing the xenobiotic in question, mineralization of substrates at low concentrations may not occur (178). Thus, many organic compounds may be inherently susceptible to biodegradation but their in situ concentration may render them recalcitrant.

Although the list of anthropogenic organic compounds which can be catabolized by microorganisms is extensive (103), the accumulation of xenobiotics in the environment demonstrates that many synthetic chemicals are recalcitrant to degradation. Recalcitrance is defined as "the persistence for extended periods in all environments tested regardless whether the compound is or is not inherently biodegradable" (3). Both environmental physical-chemical parameters and the inate characteristics of the organic compound can influence the recalcitrant

property of organic molecules. For example, anaerobiosis in aquatic sediments leads to the accumulation of polychlorinated biphenyls. Halogen substitution on organic moieties may also render aromatics and aliphatic acids less susceptible to biodegradation (4, 43). For hydrocarbon degradation, methyl branching decreases the likelihood of catabolism. Increases in the number of fused aromatic rings increases the turnover time of polyaromatic hydrocarbons in aquatic environments. Atlas (13) found that in marine sediments the turnover time is 7.1 hrs. for naphthalene, 400 hrs. for anthracene, 10,000 hrs for benz[a]anthracene, and 40,000 hrs for benz[a]pyrene.

Although the parameters affecting recalcitrance of organic compounds are extensive, Alexander (3) comprised a list of ten generalizations which will influence the biodegradation of an organic compound in the environment. These include factors such as the unavailability of the appropriate enzymes, substrate impermeability, and substrate concentrations insufficient to support growth.

Although there are many facets which limit biodegradation, microbial communities possess an extensive capacity for the degradation of a diversity of natural and anthropogenic compounds. Indeed, both domestic and industrial wastewater treatment systems often incorporate activated sludge or a fixed film microbial community into the treatment designs (84, 116, 117).

Microbial Communities and Wastewater Treatment

The *in situ* biodegradation of organic compounds is the result of the cooperative metabolic activities of diverse microbial communities. Stable microbial communities are found in most environmental

situations. The metabolic expression of these communities is not merely the sum of the individual members but is significantly affected by the interactions which occur between the members. Considerable descriptive work is available characterizing these interactions between microorganisms which include competition, commensalism, mutalism, ammensalism, parasitism, and predation (28, 74, 124).

The synergistic response is typical of the interactions between microorganisms in the catabolism of organic substrates (170). This combined catabolic response is particularly apparent in the biodegradation of higher molecular weight compounds such as lignins, cellulose, or humic acids. Since one microorganism may not contain all of the essential enzymes, degradation is often the result of a group effort (169). This group effort is described by Burns (31) who examined the catabolism of cellulose utilizing a reconstructed anaerobic community of two microorganisms. Initially, pure cultures of the cellulase producers and the cellobiose fermenters were maintained separately on cellulose as the sole carbon source. Little growth or substrate utilization was observed after 25 days. However, when the cultures were maintained as a two member community, approximately 50% of the cellulose was catabolized with substantial production of carbon dioxide, hydrogen, ethanol, and acetate.

Slater and Lovatt (170) reported similar results when they examined the catabolic activity of individual microorganisms isolated from activated sludge. Pseudomonas putida, Pseudomonas alcaligenes, Arthrobacter globiformis, and Serratia marcescens isolated from activated sludge grew poorly when maintained in pure cultures on linear

alkyl benzene sulfonates (LAS). When all four members were sustained together on LAS, the growth rate of the community was maintained at a much higher rate and significant ring cleavage occurred. Floc formation, which is typically characteristic of activated sludge, only occurred when all four members were combined.

Biological treatment of municipal and industrial wastewaters routinely includes microbial communities to improve the overall quality of the wastewater. Biological treatment is accomplished by utilizing the combined metabolic activity of diverse microbial communities such as those found in activated sludge or biofilms. By better understanding the processes which occur and the parameters which affect the growth and survival of these communities, one may better predict the ultimate fate of xenobiotics in wastewater treatment facilities.

Activated Sludge. Activated sludge is comprised of microorganisms in which Gram negative bacteria predominate. Dias and Bhat (58) examined over 300 isolates from seven different activated sludge samples and concluded that Zoogloea and Comamonas spp. were the predominant genera of microorganisms found in activated sludge. Other genera which are commonly found in activated sludge are Achromobacter, Enterobacter, Alcaligenes, Bacillus, Brevibacterium, Corynebacterium, Flavobacterium, Micrococcus, Pseudomonas and Spirillium. Microorganisms such as coliforms, which are common in sewage, rarely occur in activated sludge (58).

The ability of the activated sludge microorganisms to form flocs is one of the distinguishing characteristics of activated sludge (135). Flocculation can be defined as ". . . the aggregation of suspended bacterial cells" (25). Using ATP measurements to analyze the viability of flocs found in activated sludge, Nelson and Lawrence (135) divided the particulate matter found in flocs into three distinct groups: active or viable microbial solids, nonviable biodegradable microbial solids, and inert or recalcitrant microbial debris.

Microbial production of extracellular polymers is thought to play an important role in the flocculation process (25) and may be the determining factor in the formation of the activated sludge community (61, 73, 158). This extracellular polymer is of high molecular weight in nature and bridges the bacterial cells resulting in a three dimensional matrix. The degree of flocculation in activated sludge is directly related to the quantity of extracellular polymer produced by the microorganisms. Busch and Stumm (30) provided further evidence that this extracellular polymer was responsible for floc formation. They obtained extracts of the polymer and added it to dispersed bacterial populations or an inorganic colloidal suspension of silica. Flocculation occurred in the bacterial population and the colloidal suspension of silica upon addition of the extract.

Parameters external to the activated sludge may also influence floc size and formation. Physical factors such as increase in mixing intensity commonly results in an overall decrease in floc size. Nutrient sources such as the type of substrate and availability will affect floc size. Often flocs will predominate when the available

substrate is complex in nature (34). The substrate/biomass ratio also significantly affects floc size. Those activated sludge systems which support high substrate/biomass ratios often contain flocs of very small size (34). Thus, floc formation is regulated by external environmental factors as well as the metabolic activity of the microorganisms contained in the activated sludge.

The biochemical structural components of the extracellular polymer responsible for floc formation have been the focus of several investigations (25, 30). Brown and Lester (25) compiled a table of 15 separate published reports concerning the analysis of the extracellular polymer extracted from activated sludge (see Table 2 of reference 25). Depending on the extraction procedure, small quantities of proteins and nucleic acids were identified by some investigators. However, all 15 studies established that polysaccharides comprised the bulk of the extracellular polymer. Upon hydrolysis of the exopolysaccharide matrix sugars such as D-glucose, D-galactose, and D-mannose were commonly identified. The polymer also contained numerous uronic acids and hexosamines (25).

Extracellular polymer production by the activated sludge is responsible for many of its inherent characteristics. The polymer provides a matrix for adsorption of dissolved (50) and particulate matter (2). This matrix provides a medium for the hydrolytic enzymes synthesized by the activated sludge microorganisms and slows the diffusion of these enzymes away from the cell. The polymer may also provide protection from the protozoans which commonly graze on microorganisms contained in activated sludge.

Substrate utilization by activated sludge is often viewed as a two stage process (2, 99, 123). First, there is adsorption of the substrate onto the sludge floc and does not require any expenditure of metabolic energy on the part of the activated sludge (123). In the second stage, extracellular enzymes hydrolyze the complex substrates followed by transport and intracellular catabolism. The second stage is highly dependent on the viability of the activated sludge.

Sludge viability is reported to decrease with increasing age of the sludge (135). On a per weight basis, ATP measurements indicate that older sludge is only 50% viable. This reduced viability is probably due to the accumulation of temporarily non-degradable suspended solids (2) such as poly-beta-hydroxybutyric acid (PHB) (58) and extracellular polysaccharides. Dias and Bhat (58) examined over 300 sludge isolates and found that 50% of the isolates were capable of PHB accumulation. PHB granules are intracellular storage materials whose assimilation by bacteria is not necessarily linked to microbial growth and synthesis (60). However, the microorganisms can utilize this carbon reserve for endogenous respiration at a later time. Thus, the production of PHB granules and extracellular polysaccharide represents a carbon sink and are major mechanisms for the removal of organics substrate from wastewater by activated sludge.

Wastewater quality is often improved by activated sludge treatment since the sludge is capable of removing both dissolved and particulate matter (116, 117, 174, 190) as well as potentially toxic metal from the wastewater (25, 26, 27, 62). The kinetics of removal by activated

sludge depends upon the complexity of the substrate (190) and whether or not acclimated or nonacclimated sludge is employed (83).

Besides organic carbon removal, activated sludge is particularly efficient in removing metals from wastewaters. The efficiency of removal depends upon the characteristics of the activated sludge. Dunn and Bull (62) reported that copper was accumulated up to 30% of the dry weight of the sludge. Brown and Lester (25) compiled a list of 19 different published reports which investigated the efficiency of metal removal by activated sludge from actual wastewater treatment facilities, pilot plants and laboratory scale activated sludge units. A wide range of chemicals was efficiently removed: cadmium, calcium, chromium, copper, iron, lead, magnesium, manganese, mercury, nickel, and zinc. Iron, copper, chromium and zinc were removed with the highest efficiency while nickel, calcium, manganese and magnesium were removed with the lowest efficiency.

The extracellular polysaccharide polymers were identified as the active component responsible for the removal of metals by activated sludge (26, 27, 75). Brown and Lester (27) demonstrated that when extracellular polysaccharide production increased there was a concomitant increase in the efficiency of metal removal. The removal of the extracellular polysaccharide from the activated sludge by extraction reduced the capacity of the flocs to remove metals from solution. Prior to extraction approximately 1-10 mg liter^{-1} of cadmium, nickel, or cobalt were removed from solution. After extraction the capacity for adsorption only reached 0.01 mg liter^{-1} (26) representing a 100-1000 fold reduction.

Two components of the activated sludge system must be considered in the design of a wastewater treatment plant: the biological reactor and a phase separator (25). The biological reactor stage contains large populations of microorganisms which participate in the aerobic degradation of organic compounds contained in the wastewater. The second stage comprises settling tanks or phase separators in which the flocs of the activated sludge settle. From here the settled sludge may be returned to the reactor while the clarified effluent is discharged.

The rationale behind a two phase system is that the rate of dissolved organic carbon removal is highest when shear rate is high, under intense oxygenation, which occurs in the first stage. The efficiency of removal of suspended matter is lowest under these conditions. The optimum conditions for particulate removal by activated sludge occurs in the settling tanks which have low shear rates and slow growing biomass (2). Thus, the two stage design promotes efficient removal of both dissolved and particulate organic matter.

Other design parameters affect the efficiency of wastewater treatment by activated sludge. Older sludge is often better than younger sludge since there is less deterioration in water quality when subjected to mass loading (185). Although the growth rate of older sludge is not as great as that for young sludge, growth rate is not as critical as the storage capacity for carbon matter. This capacity is greater in older sludge. The substrate feeding pattern also affects treatment (189). Wastewater fed to the sludge intermittently as compared to continuously results in a higher substrate utilization rate

and higher oxygen consumption rates. This type of activity is indicative of a highly active microbial community.

Bacterial Attachment and Biofilm Communities. Although activated sludge possesses certain desirable characteristics as a wastewater treatment method, there has recently been a renewed interest in the fixed film biological processes (84). Furthermore, fixed film systems are less energy intensive and can maintain higher solid retention times coupled with larger volumetric flows than activated sludge systems (44).

Zobell (201) was one of the first to report that the presence of surfaces increases bacterial activity. He demonstrated that the bacterial utilization of hydrocarbons in seawater was accelerated two to ten-fold when additional surface area was provided in the form of sand or shredded asbestos. He hypothesized that microorganisms may concentrate at the surface since dilute nutrients present in seawater tended to absorb to surface layers. Surfaces also hinder the diffusion of bacterial exoenzymes away from the cell. Soluble enzymes tend to be more stable when absorbed, thus extending the life of the hydrolyzing bacterial exoenzyme. Any one or all of these phenomena could explain the stimulatory effect of surfaces on bacterial activity.

The presence of microorganisms at liquid/solid interfaces has a stimulatory effect on their growth and metabolism. Kjelleber and coworkers (102) reported that a marine vibrio would grow and divide at solid surfaces under nutrient concentrations which were too low to support growth of the microorganisms in the aqueous phase. Similar

findings were reported by Elwood et al. (65) in that the growth rate of a freshwater pseudomonad was two-fold higher when it was attached to a solid surface than when it was free-living.

The surface stimulatory effect on growth reflects an overall increase in the metabolic activity of microorganisms at solid/liquid interfaces. The metabolic activity of attached microorganisms present in 44 different aquatic environments was examined by comparing the turnover times for glucose and amino acids. Attached microorganisms had higher turnover times for both compounds even though there were comparable populations of free floating microorganisms (17). Laboratory studies also confirmed that microorganisms were often metabolically more active when attached to a substratum. Amino acid assimilation and electron transport activity of a marine pseudomonad were compared in the attached versus the free-living mode under laboratory controlled conditions. In all cases the attached pseudomonad displayed higher amino acid assimilation and electron transport activity (24).

Recently there have been numerous reports concerning microbial attachment and the processes occurring within a fixed film (i.e. biofilm) (41, 69, 128, 188). Eighmy et al. (64) defined a biofilm as an ". . . assemblage of bacterial cells that is both enclosed by and attached to a wetted surface by means of an extracellular fibrous polysaccharide containing matrix." He concluded that the biofilm represents a microbial community markedly different from unattached microorganisms.

Attachment to a surface by microorganisms is heavily influenced by the physical-chemical parameters of the immediate microenvironment as well as the physiological response of the individual microorganisms. Surface conditioning plays an important role in the attachment process. Prior to bacterial attachment the surface is preconditioned by the adsorption of an organic layer (120). This adsorption occurs almost immediately in aquatic environments. Once the organic layer is adsorbed the microorganisms begin to concentrate at the substratum. The attachment of microorganisms is hypothesized to occur in two distinct steps: reversible sorbtion and irreversible sorbtion (121). In the reversible sorbtion stage the bacteria are transported or collide with the surface and are weakly held in place through electrostatic interactions and van der Waals forces. At this point of the attachment process the microorganisms are easily dislodged from the surface. At a critical time after initial adsorption the microorganism becomes irreversibly bound. This irreversible sorbtion is mediated through somatic structures produced by the microorganism (40). Electron microscopic examination of biofilms (6, 56) have implicated numerous somatic structures of microorganisms utilized for attachment. These include pili, stalks or holdfasts, and exopolymers (71).

Although pili and stalks are important, microbial exopolysaccharides have been implicated as the primary mechanism of bacterial attachment to surfaces (180). One investigator has suggested that all natural bacterial populations are enveloped in this exopolysaccharide layer (47). This layer has been observed by direct

microscopic examination of bacterial populations in situ inhabiting the soil, freshwater sediments, marine ecosystems, bovine rumen, and human infections of the lung and bladder (47). All microorganisms observed were surrounded by this glycocoalyx.

The glycocoalyx is defined as ". . . any polysaccharide containing bacterial surface structure that is distal to the surface of the outer membrane (lipopolysaccharide containing layer) of Gram negative bacteria or to the surface of the peptidoglycan (murine) layer of Gram positive bacteria" (47). Numerous studies have reported the anionic nature of the glycocoalyx (46, 71). Ruthenium red, which stains acidic polysaccharides, is commonly employed to visualize the glycocoalyx for electron microscopy. Although 98% of the glycocoalyx is comprised of water the remaining constituents are linear or branched polymers of carbohydrates (39). The different monomeric subunits of the exopolysaccharide have been identified as fucose, glucose, galactose, glucuronic and uronic acids. However, each individual microorganism is capable of producing its own unique glycocoalyx.

A wide range of functions are attributed to the glycocoalyx of bacterial cells. Production of the exopolysaccharide is stimulated by nutrient limitation, specifically, nitrogen, sulfur, and phosphorous. Thus the glycocoalyx could serve as a carbon source when these limiting nutrients become available (39, 180). As previously mentioned the glycocalyx primary function is for bacterial attachment. The glycocoalyx is produced as a fibrous network bridging the cell to the surface. This bridging is necessary to overcome the electrostatic repulsive forces between the cell and the surface. The glycocoalyx

also serves in an ion exchange capacity and as a matrix where nutrients can concentrate (47). The anionic polysaccharide layer can also provide a protective function for the attached microorganisms such as a buffer zone between the microorganisms and their immediate environment. Bacteria within the glycocoalyx have greater resistance to phagocytosis, antibiotics or surfactants (46).

Although microorganisms are capable of attachment to solid surfaces, environmental physical-chemical parameters greatly affect this process. The extent of sorbtion of microorganisms onto surfaces is dependent on the surface properties of the suspending liquid medium, substratum, and adhering particles (microorganisms) (1).

Surface characteristics are an important variable to consider when examining bacterial attachment. Increased surface area will routinely lead to increased production of the glycocoalyx by the biofilm microorganisms (188). Attachment of microorganisms is also affected by the substratum's charge and will be less efficient the more electronegative or hydrophilic the surface (150).

In vitro studies are often employed to ascertain which parameters contribute to the attachment process of microorganisms. One important variable appears to be the nutrients present in the suspending medium. Both the type and concentration of nutrients significantly affect attachment. The presence of non-basic proteins diminishes attachment (69) while carbohydrates such as galactose stimulate glycocoalyx production (188). Biofilm development is maximum during periods of metabolic stress brought on by carbon limitations. However, individual bacterial genera have been reported to respond differently to carbon

limitation. Fletcher and McEldowney (72) reported carbon limitation promoted the attachment of a Chromobacterium species, reduced the attachment of a Flexobacter species, and had no affect on the attachment of Pseudomonas.

Culture conditions employed in laboratory studies to examine biofilm development also affect the attachment process. Molin and Nilsson (129) established the efficiency of attachment increases as the pH of the suspending medium was increased up to a pH of 7.0. Biofilm buildup can be significantly different under batch versus continuous culture conditions. In batch culture the cells in an older culture adhere much less effectively than cells in log phase (70). Under continuous culture conditions as the growth rate is increased the biomass also increases in the biofilm (125, 128).

Thus, biofilm development is a complex process influenced by a wide range of variables. These variables include factors such as the physical characteristics of the substratum and suspending medium as well as the physiological status of the attaching cells.

Microbial biofilms were first introduced into the United States as a means for biological treatment of wastes around 1889. However, the first commercial unit was not installed until 1908 in Columbus, Ohio, and Reading and Washington, Pennsylvania (11). Since the development of fixed films for biological treatment of wastewaters, numerous mathematical models have been published to help predict the substrate removal capacity for fixed films (7, 12, 182, 194, 195). (For an extensive review of the mathematical modeling for substrate removal by biofilms see reference 84). This review will not attempt to summarize

these models, however, it will cover some of the underlying assumptions incorporated into these models.

Biofilm development during wastewater treatment is limited by the incoming substrate concentration (104). If there is an increase in the substrate concentration or the hydraulic loading there will be a concomitant increase in film thickness (136). As film thickness increases there is also an increase in substrate removal rate until a critical film thickness is reached (84). Although film thickness (and thus the biomass of the microbial community) increases, the viability of the fixed film levels off. LaMotta (104) examined the ATP concentration during biofilm development and noted a direct correlation between ATP and film thickness. However, at a specific thickness (50-100 microns) there was no further increase in ATP although the biofilm continued to accumulate until it reached 200 microns in depth. These results led to the hypothesis that the fixed film community can be divided into an active and an inactive layer (84). The active layer is defined as an area where substrate removal occurs and is the layer primarily responsible for the water quality of the resulting effluent. The rate of substrate removal by the biofilm is a result of the combination of the transport rate of the substrate from the bulk liquid (187) and the reaction rate occurring within the fixed film community (84). The first step appears to be rate limiting. Thus, the current model is that substrate removal is directly proportional to film thickness up to a critical value corresponding to a depth where substrate penetration no longer occurs (84, 105).

Sufficient oxygen is necessary for the metabolic oxidation of organic wastes by a fixed film community. Oxygen diffusion within a biofilm decreases with increasing thickness of the film (84). At a thickness of about 100 microns oxygen microprobe analyses revealed anoxic conditions (29, 91). This depth corresponds approximately to the active film depth (90). Thus, substrate removal may be limited by both diffusion of substrate and oxygen in the fixed film microbial community.

Methods for Estimating Biodegradation of Organic Chemicals

Numerous assays are available to assess whether a specific organic compound or class of compounds is capable of being degraded (33, 108, 113, 183). All of these assays consist of incubating the compound(s) or wastewater of interest with a particular microbial community (pure or mixed cultures) and then attempting some analytical assessment of degradation. Methods for assessment of biodegradation can be divided into two general categories: non-specific and specific (200). Non-specific methods include biochemical oxygen demand (BOD) or assessment of organic carbon loss (COD, DOC or TOC measurements). Other non-specific measurements include determination of carbon dioxide evolution by the microbial communities during the incubation with the appropriate wastewater or xenobiotic. Specific methods for estimation of biodegradation include disappearance of parent compound or production of specific metabolites (including carbon dioxide) from the compound of interest.

Non-specific Methods. During the aerobic catabolism of organic matter oxygen is consumed by the microorganisms involved. Increased oxygen utilization is indicative of increased catabolic activity (163). Thus, oxygen consumption rates in the form of the BOD test are often employed as an indirect measure to assess the pollution potential of wastewater which contains organic matter (76). The purpose of the BOD test is to determine the dissolved oxygen (DO) demand of a particular wastewater and then reduce the demand. This reduction prevents a drain of the receiving water DO caused by direct discharge of the wastewater.

The classical method for determining the BOD is through the "dilution method" (175). This method involves the measurement of oxygen consumption by a seed inoculum incubated with the wastewater. The technique is called the dilution method since the wastewater is often diluted prior to inoculation so the limiting factor is the carbon source and not the oxygen. The classical dilution method for determination of the BOD has drawn criticism over the years and the development of respirometric techniques are touted as being superior for BOD determination (19, 130, 164, 183). The primary advantage of respirometric methods is they provide continuous measurements of oxygen consumption (183). Furthermore, the BOD measurements made by respirometric methods are routinely found to be higher (130) and the reproducibility (precision) of the measurements are far greater using a respirometer (19).

Young and Clark (197) initially described an improvement for the respirometer--the electrolysis cell. The electrolysis cell serves as a manometer for the respirometer by detecting oxygen uptake in the

sample. As oxygen is consumed the cell produces oxygen electrolytically to maintain a constant partial pressure (198). Larsons and Perry (108) employed the electrolytic respirometer to examine the biodegradation potential of anionic, cationic, and nonionic surfactants in Ohio River water. They concluded that the extent and rate of biodegradation of organic compounds can be estimated using respirometric techniques.

When the electrolytic respirometer was compared to the dilution method in both laboratory and field tests, the electrolytic respirometer proved to be superior (199). The major advantages of the electrolytic respirometer are:

1) no dilution required
2) larger samples may be analyzed
3) test can run days-weeks
4) simple to operate
5) greater precision
6) shorter analysis periods (ie. three day BOD using respirometer was equivalent to five day BOD using the dilution method)

Other non-specific methods for estimating the biodegradative potential for organic compounds include carbon dioxide evolution (nonradiolabeled) (81, 108) and DOC disappearance (106, 156). Analysis of carbon dioxide evolution provides information on the potential for ultimate biodegradation. This method alone may underestimate the true potential for biodegradation since organic chemical may be removed from solution by alternate mechanisms aside from mineralization. Analysis of DOC removal in combination with determination of carbon dioxide

evolution, the true biodegradative potential of organic compounds may be determined (106).

Rogers et al. (156) employed a carbon mass balance when examining the potential for microbial degradation of oil shale retort water. They accounted for DOC removal and carbon dioxide evolution as well as the amount of carbon assimilated into microbial biomass. They found that 30% of the organic compounds were capable of being degraded and the loss of DOC could be completely accounted for by carbon dioxide evolution and assimilation of the DOC into biomass. Estimation of mass balance (with respect to carbon) may also provide information in addition to the biodegradative potential. High DOC removal rates coupled with low carbon dioxide evolution may indicate sorbtive properties of the organic material or potential toxicity of the compounds (106).

Specific Methods. These methods for estimation of biodegradability are specific with respect to the compound or group of compounds being examined (200). With the non-specific methods any organic compound present in solution may contribute to carbon dioxide evolution, oxygen consumption, or the DOC concentration. The specific methods focus on the biodegradative fate of the individual parent compound(s) (33).

The application of these methods involves addition of a known concentration of a parent compound with a microbial population (mixed or pure). After a designated incubation interval determination of the concentration of either substrate removed or appearance of metabolites

is made (38, 112, 151, 166). Quantitation is often accomplished using thin layer chromatography (181), gas chromatography (193), ultraviolet, visible, infrared and fluorescence spectrophotometry (35), mass spectrometry (37), or any other of a host of analytical techniques (200). Often the parent substrate may be radiolabeled to facilitate detection of substrate loss (146) or metabolite production (36) which includes ^{14}C carbon dioxide (32). One of the more popular and definitive techniques to assess the biodegradative potential of an organic compound is the radioisotope assay (48, 68, 86). This technique employs the use of a ^{14}C-radiolabeled parent compound and measures the amount of ^{14}C carbon dioxide liberated after degradation. Detection of ^{14}C-CO_2 from ^{14}C-labeled substrate was originally developed by Parsons and Stickland (143) and later modified by Caparello and LaRock (33) to assess the ability of microorganisms in the natural environment to mineralize hydrocarbons. These investigators concluded that the detection of ^{14}C carbon dioxide was indicative of the mineralization potential of hydrocarbons in natural environments (33). Since the inception of this method the biodegradative fate of a wide range of organic compounds ranging from C_1 compounds (ie. carbon tetrachloride) (22) to complex carbon polymers such as lignin and lignin-cellulose (48, 68) were assessed utilizing the basic method of Caparello and LaRock. Examination of only mineralization ignores the fact that organic compounds may be transformed by microorganisms without complete oxidation to carbon dioxide (137, 147). To circumscribe this problem investigators will often include the analysis of ^{14}C carbon dioxide and the amount of parent substrate removed or transformed (22, 172).

The identification of metabolic intermediates during the biodegradation of xenobiotics not only promotes the understanding of the degradative pathways involved, but may also elucidate the potential for formation of toxic or carcinogenic intermediates. This method for assessing the biodegradation can also facilitate our understanding the mechanism of recalcitrance (38). For example, in an elegant series of reports (35, 36, 37) Cerniglia and coworkers identified the metabolic intermediates and formulated the proposed pathway for the biodegradation of naphthalene by the fungus Cunninghamella elegans. His most recent report states that fluoro substitution interferes with naphthalene metabolism by the fungus since fluoro substituents block the epoxidation of the fluoro substituted double bond (38).

Thus, examination of the disappearance of parent compounds, production of metabolic intermediates, and evolution of carbon dioxide all further our understanding of the potential for biodegradation of xenobiotics.

Kinetics of Xenobiotic Biodegradation

A qualitative evaluation of the biodegradative potential of xenobiotics is not sufficient to fully understand the ultimate fate of these compounds in the natural environment. A quantitative assessment of this potential must also be made. The development of kinetic parameters is essential to predict the rate of biodegradation of specific organic compounds in situ. The development of kinetic rate constants provides a concentration independent estimation of how fast

degradation can occur (106) and establishes a baseline for comparative purposes (15, 140, 162). One may utilize these kinetic constants to compare the degradative potential between different organic compounds (162), between the same compound under different environmental conditions (54), or as a comparison with other studies available in the literature (162).

Determination of Kinetic Constants from BOD Data. Oxygen consumption by "seed" microorganisms has historically provided kinetic parameters for the evaluation of the quality of a wastewater (63, 173). The determination of the BOD for wastewaters provides information concerning the rate of utilization of dissolved oxygen (76). While the oxygen is being consumed during the course of the BOD experiment the rate at which the residual oxidizable material decreases becomes increasingly less eventually reaching some lower limit. Mathematically the BOD for a wastewater is described by the first order decreasing rate curve (77) which can be represented by the following equation:

$$Y_t = L\ (1-10^{-kt})$$

Where Y_t is the BOD for any time period t, L is the ultimate BOD for the particular wastewater, and k is the velocity rate constant. This equation was first formulated in 1927 (184) to describe the kinetics of oxygen consumption by a wastewater. Numerous methods are now available to calculate k and L from the BOD data (134, 165, 186). (For a review of these methods see reference 77 and 19.)

Kinetic expression of oxygen consumption does not provide estimates of the true organic load of a wastewater since certain xenobiotics may be slowly oxidized or completely recalcitrant (5). However, Larsons (106) found the rate of surfactant degradation, as well as the extent of degradation, were comparable whether oxygen consumption or carbon dioxide evolution was employed to determine biodegradation rates. Thus, BOD kinetic parameters can be used as a measure of the organic load of a wastewater but should be done with caution (14).

Kinetic Models. A first order kinetic approach with respect to substrate concentration and biomass concentration is often employed to describe the rate of xenobiotic biodegradation in the environment (15, 107). To develop rate constants many investigators utilize a modified Monod expression (139, 140, 141, 168) described by the following equation:

$$-d[S]/dt = u_{max}\ [S][B]/Y\ (K_s + [S]) \qquad \text{(eq. 1)}$$

This equation states that the substrate disappearance (-d[S]/dt) is dependent upon the substrate ([S]) and biomass ([B]) concentration as well a the yield factor (Y = the number of bacteria produced per milligram of substrate), K_s (one-half saturation constant), and u_{max} (maximum growth rate) (140, 141). At low substrate concentrations where $K_s >> [S]$ the expression is modified to:

$$-d[S]/dt = (u_{max}/Y\ K_s)\ [S]\ [B] \qquad \text{(eq. 2)}$$

$$= k_2\ [S][B] \qquad \text{(eq. 3)}$$

This expression states that substrate disappearance is a first order process with respect to [S] and [B] but second order overall (140). If the biomass is constant the first order rate constant (k_1) can be calculated by plotting the log [S] versus time. These data are analyzed by the least squares method (171) and the regression equation for this line then becomes:

$$\log [S] = \log [S]_o - k_1 t \qquad \text{(eq. 4)}$$

where $[S]_o$ is the initial substrate concentration and k_1 is the first order rate constant (107). The k_1 is often normalized to the biomass concentration to obtain the second order rate constant, k_2 (107, 139, 140, 141, 142).

Although k_2 is routinely determined by normalizing k_1 to biomass concentration, Paris et al. (139) calculated k_2 for the biodegradation of the pesticide malthion by measuring u_{max}, Y, and K_s since by equation 3:

$$k_2 = u_{max}/[(Y)\ (K_s)]$$

He concluded that both methods yielded similar results thus substantiating the validity of determining rate constants by the least squares method.

Since the inception of the second order kinetic model to describe biodegradation, numerous reports are available substantiating that biodegradation of xenobiotics is first order with respect to substrate and biomass concentration (15, 139, 140, 141, 162). Paris and coworkers (140) examined the transformation of malathion, chloropropham, and the butoxyethyl ester of 2,4-dichlorophenoxyacetic acid in natural waters from 40 separate locations around the U.S. In

all cases examined the rate of disappearance of these compounds from natural waters fit the second order kinetic model.

The linear transformation of non-linear data in order to determine rate constants for xenobiotic degradation introduces a certain amount of bias into the mathematics (51, 107). The occurrence of lag phases and asymptotes in the course of biodegradation cause difficulty when the data is fit with conventional linear regression techniques. Some investigators propose developing kinetic parameters using non-linear methods (107, 168). However, the utility of this approach over the linear regression method are yet to be firmly established.

III. GOALS AND OBJECTIVES

The overall goal of this investigation was to determine the potential for biological treatment of coal slurry waters. Since previous reports (55, 152, 153) have documented that significant levels of organic contaminants leached from the coal into the transport waters during the coal slurry process, the biologically mediated removal of these compounds was examined. Specific objectives include:

1) Developing predictive capabilities to aid in assessing the removal of DOC from slurry waters.
2) Determining the fate of specific contaminants (or classes of contaminants) comprising the DOC of the slurry waters.
3) Examining the affect chlorination has on the biological treatment of slurry waters.
4) Analyzing the growth and development of a biofilm community and its ability for biological treatment of pipeline slurry wastewaters.

IV. MATERIAL AND METHODS

Since the use of coal slurry pipelines for coal shipment will employ large volumes of water, methods were developed to assess the potential for biological treatment to improve the quality of the resulting slurry wastewater. Initial studies focused upon the development of kinetic parameters for DOC removal by biological treatment to describe the overall improvement of the slurry wastewater quality. During this stage of the investigation the response of mixed microbial populations to a heterogeneous substrate mixture (slurry wastewater) was examined. The second phase of the investigation explored the growth and development of microorganisms in a biofilm nourished by slurry wastewaters.

Preparation of Coal Slurry Wastewaters

Two distinct classes of slurry wastewater were examined: laboratory generated slurry waters and a wastewater collected from an operational slurry pipeline, courtesy of Mr. Greg Hoxing (Black Mesa Pipeline Company, Laughlin, NV). Laboratory generated slurry wastewaters were prepared from Wyodak coal. Wyodak coal is a surfaced mine, low sulfur, sub-bituminous coal and was obtained from Powder River Basin in northeastern Wyoming. All samples were obtained from and stored at Oak Ridge National Laboratory (ORNL), Oak Ridge, Tennessee, in sealed 55 gallon drums for approximately two to four years prior to use. The coal was prepared by crushing with a laboratory scale Wiley Mill to pass through a #40 mesh screen (<0.29 mm). The coal was then mixed with lake water (Melton Hill Reservoir,

Oak Ridge, TN) in a 1:1 (w/v) ratio in a 12 liter glass vessel which had been shielded from any light. Any head space in the container was purged with nitrogen prior to mixing in order to simulate anaerobic conditions found in the actual coal slurry pipelines. The coal/water mixture was then stirred for three days by attaching a glass stir rod with a teflon propeller to a Sears Craftsman® Reversible variable speed drill (3/8").

After three days of mixing, coal/water separation was achieved by centrifugation at 10,000 rpm for 10 minutes in a Sorvall RC2-B centrifuge (Ivan Sorvall Inc., Norwalk, CT). The supernatant was then filtered through a 0.4 μm Nucleopore (Nucleopore Corp., Pleasonton, CA) filter to remove coal fines and the resulting slurry water was stored at 4°C until needed. Any organic carbon material remaining in the slurry water was considered dissolved organic carbon (DOC). The process conditions are an approximation of actual transport conditions designed to simulate anaerobic transport time, temperature, and mixing conditions in the pipeline transport, yet allow experimental control and standardization of laboratory based research. The Black Mesa Centrate (BMC) wastewater was treated in a similar fashion. Upon receiving the BMC from the Mohave Power station it was stored at 4°C until needed. Since the BMC still contained appreciable amounts of coal fines it was also subjected to filtration (0.4 μm filter) prior to use.

Wastewater Chlorination

One industrial reuse of slurry transport waters is as cooling waters. Since cooling waters are often subjected to chlorination to prevent biofouling (88), studies were undertaken to determine the effect of chlorination on biological treatment of slurry waters. Chlorinated slurry wastewater was prepared by adding hypochlorous acid (Clorox®, Clorox Co., Oakland, CA) to the filtered wastewater at a 2:1 chlorine to carbon ratio. The chlorine content of the Clorox® was determined by the iodometric method as described in Standard Methods (175). Chlorine contact time was 48 hours at room temperature in the dark. The chlorination reaction was quenched with sodium thiosulfate, added at sufficient concentrations to neutralize at least twice the amount of chlorine. Control slurry wastewaters for chlorination experiments were treated in a similar fashion except the hypochlorous acid was quenched with sodium thiosulfate prior to addition to the slurry wastewater.

Activated Sludge

Activated sludge samples were collected from aerobic treatment ponds located at the Knoxville Municipal Waste Treatment facility or American Ekna Company. The American Ekna Company is located in Lowland, TN and produces man-made fibers such as nylon, rayon, and polyester. Once collected the sludge was immediately transported back to the laboratory and maintained in the presence of coal slurry wastewater (pH 7.0-7.2) as their sole carbon and energy source.

Acclimation of the activated sludge was accomplished using the fill and draw technique (50). Approximately 300 ml of the activated

sludge were transferred to a 500 ml Erlenmeyer flask and shaken on a rotary shaker (150 rpm). The flask was shaken in order to maintain oxygen saturation within the vessel and also to maintain the flocs in suspension. After 48 hours the flask was removed from the shaker and the solids (flocs) were allowed to settle to approximately 1/3 of the working volume. One hundred ml of the clarified supernatant was removed and then replaced with 100 ml of fresh slurry water. The flask was returned to the shaker and this cycle was continually repeated to ensure acclimation.

During the course of the investigation the fill and draw reactors were utilized to examine the substrate removal rate in a semi-continuous system. Duplicate fill and draw reactors were prepared with the activated sludge and Wyodak slurry wastewaters. Two separate sets of reactors were prepared with the only difference between the two being the concentration of the activated sludge. One set of reactors contained twice the biomass level as compared to the other set (1,836 mg $liter^{-1}$ vs. 3,525 mg $liter^{-1}$). Again, the reactors were subjected to one month of the fill and draw cycle as previously described. The biomass concentration remained the same throughout the study and there was no sludge wasting during the course of the investigation.

The rate of substrate (DOC) utilization was viewed on the basis of a mass balance approach which is the same as that reported for a completey mixed activated sludge treatment plant (179) using the following equation:

$$v = dS/dt = So-Se/t$$

where So is the initial substrate concentration in the reactor's supernatant and Se is the substrate concentration in the supernatant after time t (usually 48 hours).

Biofilm Growth on Coal Slurry Water

For the final phase of this research the potential for wastewater treatment utilizing a microbial biofilm (fixed film) was investigated. Although biofilms are routinely employed in wastewater treatment designs, the microbial processes occurring in the biofilm are poorly understood. Thus, the purpose of this stage of the investigation was two-fold:

1) examine the biodegradation of a model compound which represent the structural unit of humic acid compounds
2) examine the microbial processes occurring during the formation of a microbial biofilm when grown on Black Mesa Centrate.

A microbial biofilm was grown on cellulose acetate strips using a modified bench top chemostat (Bioflo, New Brunswick Science Co., Edison, NJ). Sterile BMC (pH 7.0-7.2) was introduced into the growth vessel (see Figure 1 and 2) at a specified dilution rate (0.9 day^{-1}). At designated time intervals activated sludge was introduced into the growth vessel. To insure equal colonization of the acetate strips by the activated sludge, the strips were cut into sections which were 3.5 cm in width and 115-120 cm in length and threaded onto stainless steel developing reels (size 220) (Figure 3) (Samigon Division Argraph Corp., Carlstadt, NJ). This method permitted maximum exposure of the surface area of the strips to the wastewater/

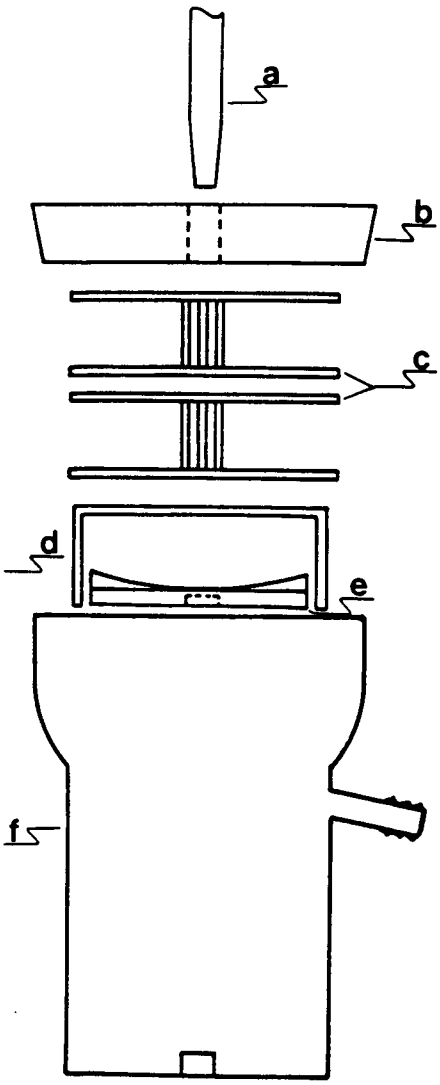

Figure 1. Exploded view of the biofilm continuous flow reactor. a) influent and aeration source, b) rubber support, c) stainless steel reels for support of cellulose acetate strips, d) glass table support, e) stirring mechanism, f) reactor chamber.

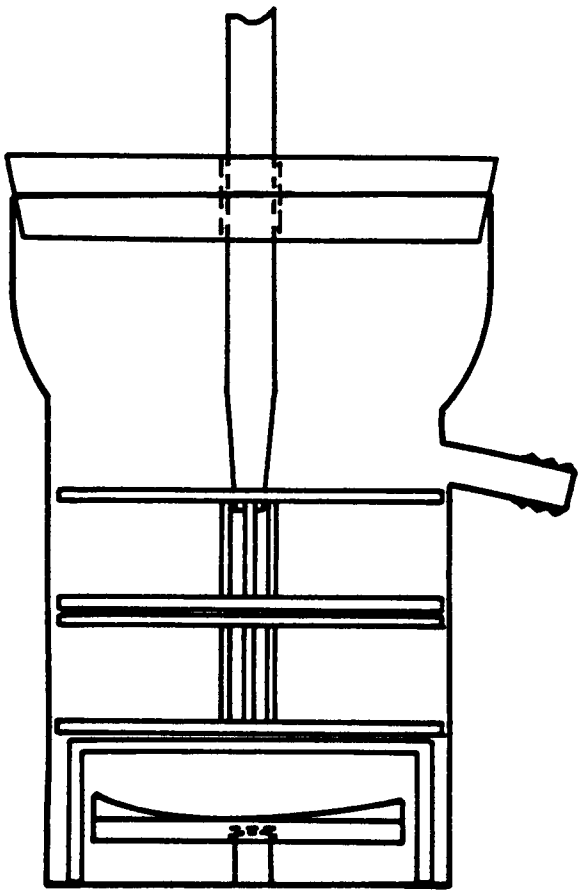

Figure 2. Assembled biofilm continuous flow reactor.

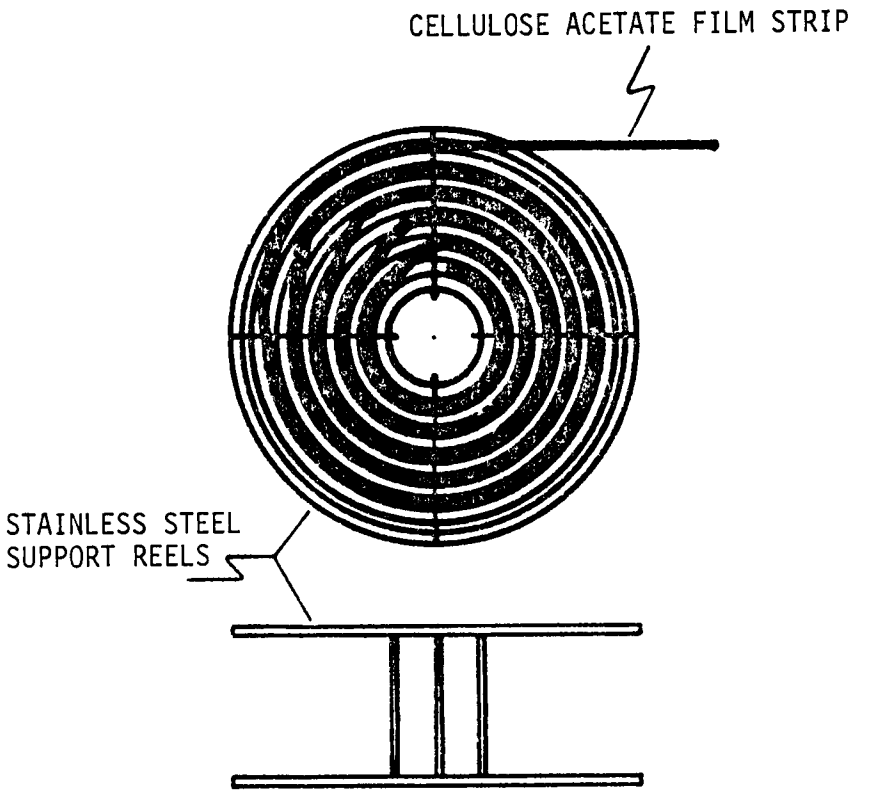

Figure 3. Top and side view of the stainless steel support for the cellulose acetate strips.

sludge and still allowed a homogeneous solution of the sludge/slurry wastewater mixture.

Biofilm Sampling

Subsections of the cellulose acetate strips were routinely taken for analysis during the course of biofilm development. To collect a sample, the stainless steel developing reel was removed from the growth chamber and the desired length of the cellulose acetate strip was unwound and cut from the reel. The sample strip was immediately and gently submerged into 50 ml of phosphate-buffered saline (pH 7.6) (PBS) to prevent water loss from the biofilm.

The biofilm sample was then cut into subsections, 3 cm x 3.5 cm rectangles, and each rectangle was washed in PBS to remove superficially attached microorganisms. Washing was accomplished by placing the 3 x 3.5 cm rectangular strip into a test tube containing 30 ml PBS. The tube was then placed in a horizontal position on a Kraft wrist action shaker (Kraft Apparatus Inc., Minneola, NY) and shaken for 10 seconds with a 4 cm arc at an approximate speed of 130 shakes/min. The sample was then removed from the wash solution and subjected to the appropriate analysis.

Biochemical Oxygen Demand (BOD) and Oxygen Uptake

Biochemical oxygen demand for slurry waters was determined using the E/BOD electrolytic respirometer system (Oceanography International, College Station, TX). In this system both oxygen and carbon dioxide are removed from the sample. Oxygen was removed chemically or biologically and carbon dioxide was removed from the head space by an

alkali CO_2 (2N KOH) trap creating a drop in pressure in the head space of the respirometer vessel. The pressure drop changed the level of the electrolyte (2N H_2SO_4) in the electrolysis cell completing the circuit. Oxygen was produced electrolytically and simultaneously measured by integration of the amperage over the cross sectional area of the electrode.

Since the E/BOD respirometer contains only three vessels, duplicate samples were routinely run with the third vessel serving as a control for endogenous respiration. In the chlorination studies the third vessel contained the control chlorinated slurry water. Prior to inoculation phosphate buffer (1.0 ml $liter^{-1}$) was added to the coal slurry water and the slurry water was adjusted to a pH 7.0-7.2 with NaOH or concentrated HCl. Approximately 500 ml of slurry water were then added to duplicate vessels while lake water (pH 7.0-7.2), which was used to prepare the slurry water, was added to the third vessel. Control values for endogenous oxygen comsumption were routinely subtracted from the experimental values. Each sample was equilibrated to the incubation temperature (25°C) prior to inoculation.

After equilibration, activated sludge or microorganisms acclimated to slurry water were used to inoculate each vessel. Prior to inoculation the inoculum was washed twice in PBS (pH 7.6) and then resuspended in 10-20 ml of PBS. Each vessel was then inoculated with equivalent amounts of the suspension. Final concentration of seed inoculum was approximately 1.0×10^7 to 1.0×10^8 bacteria ml^{-1}. An alkali trap containing 5.0 ml of 2N KOH was placed at the top of each vessel and then sealed with an electrolysis cell containing

30-40 ml of electrolyte (2N H_2SO_4). The vessels were then connected to the E/BOD respirometer to measure oxygen consumption over a six to 20 day period. During this time the samples were continuously stirred utilizing magnetic stir bars. Theoretical BOD curves were constructed from the estimated velocity constant (k) and the ultimate BOD (L) contained in the following equation:

$$Y_t = L\ (1-10^{-kt})$$

where Y_t is the BOD at any time t. Since the above equation contains two unknown variables (k and L), the Thomas Graphical method (186) was used to determine both variables. This method estimates both k and L by regression analysis of the data obtained from the BOD study since these variables are related to the slope and intercept of the regression line. Once k and L were determined, the above equation was utilized to generate the theoretical BOD curves.

In a separate series of experiments the activated sludge's rate of oxygen utilization was monitored in the presence of four separate molecular weight fractions isolated from the Wyodak slurry water's DOC [less than 5,000 MW (<5,000 MW), greater than 5,000 MW (>5,000 MW), less than 1,000 MW (<1,000 MW), and greater than 1,000 MW (>1,000 MW)]. The oxygen uptake of acclimated activated sludge was monitored utilizing a YSI Model 53 Biological Oxygen Monitor method (Yellow Springs Instruments Co., Yellow Springs, OH). Again, all samples were run in triplicate. The basic procedure involves adding 1.0 ml of sludge to the sample chamber of the Model 53 Biological Oxygen Monitor followed by the addition of 2.0 ml of slurry water of the appropriate

molecular weight fraction. An oxygen probe was then inserted into the sample chamber and the oxygen consumed by the sample mixture was monitored over a period of six to 10 minutes. From these measurements one can calculate the moles of oxygen consumed.

The inoculum used in this experiment was activated sludge acclimated to Wyodak slurry water. Forty-eight hours prior to inoculation the sludge was washed twice with PBS and stored at 4°C until needed. Approximately one hour before use, the sludge was equilibrated to room temperature. Fractionation of the slurry wastewater DOC was accomplished by ultrafiltration.

Dissolved Organic Carbon (DOC) Determinations

DOC determinations of the slurry waters were performed using a Beckman Model 915 Total Organic Carbon Analyzer (Beckman Instruments, Fullerton, CA) with direct injection capabilities. An aliquot (3-5 ml) of the slurry water sample was removed and centrifuged (15,000 rpm) for three to five minutes in an Eppendorff Microfuge model 5414 (Brinkman, Westbury, NY). The supernatant was collected and then acidified with concentrated phosphoric acid to convert any carbonate or bicarbonate to carbon dioxide. The acidified sample was purged for five minutes with compressed gas (nitrogen or oxygen) prior to DOC analysis. Standard DOC solutions prepared with potassium phthalate accompanied each determination.

Carbon Dioxide Determinations

At the termination of each respirometry study the carbon dioxide respired by the inoculum was determined. The alkali trap was removed

from the sealed respirometer vessel and immediately transferred to serum vials. These vials were immediately sealed to prevent further CO_2 absorption. Carbon dioxide levels were determined by injecting 20-50 µl of the alkali trap into a 0524-HR carbon analyzer (Oceanography International, College Station, TX). Standard sodium carbonate solutions were also run with each set of determinations. These standards facilitated the calculation of the CO_2 concentration in the alkali trap. Values for endogenous respiration were subtracted and results presented as mg of carbon respired.

Determination of Kinetic Constants

Both first and second order kinetic constants were determined for biologically mediated DOC removal from slurry waters. Kinetic constants for the respirometer studies were determined by monitoring the DOC removal over the entire study. Five milliliter aliquots of slurry/sludge were removed from each vessel at regular intervals during the course of the study. DOC determinations were then performed as previously described.

DOC removal was described using the following expression:

$$-d\,[DOC]/dt = k_1\,[DOC]$$

where k_1 represents the pseudo-first order rate constant. The k_1 was determined by plotting the log [DOC] remaining at time t versus t. The slope of the line was taken to be the pseudo-first order rate constant k_1. For comparative purposes all first order kinetic constants were normalized to the activated sludge concentration (dry

weight) and second order kinetic constants (k_2) were obtained (107).

Molecular Weight Determinations Using Ultrafiltration

The molecular weight distribution of the DOC contained in the slurry waters was determined before and after each respirometry study. Ultrafiltration studies were conducted in order to determine the molecular weight distribution of the DOC present in the slurry waters. Aliquots of the slurry water were removed and then filtered through a 0.4 μm Nucleopore filter to remove any of the biomass. Ultrafiltration was then performed using Amicon YM (Amicon Corp., Lexington, MA) ultrafiltration membranes and an Amicon Model 52 stirred cell with an operating pressure of 40 psi (nitrogen). Slurry water samples were then added to the stirred cell assembly which utilized either a YM 5 or a YM 2 membrane with molecular weight cutoffs of 5,000 MW and 1,000 MW respectively.

Since during operation of the stirred cell the ultrafiltration membranes can leach endogenous DOC material, these membranes were washed extensively in doubly distilled water prior to use. After washing, doubly distilled water was passed through the membranes and the DOC level of the filtrate was checked to insure that there was no leaching from the membrane of endogenous DOC.

The filtrate from the slurry water which passed through the membrane was recovered and analyzed for DOC. By employing the use of the YM 2 and YM 5 membranes the DOC found in the slurry waters were

classified into three molecular weight fractions: >5000 MW, 1000-5000 MW, and <1000 MW.

Biomass Estimations and Microbial Enumeration

Microbial biomass and population densities were determined by dry weight measurements, ATP determinations, and direct cell counts. Dry weight determinations were performed by filtering 10-30 ml of the desired sample through a previously washed (in distilled water), dried (105°C), and tared filter (0.2 μm Nucleopore). The filters were then placed at 105°C and dried to a constant weight (24-48 hours). The difference between the final weight and the tared filter weight was recorded as the dry weight. All determinations were performed in triplicate and dry weight values were recorded as mg of sludge $liter^{-1}$. Cellular carbon was estimated as 50% of the dry weight determinations (115).

Acridine orange direct counts (AODC) were performed by the method described by Hobbie et al. (92). An aliquot of the sample to be counted was removed and fixed with 2% formaldehyde (v/v)-PBS solution. In order for the AODC procedure to yield valid results, a homogeneous suspension of cells must exist in the sample. Since activated sludge form visible flocs, each sample was homogenized prior to staining using a glass tissue grinding vessel (Potter Elvehjen type) with a teflon pestle connected to a 3/8" variable speed drill. With the drill in the on position, each sample was given 10 strokes for maximum homogenization of the sludge, since previous results indicated that this procedure gave maximum counts.

A 2.0 ml aliquot of the inoculated slurry water was then stained for five minutes with 0.01% (final concentration) solution of a filter-sterilized acridine orange dye (Sigma Corp., St. Louis, MO). The stained sample was then passed through a 0.2 μm Nucleopore membrane filter supported by a cellulose acetate filter (Millipore Corp., San Francisco, CA) to insure even distribution of the sample. All filters were stained prior to use by soaking in a filter-sterilized solution of 2% acetic acid, irgalan black (2 g $liter^{-1}$) (acid black 107, Union Color and Chemical Co., Boston, MA) dye. All filters were rinsed in filter-sterilized distilled water prior to use.

As the last drops of the sample were passed through the filter, 5.0 ml of filter-sterilized distilled water were added and allowed to pass through the filter. This distilled water rinse is reported to increase the fluorescence of the stained bacterial cells (92). The damp filter was then placed upon a previously cleaned microscope slide containing one drop of a nonfluorescing immersion oil (Olympus Optical Company, LTD, Japan) on its surface. The filter was layered onto the oil/glass surface and then another drop of immersion oil was placed on the filter surface.

The stained preparation was counted using a microscope (American Optical, Buffalo, NY) equipped with an epifluorescent illumination system that consisted of a 100-W high pressure mercury vapor lamp. The number of bacteria per milliliter was estimated by counting at least 10 randomly chosen microscopic fields, or a minimum of 100 to 200 bacteria. The average number of bacteria per field was divided by the fraction of the filter observed in one field. This determination

yielded the bacteria/filter. The number of bacteria per filter was then divided by the amount of sample filtered (ml) to obtain the number of bacteria per ml.

Fixed film microorganisms were enumerated in a similar fashion with minor modifications. Triplicate 1 cm x 1.5 cm strips were fixed with 10.0 ml of filter-sterilized 2% formaldehyde in PBS with 1% Tween 80. The biofilm was then manually scraped off until there was no visible growth remaining on the strip. The samples were then treated as previously described.

Direct counts were used to ascertain the number of bacteria in the biofilm but this method alone does not assess the relative viability of the microorganisms present. Several published reports (84, 90, 104) state that many of the microorganisms present in a fixed film community may be inactivate or non-viable. ATP measurements were employed to determine the viability of the microorganisms in a biofilm. ATP measurements were performed using a DuPont 760 Luminescence Biometer (DuPont Co., Wilmington, DE) and by following the manufacturer's instructions. Basically, 10 μl of the ATP extract were injected into buffered reaction mixture of a luciferin/ luciferase (Luminescence Biometer Reagent Kit, DuPont Instrument Co., Wilmington, DE) resulting in a light response which the biometer converts to ATP ml^{-1}. The buffered reaction mixture consisted of 0.01 M morpholinopropane sulfonic acid (MOPS) pH 7.4, 0.01 M anhydrous $MgSO_4$, 0.71 M luciferin, and purified luciferase (>100 units).

Prior to injection into the biometer, ATP must first be extracted from the microorganisms. There are numerous extraction procedures

reported in the literature (20, 100, 101, 109) and in this study two different ATP extraction procedures were compared to determine which procedure yielded maximum ATP values for the biofilm system. These two procedures were NRB (Nucleotide Releasing Reagent for Microbial ATP, Lumac Medical Products Division/3M, St. Paul, MN) and the boiling tris procedure.

For the boiling tris procedure three 1 x 1.5 cm strips were removed from the biofilm and placed into 11.0 ml of ATP diluent (0.001 M EDTA, 0.01 M anhydrous $MgSO_4$, and 0.01 M MOPS). The biofilm was then removed from the substratum by manually scraping the microorganism into the ATP diluent combined with vigorous agitation. Triplicate aliquots of each sample were then frozen in liquid nitrogen and stored at -20°C until the sample was extracted. Prior to extraction the samples were thawed at room temperature and then placed on ice. Each thawed sample was separately passed through a 0.2 μm Nucleopore membrane (27 mm in diameter) and then the filter was extracted.

Filtered samples extracted by boiling tris buffer were placed into a beaker containing 10.0 ml of sterile boiling tris (0.02 M, pH 7.7) and extracted for 10 minutes. After the extraction the supernatant was removed and aliquots were immediately frozen in liquid nitrogen and stored at -20°C until assayed for ATP.

For the NRB extraction triplicate biofilm samples (1 cm x 1.5 cm) were removed and placed into 1.8 ml sterile vials containing 400 μl of ATP diluent. Two-hundred μl of NRB reagent were added to each vial and placed on a horizontal hand shaker (130 shakes min^{-1}) for one minute. The extract was then centrifuged for one minute (15,000 rpm)

to remove any particulate matter, frozen in liquid nitrogen, and stored at -20°C until assayed.

Mineralization Assays

To assess the heterotrophic biooxidation potential of the developing biofilm community, the ability of the fixed film microorganisms to mineralize radiolabeled organic compounds was examined. Two organic compounds were chosen: glucose and para-hydroxybenzoic acid. These assays were performed by removing a previously washed 1 cm x 1.5 cm rectangular strip and placing it into a scintillation vial containing 5.0 ml of sterile Black Mesa Centrate (pH 7.2). Ten microliters of labeled compound was added to each vial. The labeled compound was either p-hydroxybenzoic acid-ring-U1-^{14}C (Pathfinder Laboratories, St. Louis, MO) or glucose-D-[^{14}C (U)] (New England Nuclear, Boston, MA) which contained approximately 100,000 counts per minute. The specific activities were 346 mCi/mmol for glucose and 7.74 mCi/mmol for para-hydroxybenzoic acid. Following addition of the label, 0.5 ml of an alkali trap (0.2N KOH) was added to a Kontes center well container containing a folded piece of a #1 Whatman paper (1 x 5 cm). The alkali trap was inserted into the vial cap and suspended in the head space over the mineralization mixture. The vials were tightly capped and incubated in the dark at 25°C. Control vials were constructed in an identical fashion with the following exception. To one group of samples (three vials per group) no biofilm strips were added while 0.2 ml of 37% formaldehyde (final concentration of 2%) were added to the second set.

After incubation the biofilm, the KOH trap, and 1.0 ml aliquot of the mineralization mixture were analyzed for radioactivity for mass balance calculations. The cellulose acetate strips containing the biofilm were placed into 10 ml of scintillation cocktail (Beckman Ready Solution Hp/b, Beckman Instruments Co., Fullerton, CA) and 0.5 ml of 2N H_2SO_4 were added. The vials were shaken for two hours at room temperature to insure that all the carbon dioxide was captured by the alkali trap. After shaking, the Kontes center well and 1.0 ml aliquot of the mineralization mixture were placed separately into 10 ml of scintillation cocktail. The ^{14}C in each of the samples was then measured by liquid scintillation counting. The cpm for the control samples were subtracted from the test samples.

Electron Microscopy

Electron micrographs were made of the biofilm microorganisms utilizing two different procedures. A section of the colonized cellulose acetate strips (3 cm x 3.5 cm) was fixed with 3% glutaraldehyde for one hour and then placed in 0.1 M phosphate buffer (pH 7.6). The sample was dehydrated by soaking in an ethanol series (25%, 50%, 75%, 100%) for 15 minutes at each concentration. The alternate procedure utilized liquid nitrogen, eliminating the glutaraldehyde and ethanol steps. Once removed from the growth reactor the cellulose acetate strip was immediately immersed in liquid nitrogen. From this point both samples were treated identically.

The samples were then loaded into a Ladd critical point dryer and the temperature and pressure were raised to 42°C and 1300 psig. The pressure was then lowered at a rate of 100 psig/minute until ambient pressure was reached. The sample was mounted and loaded into a Denton Vacuum DV-515 automatic evaporator. A vacuum at 5.0×10^{-5} Torr was applied and 6" of gold wire (0.008 mil) was coated onto the sample. The sample was then examined with a ETEC autoscan, scanning electron microscope, and photographed on type 55 P/N polaroid film.

V. RESULTS

Previous reports have indicated that the coal slurry process will result in the leaching of variable but significant concentrations of organic contaminants from the coal into the transport water. The composition of this DOC has been determined to be primarily of high molecular weight, water soluble, polar organic compounds derived from the humic constituents of coal (152, 153). The organic contamination results in deterioration of transport water quality and can also serve as precursors for the formation of trihalomethanes (55) and other halogenated organic compounds (126). Therefore, biological treatment processes were examined for their applicability to the renovation and release and/or reuse of the transport wastewaters. For this purpose Wyodak and Black Mesa coal slurry wastewaters were utilized as model slurry wastewaters.

Wyodak Coal Slurry Wastewater: Biochemical Oxygen Demand

The BOD of the Wyodak slurry wastewater provided evidence that biological treatment may be an affective mechanism in reducing the organic load of the slurry wastewaters. The BOD results of five separate samples with Wyodak slurry wastewaters are shown in Table 3. Activated sludge was used as the inoculum in all runs except for sample four in which the bacteria associated with the coal was employed as the inoculum. As indicated by these data the DOC ranged from 114-298 mg of carbon liter^{-1} with an average DOC of 188 $\pm$ 81 mg of carbon liter^{-1}.

Table 3. DOC Concentrations and BOD Constants for Wyodak Slurry Wastewaters

Wyodak Sample	Initial DOC (mg of carbon $liter^{-1}$)	BOD_{20} (mg $liter^{-1}$)	$\frac{BOD_{20}}{DOC}$	k (day^{-1})
1	114	78	0.68	0.09
2	298	216	0.72	0.10
3	197	108	0.54	0.11
4	ND[a]	152	ND	0.18
5	142	52	0.35	0.08
Mean	188 $\pm$ 81		0.57 $\pm$ 0.16	0.11 $\pm$ 0.4

[a]Not determined.

The rate of the BOD reaction is assumed to be a first order process and the rate is proportional to the concentration of oxidizable organic material. This relationship is described by the following equation:

$$\frac{dL}{dt} = kL \qquad \text{equation 1}$$

L represents the ultimate demand at the start of the time interval t and k is the rate constant for the reaction. L is interpreted in terms of oxygen used. Integration of equation 1 yields:

$$Y_t = L\ (1\text{-}10^{-kt}) \qquad \text{equation 2}$$

where L is the ultimate BOD, Y_t is equal to the BOD at any time t, and k is the velocity rate constant. Equation 2 describes the theoretical BOD curve for a wastewater.

Since equation 2 contains two variables (k and L), the Thomas graphical method (186) was used to determine both variables. Once k and L are determined by this method, equation 2 was then used to generate the theoretical BOD curve. The experimental curves as well as the theoretical curves for three separate Wyodak samples are depicted in Figure 4. The theoretical curves predicted by the equation two were almost superimposed on the experimental curves, further supporting the use of this equation to describe the BOD of the Wyodak slurry wastewater.

The calculated velocity rate constants (k) indicated biological treatment was successful in reducing the biologically oxidizable organic material in the slurry wastewaters. The velocity rate constants ranged from 0.08-0.18 day^{-1} with an average of 0.11 $\pm$

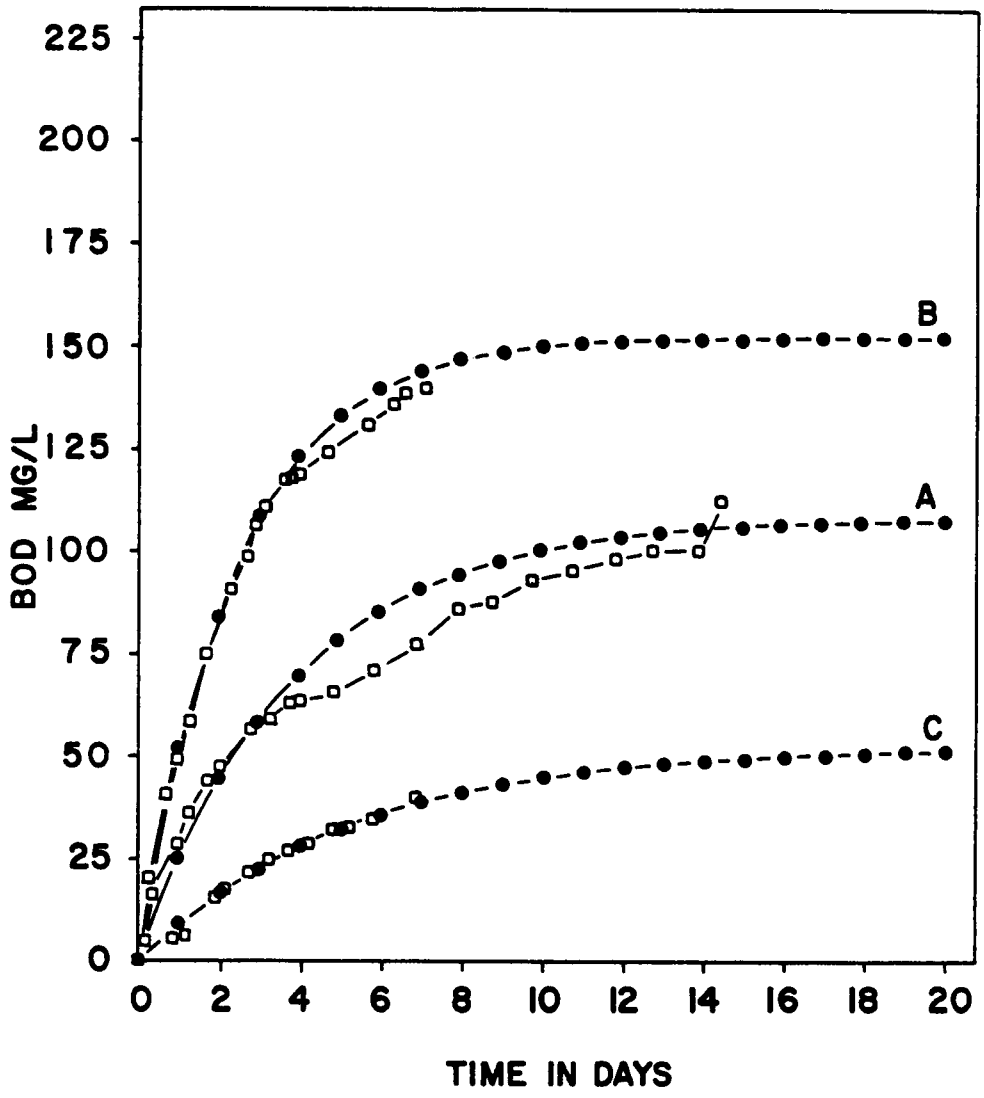

Figure 4. Experimental (□) and theoretical (●) oxygen demand exerted during respirometry studies. A) Wyodak sample 3, B) Wyodak sample 4, C) Wyodak sample 5.

0.04 day^{-1}. These values compare favorably with other values reported for wastewaters which are amenable to biological treatment such as primary and secondary effluent with reported velocity rate constants between 0.10 and 0.15 day^{-1} (63).

The BOD_{20}, which was also determined using the Thomas graphical method, was related to the initial DOC concentrations of the slurry wastewater (Table 3). Wyodak samples 2 and 3 contained the highest initial DOC concentrations (298 and 197 mg of carbon $liter^{-1}$) which corresponds with the highest BOD_{20} (216 and 108 mg $liter^{-1}$ respectively). Similar trends were observed with samples 1 and 5 in that they exerted the lowest BOD_{20} (78 and 52 mg $liter^{-1}$) and also contained the least amount of organic carbon (114 and 142 mg of carbon $liter^{-1}$, respectively). Others have also suggested that oxygen consumed by microorganisms is directly related to the organic concentration. Larsons and Perry (108) reported that the oxygen consumed by microorganisms indigenous to Ohio River Water was directly related to the organic concentration of simulated industrial wastewaters.

Although oxygen consumption was related to theoretical DOC levels of the slurry wastewater, there was not a direct correlation between the two parameters. This was examplified by Wyodak samples 1 and 5 where the BOD_{20} was higher for sample 1 (78 vs. 52 mg $liter^{-1}$-- Table 3) but the DOC concentration was lower for this sample (114 vs. 142 mg of carbon $liter^{-1}$). These results suggest that Wyodak slurry wastewaters contained organic compounds which were not completely catabolized by the microorganisms. Further evidence to

support this hypothesis was demonstrated by the BOD_{20}/DOC ratios in Table 3 which ranged from a low of 0.35 to 0.72. These values were relatively low with typical BOD_{20}/DOC values ranging between 1.0-2.0 for wastewaters in which biological treatment has proven successful (14, 63). These low ratios suggest incomplete biological oxidation of the organic contaminants in the slurry wastewater.

DOC Removal from Wyodak Slurry Waters

Further evidence that biological treatment can improve the overall quality of slurry transport waters was provided by examining DOC removal during the respirometer studies. At the termination of each E/BOD study the percentage of the DOC removed from the slurry wastewater was determined (Table 4). A 51% to 74% reduction in the total DOC levels with an average of 60% reduction was demonstrated for the four studies. Although a reduction in DOC was noted, there always remained a residual DOC (28-40.1 mg of carbon $liter^{-1}$) in the slurry wastewater regardless of the length of time of the study (7-16 days). This residual DOC averaged 33.5 $\pm$ 6.4 ppm for the four studies. However, it cannot be determined from these results whether the residual carbon was unmodified or the result of microbial transformation products. The residual DOC indicates that even after extended (16 days) biological treatment there may remain elevated (>33 mg of carbon $liter^{-1}$) levels of DOC in the slurry wastewaters which is not amenable to removal by microorganisms. This does not rule out the possibility that these organic compounds may require extended time periods (months-years) for degradation.

Table 4. DOC Removed During Respirometer Studies

Wyodak Sample	BOD Interval (Days)	DOC[a]		
		Initial	Final	% Removal
1	7	57.0	28.0 ± 1.1[c]	51
2	16	149.0 ± 2.1	38.0 ± 2.2	74
3	15	98.0 ± 7.4	40.1 ± 4.2	59
5	6	72.0 ± 4.5	27.9	61
Mean				61 ± 10

[a]Values represent mg of carbon present.

[b]Coal slurry water made with distilled water all others prepared with lake water.

[c]± one standard deviation.

The respirometry studies also furnished information which would aid in providing predictive capabilities for biologically mediated DOC removal from Wyodak slurry wastewaters. By monitoring the residual DOC over the time course of the respirometry studies, kinetic parameters for DOC removal were developed.

To describe the rate of substrate removal in biological wastewater treatment systems, Michaelis-Menten relationships are often used (63, 161). This relationship can be expressed in the following form:

$$V/[B] = V_{max} [S]/([S] + K_s)$$

where the rate of substrate utilization (V) per unit biomass [B] is dependent on the substrate concentration [S], K_s (one-half saturation constant), and the maximum rate (V_{max}) of substrate utilization at infinite [S]. At low [S] where $K_s > [S]$ this equation simplifies to $V/[B] = (V_{max}/K_s) [S]$ where V_{max} and K_s are constants for a specific wastewater and can be replaced by the average kinetic constant k. Since V is equal to the rate of substrate utilization the equation can be written as:

$$-dS/dt = k[S] [B]$$

The substrate removal can be approximated by a first order kinetic model (63, 161) with respect to substrate concentration and biomass concentration but second order overall.

First order kinetic constants (k_1) were determined by least squares analysis of the linear portion of the curve when the logarithm of the residual DOC was plotted versus time (days). Table 5 lists k_1 for experiments 1, 2, 3, and 5. An exponential decrease in the DOC concentration occurred with time and this linear relationship was

Table 5. First and Second Order Rate Constants for Removal of DOC from Slurry Wastewaters

Experiment	k_1 (day^{-1})	r^2	Dry Weight (mg $liter^{-1}$)	k_2 liter day^{-1} (mg of $sludge^{-1}$)
1	0.020	0.95	115	1.74×10^{-4}
2	0.028	0.94	161	1.74×10^{-4}
3	0.044	0.98	ND[a]	ND
5	0.049	0.97	213	2.30×10^{-4}
Mean				1.93×10^{-4}

[a]Not determined.

demonstrated by the high values obtained for the coefficient of determination (r^2) listed in Table 5. The first order kinetic constants (k_1) varied by over 2.5 fold (0.020-0.049 day^{-1}). However, when k_1 values were normalized to the activated sludge concentrations used in each respirometry study the difference in the second order kinetic constants fell to less than 25% (1.74×10^{-4} vs. 2.30×10^{-4} liter day^{-1} [mg of sludge]$^{-1}$).

For the five samples examined in the Wyodak BOD studies, carbon material balances were calculated from the data on DOC concentrations (initial and final), cellular carbon, and respired carbon (Table 6). As seen in this table, 61% to 97% of the DOC removed could be accounted for as biomass accumulated or respired as carbon dioxide indicating microbial transformation of the DOC contained in the Wyodak slurry wastewater. Mass balance determinations indicate wide variations in the amount of DOC removed, respired, and accumulated into biomass for the five samples (Table 6). For samples 1, 2, and 3 comparable patterns were observed. Although there was an approximate four-fold difference in the net DOC removed (29 vs. 111 mg of carbon liter^{-1}--Table 6), the percentage respired (36 to 41%--Table 6), and accumulated into biomass (25 to 47%--Table 6) were similar. These similarities between the three samples were further indicated by the narrow range of the carbon respired:biomass carbon ratios (0.9 to 1.4--Table 6).

In contrast, Wyodak samples 4 and 5 were markedly different. Comparable levels of DOC were found in the net biomass accumulated plus CO_2 respired fractions for samples 4 and 5 (39.9 vs. 42.7 mg of

Table 6. DOC Respired and Accumulated Into Biomass During Respirometer Studies

Wyodak Sample	Net DOC Removed[a]	Biomass		Respired CO_2		% of DOC Removed in Biomass plus Respired	Carbon Respired Carbon Accumulated in Biomass
		Net Accumulation[a]	% of Total DOC Removed	Net CO_2 Respired[a]	% of Total DOC Removed		
1	29.0	7.2 ± 0.6	25	10.3 ± 1.4	36	61	1.4
2	111.0	34.0 ± 1.5	31	44.6 ± 0.7	40	71	1.3
3	57.9	27.5 ± 4.1	47	24.0 ± 1.7	41	88	0.9
4	ND[b]	7.9 ± 0.1	ND	32.0	ND	ND	4.1
5	44.1	33.5 ± 2.1	76	9.2 ± 0.7	21	97	0.3

[a]All values recorded as mg of carbon present.

[b]Not determined.

carbon--Table 6). However, the carbon respired:biomass carbon ratios were 4.1 and 0.3 for samples 4 and 5 respectively. These ratios indicate that a greater percentage of the DOC removed was mineralized to CO_2 for Wyodak sample 4 as compared to sample 5.

The amount of carbon respired as CO_2 provides a basis for the difference in plateau heights (BOD_{20}) achieved by the BOD curves for samples 3, 4, and 5 in Figure 4 (page 65). On a per milligram basis microbial mineralization utilizes more oxygen than when the same compounds are transformed into biomass. Furthermore, the amount of oxygen consumed directly affects the ultimate height (BOD_{20}) of the BOD curves. The relationship between BOD_{20} and the quantity of carbon respired was demonstrated by samples 3, 4, and 5. Although the net DOC removed varied by only 30% for these three samples (39.9 to 57.9 mg of carbon liter^{-1}--Table 6) the total amount respired varied over four fold range (21% to 80%). For sample 4 the microorganisms respired 32 mg of carbon while only 24 mg and 9.2 mg of carbon was respired for samples 3 and 5 respectively. These relative decreases in mineralization were reflected in a concomitant decrease in the BOD_{20} for the three samples. Sample 4 had the highest level of mineralization and the highest BOD_{20} (150 mg liter^{-1}). In contrast, the BOD_{20} for samples 3 and 5 was 100 and 50 mg liter^{-1} respectively, corresponding to lower levels of carbon respired.

The first and second order kinetic constants were not affected by the amount of carbon accumulated or carbon respired. The kinetic

parameters were based only on DOC removal. Regardless whether the organic compounds were mineralized or accumulated, the rate constants were not altered.

The results indicated that the microorganisms employed in these studies were capable of mineralizing the Wyodak slurry wastewater's DOC to CO_2 as well as accumulating the organic compounds into additional biomass. However, there were variations between samples as to whether the DOC removed was catabolized to CO_2 or accumulated into biomass. Therefore, both the initial DOC concentrations as well as the percentage of the DOC removed, which was mineralized to CO_2, affect the ultimate height of the BOD curves. In contrast, only the total carbon removed had any influence on the first and second order kinetic parameters developed for DOC removal from Wyodak slurry wastewaters.

Molecular Weight Fractionation

The previous results demonstrate significant DOC removal from the Wyodak slurry water by biological treatment. The kinetic parameters (first and second order kinetic constants) provide the capacity to predict the time required for successful biological treatment. Since the DOC of these slurry waters are comprised of a heterogeneous mixture of organic compounds (152), these kinetic constants represent only an average value based on the removal rates for each of the individual organic components. These kinetic parameters do not take into account the various organic constituents of the slurry water may be removed at

significantly different rates by the activated sludge. Furthermore, DOC removal was not complete indicating the recalcitrance of a fraction of the slurry wastewater DOC or the production of stable end products from biological metabolism.

To more closely examine the biological specificity of substrate removal and utilization, the DOC in Wyodak slurry water was fractionated by ultrafiltration into three molecular weight classes before and after each respirometry study: >5,000 MW, 1,000-5,000 MW, and <1,000 MW. Table 7 summarizes the percentage of the DOC removed from each of the three fractions (expressed as percentage of DOC removed). There was an average of 61% $\pm$ 10% reduction of the original DOC at the termination of the four respirometry studies. Table 7 shows greater than 80% of the total DOC removed was from the <1000 MW fraction reflecting a preferential utilization of the <1000 MW organic compounds.

The substrate removal pattern changes as the molecular weight of the DOC increases. There was a net removal of DOC from the 1,000-5,000 MW fraction of 19.0 $\pm$ 16.4%; however, the percentage removed is far less than that of the <1000 MW fraction. One reason for the lower removal of DOC in the mid-range organic fraction is the physical size of the organic compounds renders them less susceptible to uptake and metabolism. An alternate, but equally plausible explanation, is the removal rate of the DOC contained in the mid-range fraction was comparable to the <1,000 MW fraction, but there was an influx of DOC from the >5,000 MW class due to degradation.

Table 7. Molecular Weight Distribution of DOC from Wyodak Slurry Water: Percentage Removal from Each Molecular Weight Fraction

Respirometer Study	Greater than 5000 MW %	5000-1000 MW %	Less than 1000 MW %
1	-21.5[a]	+8.4	+113.0
2	+4.0	+36.0	+60.0
3	-20.0	+29.6	+90.6
5[c]	+27.6	+2.0	+70.5
Mean	-2.5 ± 23.2[b]	+19.0 ± 16.4	+83.5 ± 23.4

[a]Negative sign indicates net gain in DOC and each value represents the % of total DOC removed.

[b]± one standard deviation.

[c]Acclimation time was greater than one month for study 5 and five to seven days for studies 1, 2, and 3.

Less straightforward results were obtained from the higher molecular weight (<5,000 MW) fraction. Here (Table 7) there was an overall 2.5% gain in DOC for the four samples, although this average is somewhat misleading. For samples 1, 2, and 3 little net reduction in the >5000 MW fraction was noted. However, there was a 27.6 reduction in the >5000 MW fraction for sample 5. The reduction can be attributed to the increased acclimation time for the activated sludge used for study 5 (greater than one month vs. five to seven days for studies 1, 2, and 3). The net increases in DOC for samples 1 and 3 was likely caused by microbial metabolism which transformed the DOC compounds from the midrange (1,000-5,000 MW) and lower molecular weight classes into higher molecular weight compounds (>5,000 MW). Production of high molecular weight compounds was not unexpected since other investigators have implicated microbial byproducts as important DOC components of activated sludge treated wastewater (16, 53, 155).

Due to preferential substrate utilization by the microbial communities involved, the molecular weight profile of Wyodak slurry water changes after biological treatment. Figure 5 represents the average molecular weight profile before and after treatment. Initially, approximately 60% of the DOC contained in Wyodak slurry water is of low molecular weight (<1,000 MW) while only 15% of the DOC is >5,000 MW. After biological treatment of the Wyodak slurry water the higher molecular weight fractions (>1,000 MW) contains the majority (65%) of the DOC. Due to the selective removal of organic compounds by the activated sludge the <1,000 MW fraction only contains 35% of the residual DOC.

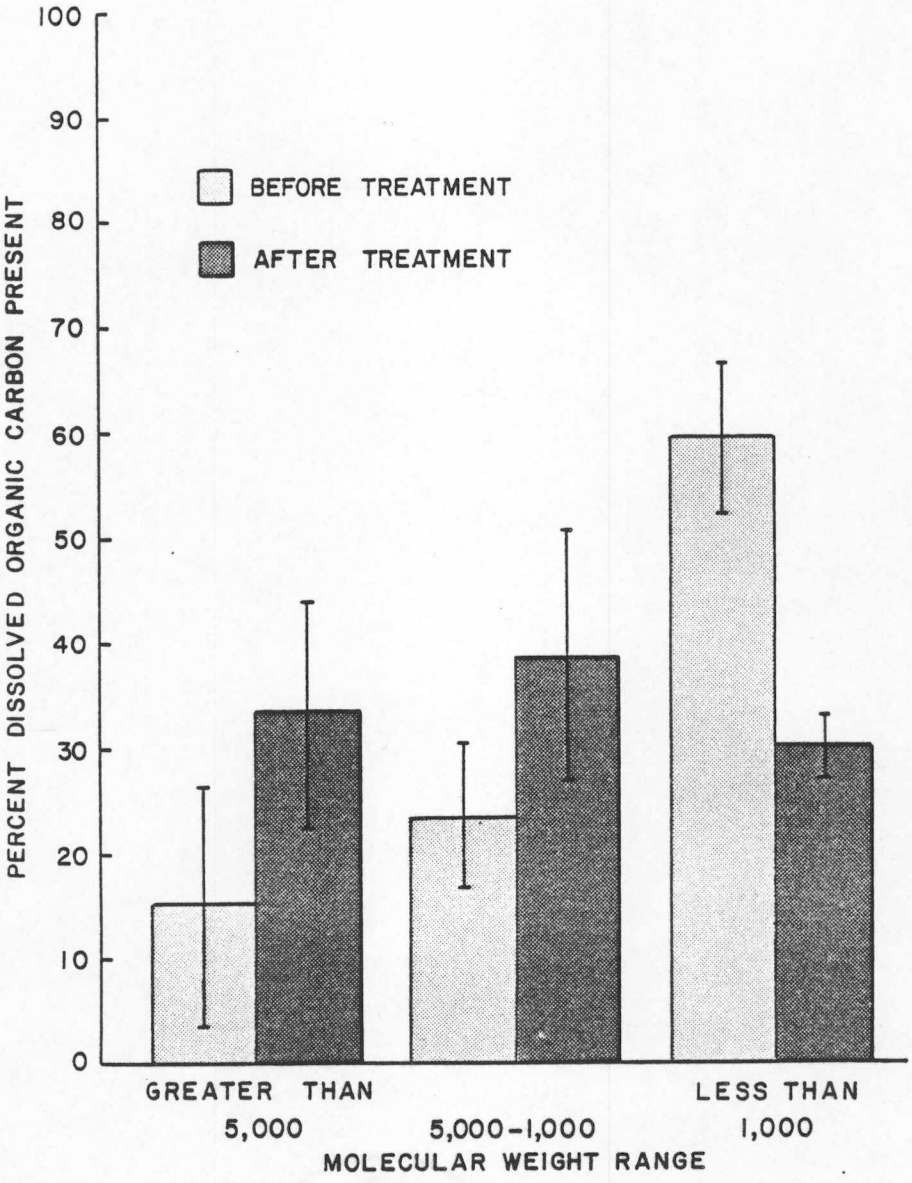

Figure 5. Molecular weight profile of Wyodak slurry wastewater before and after biological treatment. This figure represents the average value of four separate respirometry studies.

Thus, the removal rate of DOC from slurry wastewaters is dissimilar for each of the three molecular weight fractions. The lower molecular weight components (<1,000 MW) appear to be readily removed while there is little net reduction of the higher (>1,000 MW) molecular weight compounds. As a result, the molecular weight profile of the slurry water shifts after biological treatment from a wastewater initially dominated by lower molecular weight organic carbon to a wastewater dominated by higher (>1,000 MW) molecular weight compounds.

Oxygen Uptake

The molecular weight characterization of the Wyodak slurry wastewaters indicate the higher molecular weight organic compounds are more resistant to biological removal than the smaller DOC compounds. This explanation implies the organic compounds in the <1,000 MW fraction are more susceptible to transformation by the sludge microorganisms. A corollary to this hypothesis is the organic compounds in the lower molecular weight fractions should stimulate greater microbial activity. To determine which fraction had the greatest stimulatory affect on the activated sludge, the DOC in four different molecular weight fractions were separated using ultrafiltration. The Wyodak slurry wastewaters which passed through the YM5 and YM2 ultrafiltration membrane were classified as <5,000 MW and <1,000 MW respectively. The >5,000 and >1,000 MW fractions retained by the membranes still contained significant levels of DOC from the lower molecular weight fractions. To reduce contamination the

higher molecular weight fractions were diluted with doubly distilled water until the lower molecular weight DOC represented $\leq$13% of the total DOC. All fractions were then diluted to approximately 108 $\pm$ 6 mg of carbon liter^{-1}.

The DOC in each of the four molecular weight classes (>5,000 MW, <5,000 MW, >1,000 MW, <1,000 MW) and raw Wyodak slurry wastewater were added to activated sludge and the rate of oxygen utilization was measured in order to assess microbial activity. The results of this experiment are presented in Table 8. Raw Wyodak slurry water stimulated the highest rate of oxygen utilization (15.8 μg ml^{-1} hr^{-1}) while the >5000 MW fraction exhibited only endogenous respiration rates (0 μg ml^{-1} hr^{-1}). The <1000 MW fraction stimulated activity greater than any of the four enriched fractions (13.8 μg ml^{-1} hr^{-1}) and approached 90% of the activity exhibited by the raw slurry water. These results were in agreement with the previous results. The organic compounds in the <1000 MW fraction of the Wyodak slurry wastewater were the most susceptible to microbial attack. Furthermore, the higher molecular weight DOC compounds appear to be more resistant to microbial attack since they fail to stimulate any detectable oxygen uptake in the activated sludge.

Semi-Continuous Reactors

BOD analysis and DOC removal kinetic determinations were previously conducted using batch reactor conditions (E/BOD respirometer). Such analyses may suffer from the lack of steady state

Table 8. Oxygen Uptake by the Activated Sludge Using Different Molecular Weight Fractions of Wyodak Slurry Wastewater

Molecular Weight Fractions	DOC (mg of carbon $liter^{-1}$)	Oxygen Uptake ($\mu g\ O_2\ ml^{-1}\ hr^{-1}$)
greater than 5000 MW	107	0
greater than 1000 MW	104	2.6
less than 1000 MW	110	13.8
less than 5000 MW	102	14.7
raw coal slurry water	118	15.8

maintenance of the microbial community during prolonged incubations. Consequently, kinetic parameters describing removal of DOC during biological treatment may not realistically predict process efficiency. For these reasons kinetic parameters for DOC removal from Wyodak slurry wastewaters were evaluated for activated sludge maintained in a fill and draw mode (semi-continuous maintenance). Furthermore, two different concentrations of activated sludge were employed to examine the affect of the biomass concentrations on the kinetics of DOC removal.

Initial substrate concentrations in the reactors ranged from approximately 90 to 220 mg of carbon liter^{-1}. Figure 6 represents the linear relationship between the DOC removal rates and the concentration of DOC in the fill and draw reactors. As expected, as the initial DOC concentration increases the rate of DOC removal also increases. A two-fold difference in the k_1 (slope) between the two reactors was calculated (0.27 vs. 0.49 day^{-1}). This difference disappeared when k_1 was normalized to the biomass concentrations since there was also a two-fold difference in sludge concentration for reactors (1,836 vs. 3,525 mg liter^{-1}). The resulting second order kinetic constants constants were 1.47×10^{-4} and 1.39×10^{-4} liter day^{-1} (mg of sludge)$^{-1}$. These results indicate two important points: 1) the importance of considering the concentration of the activated sludge to accurately predict the rate of DOC removal from slurry wastewaters, 2) the second order kinetic parameter (k_2) developed for the fill and draw reactors systems were comparable to those formulated using the respirometer batch reactor systems.

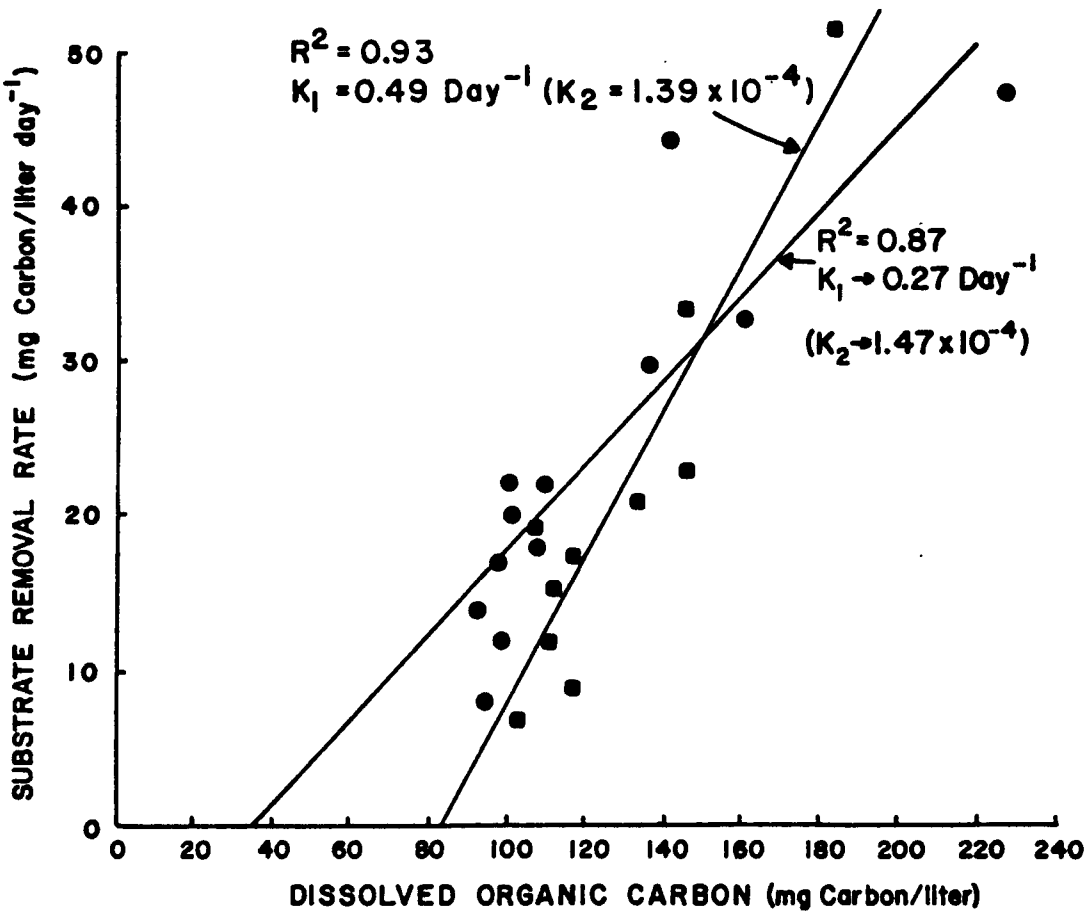

Figure 6. Rate of DOC removal by an activated sludge population in a semi-continuous (fill and draw) reactor. Each observation represents the average value from duplicate reactors.

DOC Removal as a Function of the Biomass Concentration

The previous results demonstrate the importance of the activated sludge concentration on the removal of DOC from the slurry wastewaters. Therefore, the removal of DOC from Wyodak slurry wastewaters was examined as a function of the biomass concentration. Figure 7 represents the results of DOC removal as a function of the sludge concentration over a four day period.

As expected there was no net DOC removal from the Wyodak slurry water in the killed cell samples. These results indicate that physical adsorption is not a mechanism for DOC removal by the sludge. This lack of removal is not surprising since lipophilic molecules are more susceptible to removal by adsorption and the organic compounds in the slurry waters have been characterized as polar, water soluble polymers of moderate but variable molecular weight most likely originating from humic and fulvic acids present in the parent coal (153). Thus, all net DOC removal was the direct result of the biological activity of the activated sludge.

The relationship of DOC removal rates and their dependence on the biomass concentration is demonstrated in Figure 7. As the concentration of sludge increases there was a concomitant increase in the DOC removed. However, little reduction in DOC (0 to 15%--Table 9) occurred from day 4 to day 7. Again, these results indicate the presence of a residual DOC concentration in slurry wastewaters remaining after biological treatment.

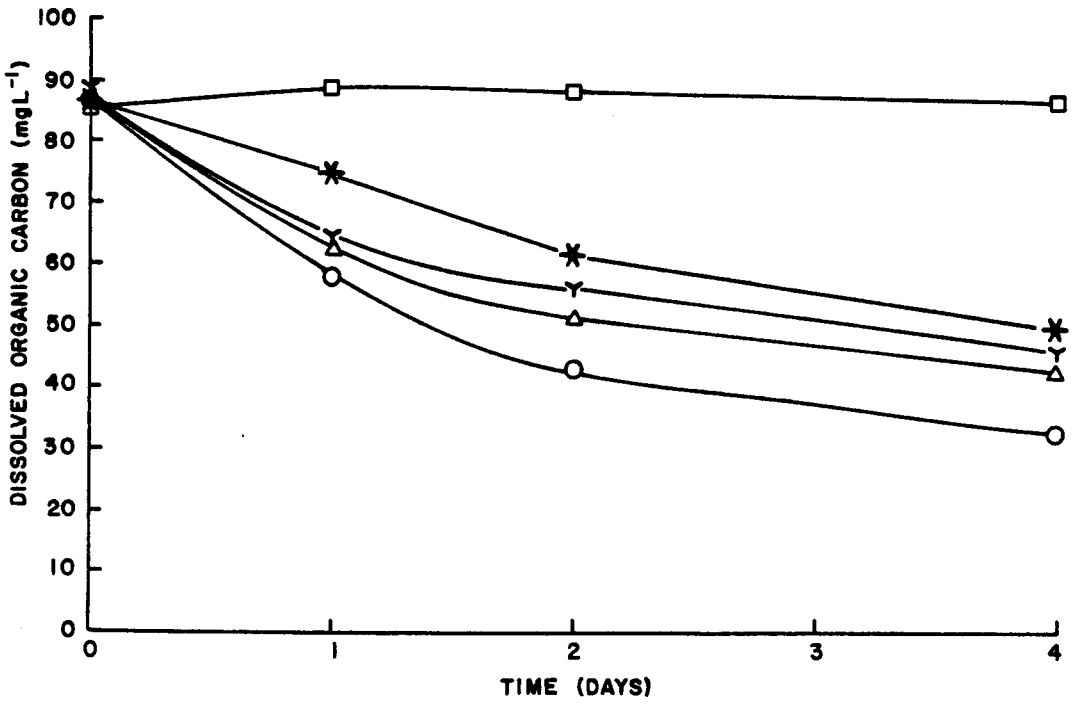

Figure 7. Removal of DOC from Wyodak slurry wastewater as a function of the activated sludge concentration. Biomass levels were determined by AODC and are recorded as bacteria ml^{-1}. O--6.43 x 10^7, Δ--1.06 x 10^7, Y--4.56 x 10^6, ✱--1.18 x 10^6, □--killed cells, 6.89 x 10^7.

Table 9. Residual DOC in Wyodak Slurry Wastewater After Treatment With Activated Sludge

Biomass Concentration[a]	DOC Concentration[b]			% Decrease from Day 4 to Day 7
	Initial	Day 4	Day 7	
X	87.7 ± 1.8	32.3 ± 1.2	32.9 ± 0.7	No Decrease
1:5 X	86.1 ± 0.5	42.4 ± 1.4	38.3 ± 1.2	10
1:10 X	87.7 ± 0.6	45.8 ± 2.3	38.9 ± 1.5	15
1:100 X	85.4 ± 1.0	49.7 ± 1.6	45.8 ± 1.1	8
Killed Cells	84.9 ± 1.5	86.2 ± 2.4	84.0 ± 4.3	3

[a]Initial concentration of biomass as determined by AODC (bacterial/ml). X = 6.43×10^7, 1:5 - 1.06×10^7, 1:10 x - 4.56×10^6, 1:100 - 1.18×10^6, killed cells - 6.89×10^7.

[b]All DOC values recorded as mg of carbon $liter^{-1}$.

Biodegradation of Chlorinated Slurry Wastewaters

Coal pipeline slurry transport water is reused for cooling waters at the power plant facility located at the terminus of the pipeline. A common practice to prevent biofouling in the cooling towers is chlorination of the cooling waters. A previous report indicated that chlorination of slurry wastewaters can result in high levels of trihalomethanes (THM) because of the significant quantities of fulvic acids (greater than 50% of the total DOC) which leach from the coal into the transport waters during slurry preparation (55). In addition, the chlorination of cooling waters obtained from power plant facilities produce other organic halocarbons besides THM (88). Jolley and coworkers (98) chlorinated freshwaters obtained from power plant facilities and identified over 50 different halogenated organic compounds including chlorinated phenols and aromatic acids. Because of the high organic content of the slurry wastewaters, numerous chlorinated organic compounds should result from the chlorination of the slurry transport waters. The objective of this stage of the investigation was to determine the affect of chlorination on the biologically mediated removal of DOC from the Black Mesa Centrate (BMC) and Wyodak slurry wastewaters. The affects of the chlorination process on the composition of wastewater DOC was also examined.

Molecular Weight Fraction of Chlorinated Wastewater. The addition of hypochlorous acid (Clorox®) to Wyodak and BMC slurry waters did not result in a reduction of the DOC concentrations. However, this addition did cause a shift in the molecular weight distribution of the soluble organic compounds. After a 48 hour chlorination reaction time

the yellow color of the slurry waters disappeared indicating oxidation of the DOC compounds present.

When the molecular weight profile of the slurry wastewater's DOC was examined using ultrafiltration, an increase in the <1000 MW fraction was noted for both the Wyodak and BMC. Figure 8 represents the mean (two separate experiments) increase in the <1000 MW DOC in Wyodak and BMC slurry waters. After chlorination the <1000 MW DOC of the Wyodak slurry water increased approximately 39% (21 mg of carbon $liter^{-1}$) while the BMC wastewater <1000 MW fraction only increased by 9% (1.5 mg of carbon $liter^{-1}$). These increases in DOC in the lower molecular weight fractions were due to the oxidative degradation of the higher molecular weight DOC constituents by the hypochlorous acid.

DOC Removal from Chlorinated Slurry Waters. The ability of activated sludge to remove the DOC from chlorinated slurry wastewaters was examined under batch culture conditions. In these studies the activated sludge employed had been previously acclimated to chlorinated slurry wastewater (BMC and Wyodak) for approximately one month.

Kinetic rate constants for DOC removal for chlorinated and unchlorinated slurry waters were determined as previously described (Table 10). For Wyodak and BMC slurry wastewaters the kinetic constants for the DOC removal were higher for the chlorinated versus the unchlorinated wastewaters. The rate of DOC removal from chlorinated Wyodak slurry waters was three times greater than from unchlorinated slurry waters (Table 10). Since the biomass

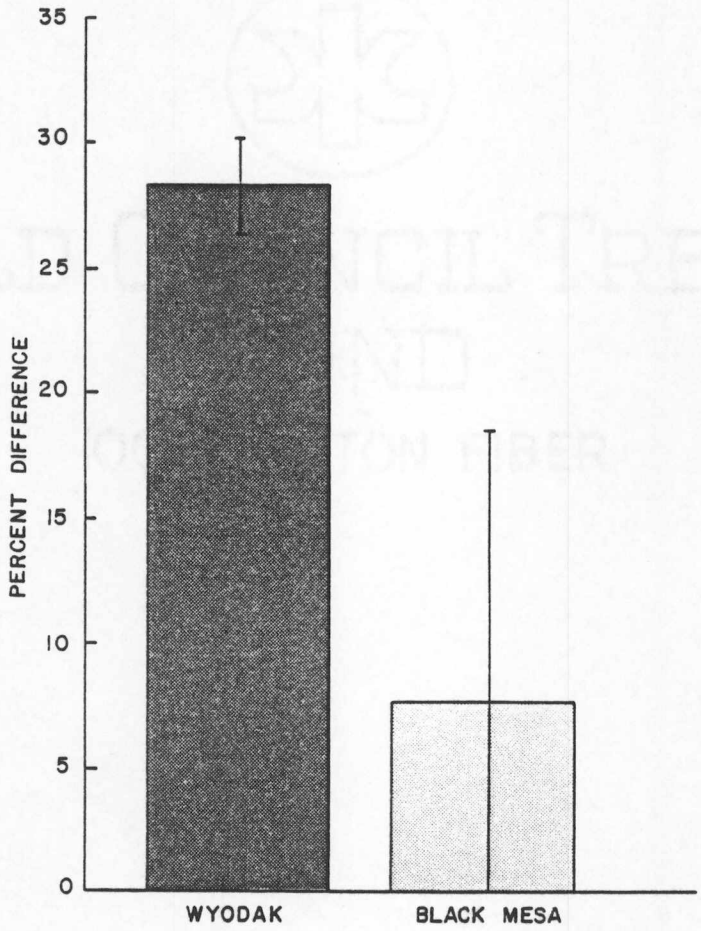

Figure 8. Increase in the <1000 MW DOC as a result of chlorination. A mean of two separate experiments.

Table 10. Kinetic Constants for DOC Removal from Chlorinated and Unchlorinated Slurry Wastewater by Activated Sludge

	k_1 (day^{-1})		k_2 (liter day^{-1} [mg of $sludge^{-1}$])	
Coal Type	Chlorinated	Unchlorinated	Chlorinated	Unchlorinated
Wyodak	$2.05 \times 10^{-2} \pm 1.3 \times 10^{-3}$	$6.20 \times 10^{-3} \pm 1.40 \times 10^{-3}$	$6.90 \times 10^{-5} \pm 1.54 \times 10^{-5}$	$2.41 \times 10^{-5} \pm 4.7 \times 10^{-6}$
Black Mesa Centrate	$1.17 \times 10^{-2} \pm 2.6 \times 10^{-3}$	NC[a]	$4.88 \times 10^{-5} \pm 1.10 \times 10^{-5}$	NC

[a]NC = No net change in DOC levels.

concentration was approximately the same for both the chlorinated and unchlorinated waters the second order kinetic constant (k_2) also had the same relationship (6.90×10^{-5} vs. 2.41×10^{-5} liter day^{-1} [mg of sludge]$^{-1}$). The difference between chlorinated and unchlorinated slurry wastewater DOC removal was even more dramatic when the BMC slurry wastewaters were exmined. Here the k_2 for the BMC chlorinated slurry wastewater DOC was 4.88×10^{-5} liter day^{-1} (mg of sludge)$^{-1}$ while there was no net DOC removal from the unchlorinated BMC slurry water.

The pattern revealed by the kinetic parameters was also reflected in the percentage of DOC removed from the chlorinated Wyodak and BMC slurry wastewaters. The net DOC removed from chlorinated wastewaters was nearly identical (38% vs. 36% respectively, Figure 9). In contrast, only 17% of the DOC was removed from unchlorinated Wyodak slurry, and none from the BMC slurry water. Thus, the activated sludge removed a greater amount of DOC at a higher rate when the slurry wastewaters were chlorinated, provided the activated sludge was first acclimated to chlorinated slurry water.

The development of kinetic rate constants for the biodegradative potential of wastewater often provides a baseline for comparative purposes (140, 162). The DOC removal rates from both chlorinated Wyodak and BMC slurry wastewaters were similar, with the k_2 of Wyodak being slightly higher (6.90×10^{-5} vs. 4.48×10^{-5} liter day^{-1} [mg of sludge]$^{-1}$). However, these second order kinetic constants are lower than those determined for unchlorinated slurry waters using activated sludge acclimated to the unchlorinated slurry

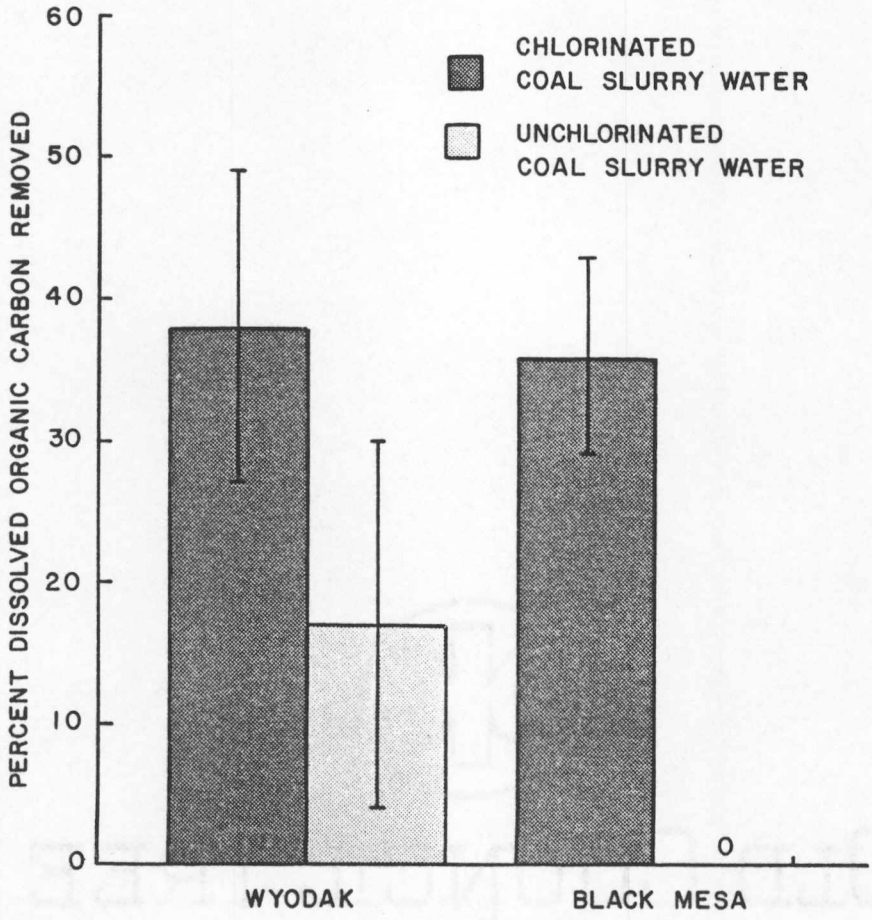

Figure 9. Comparison of the DOC removed from chlorinated and unchlorinated slurry wastewater by activated sludge.

waters (Table 5, page 70). The average k_2 (1.93×10^{-4} liter day^{-1} [mg of sludge]$^{-1}$) for the three studies listed in Table 5 (page 70) was two to three-fold greater than the k_2 for chlorinated Wyodak slurry waters (6.90×10^{-5} liter day^{-1} [mg of sludge]$^{-1}$--Table 10). Hence, the chlorination of slurry wastewaters results in a reduction of the total DOC removed as well as the rate of DOC removal by the activated sludge.

Molecular Weight Distribution of Chlorinated Slurry Waters After Biological Treatment. Molecular weight distribution profiles were developed for chlorinated Wyodak and BMC slurry waters before and after biological treatment. As with the unchlorinated slurry waters, each of the three molecular weight fractions (>5,000 MW, 1000-5000 MW, <1,000 MW) of the chlorinated slurry wastewaters displayed different susceptibilities to removal by the activated sludge (Table 11). As expected, the greatest reduction in DOC was in the lowest molecular weight fraction (<1000 MW): 76% (38 mg of carbon $liter^{-1}$) from Wyodak and 59% (6.1 mg of carbon $liter^{-1}$) for BMC. Similar trends were previously reported with the unchlorinated slurry waters. Unanticipated results were obtained for the >5,000 MW fraction. In earlier experiments a significant reduction in DOC in the >5000 MW fraction was seen in only one sample (#5, Table 7, page 76). For the chlorinated slurry wastewater studies there was a 42% and 32% reduction in the DOC from the chlorinated Wyodak and BMC slurry water originating from the >5,000 MW fraction.

Table 11. Molecular Weight Fractionation of the DOC Present in Slurry Wastewater Before and After Biological Treatment

	Initial[a]	Final	Percent of Total DOC Removed
Wyodak			
Greater than 5000 MW	33.8 ± 14.0[b]	12.8 ± 5.0	+42
5000 - 1000 MW	11.8 ± 3.0	21.3 ± 1.1	-20[c]
Less than 1000 MW	75.2 ± 21.6	37.0 ± 11.0	+76
Black Mesa Centrate			
Greater than 5000 MW	7.0 ± 2.7	3.7 ± 3.2	+32
5000 - 1000 MW	13.3 ± 4.7	12.3 ± 7.0	+10
Less than 1000 MW	12.4 ± 1.2	6.3 ± 2.3	+59

[a]All values represents the average of two separate experiments.

[b]All values are reported as mg of carbon liter^{-1} ± one standard deviation.

[c]Negative value represents a net gain in DOC.

Biological treatment of the chlorinated slurry wastewater produced an alteration in the molecular weight profile of the DOC (Figure 10). The percentage of DOC present decreased for the >5,000 MW and <1,000 MW fractions. This occurred in the chlorinated slurry wastewaters for both coal types. However, there was a concomitant rise in the percentage of the DOC contained in the mid-range fraction (1000-5000 MW) probably resulting from the influx of DOC from the >5,000 MW fraction.

One can conclude from these results that the activated sludge exhibits a preferential removal of the lower molecular weight compounds in the chlorinated slurry waters. This selective utilization pattern shifts the molecular weight profile of the chlorinated slurry water to one in which the smaller organic constituents (<1,000 MW) comprise less of the total DOC after biological treatment.

Biofilm Growth and Development on Black Mesa Centrate Slurry Water

In the final phase of the investigation the use of a fixed population of microorganisms on a solid surface, a biofilm, was examined as a mechanism for the biological treatment of BMC slurry wastewaters. Although biofilms are routinely employed in wastewater treatment designs (11), there is a paucity of information concerning the development and microbial processes of a biofilm community. To gain a better understanding of these processes, a qualitative and quantitative assessment of biofilm growth and development on BMC slurry water was initiated.

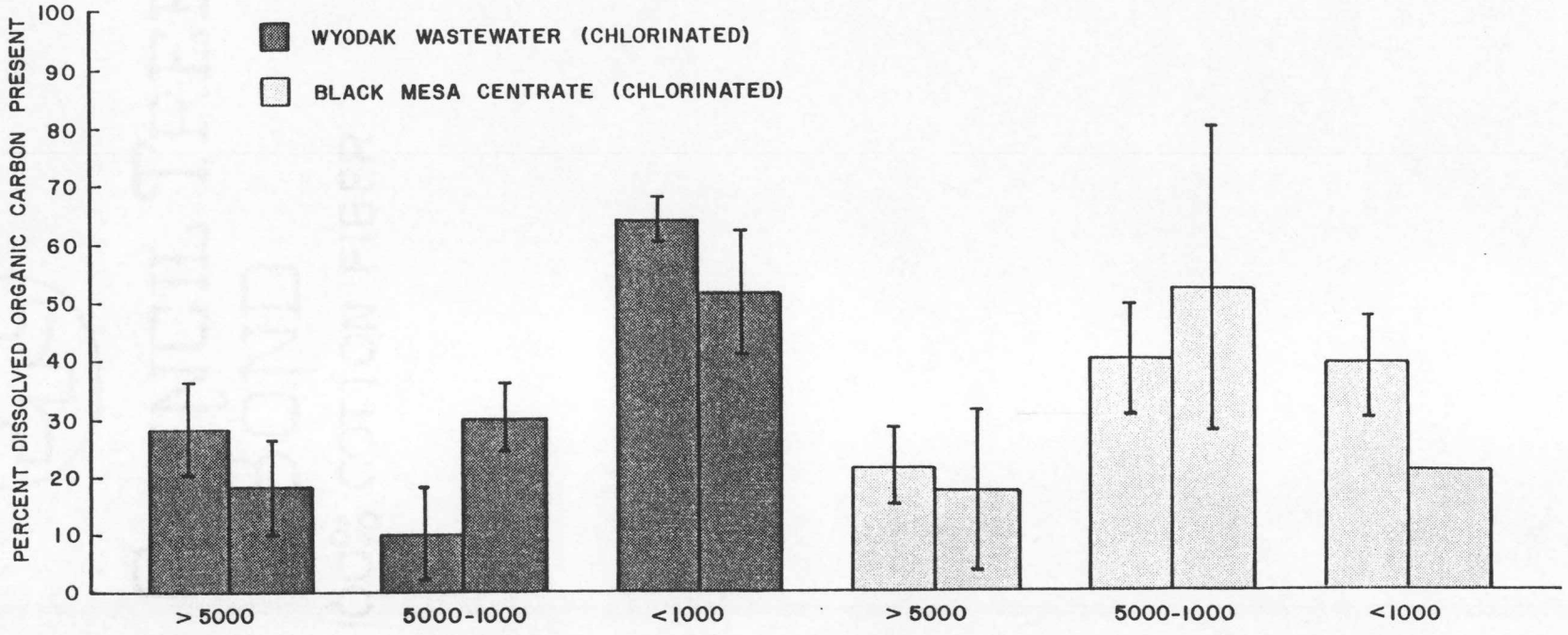

Figure 10. Molecular weight profile of chlorinated slurry wastewater before and after biological treatment. Bars are presented in pairs for each molecular weight fraction. The bar on the left represents the DOC present before treatment while the bar on the right is the percentage DOC after treatment.

Biofilm Growth. The substratum examined for colonization consisted of one set of cellulose acetate strips (3.5 cm x 115 cm) individually wrapped around stainless steel developing reels (Figure 3, page 48). For each study the growth chamber (Figure 2, page 47) consisted of two reels, each containing one set of cellulose acetate strips. Since sections of the strips were to be removed as representative subsamples of the entire substratum, the underlying assumption was that each section of the strip was colonized at similar rates.

This hypothesis was examined by determining the population densities and biomass concentration at five to six different locations at equal distance from each other along the acetate strips. After seven days of colonization time by the activated sludge, triplicate sub-sections at each site were taken and the ATP and AODC measurements were made for each site. The results were analyzed by Analysis of Variance (ANOVA) test to determine if there was any significant difference between colonized sites (171). The results of the ANOVA for both AODC and ATP measurements are listed in Tables 12 and 13. The fact that F_s (calculated) was less than F_{table} for both measurements (ATP and AODC) shows that there was no significant difference in biomass between sites. Thus all sites along the cellulose acetate strips were colonized at equal rates.

Biomass Estimations. The BMC slurry wastewater was capable of supporting a biofilm community on the substratum provided. The biofilm community was visualized by scanning electron microscopy and the

Table 12. Analysis of Variance (ANOVA) of the Colonization of the Cellulose Strips--AODC

Source of Variation	Degrees of Freedom (df)	Sum of Squares (SS)	Mean Squares (MS)	F_s
Between sites	5	2.0×10^{16}	4×10^{15}	1.2
Within sites	6	2.0×10^{16}	3.3×10^{15}	
Total	11	4.0×10^{16}		
		$*F_{0.05\ [5,6]} = 4.39$		

*Since $F_s < F_{0.05\ [5,6]}$ there is no significant difference between sites.

Table 13. Analysis of Variance (ANOVA) of the Colonization of the Cellulose Strips--ATP

Source of variation	Degrees of Freedom (df)	Sum of Squares (SS)	Mean Squares (MS)	F_s
Between sites	4	1.90	0.48	1.63
Within sites	9	2.94	0.29	
Total	13	10.85		
	$*F_{0.05\ [4,9]} = 4.39$			

*Since $F_s < F\ 0.05\ [4,9]$ there is no significant difference between sites.

infrastructure was directly affected by the sample preparation procedure (154). Two distinctly different procedures were utilized in this study (see Materials and Methods). Figures 11 and 12 are scanning electron micrographs of the fixed film microorganisms on the cellulose acetate strips. In Figure 11 the microorganisms were barely discernible since a slime layer covered most of the microorganisms preventing direct visualization. In Figure 12 the microorganisms were easily seen and were connected by fibrillar like structures. Both the slime layer and the fibrillar structures have been identified by other investigators (47, 71, 154) as the glycocalyx or extracellular polysaccharide produced by the biofilm community. Richard and Turner (154) examined the affect of sample preparation on the biofilm infrastructure and concluded that the biofilm microorganisms were immersed in a hydrated slime layer and the fibrillar structures were an artifact of sample preparation. The fibrillar structures were a direct result of the dehydration of the slime layer caused by the alcohol dehydration treatment.

After initial colonization of the strips, the concentration of microorganisms steadily increased over a two week period as indicated by the AODC values (Figure 13). Initial (day 1) concentration of microorganisms was 4.80×10^7 bacteria cm^{-2} and increased by over three-fold by day 16 to approximately 1.80×10^8 bacteria cm^{-2}. However, the AODC values include all organisms within the biofilm community which may include attached, moribund, and dead cells. Since ATP levels are often provided as an indication of the potential of cellular activity (101), ATP measurements were performed on the biofilm

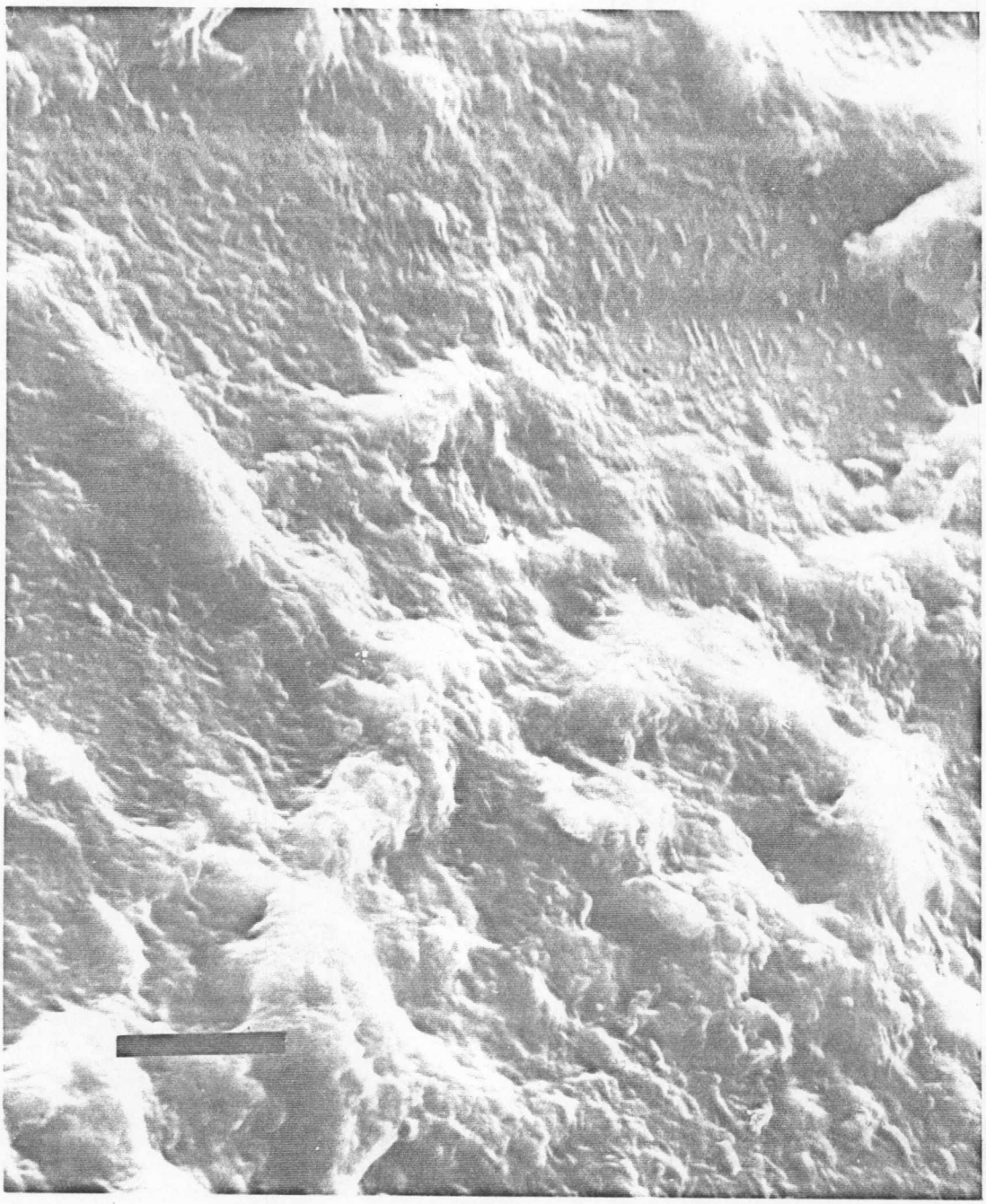

Figure 11. Electron micrograph of the biofilm community--sample A. Sample was prepared using liquid nitrogen. Bar represents 10 microns. Total magnification is 2400x.

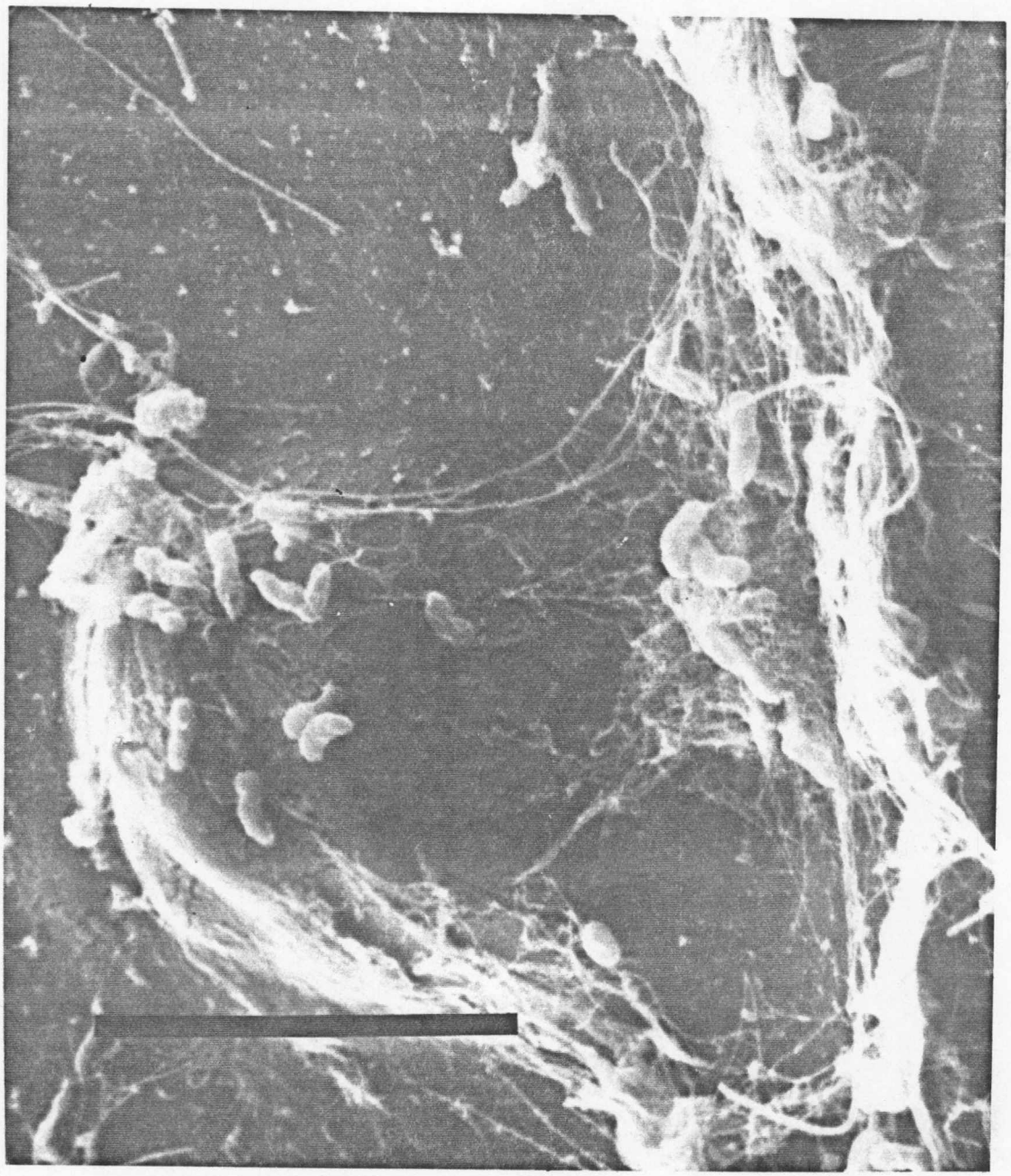

Figure 12. Electron micrograph of the biofilm community--sample B. Sample was prepared using glutaraldehyde and ethanol dehydration steps. Bar represents 10 microns. Total magnification is 6200x.

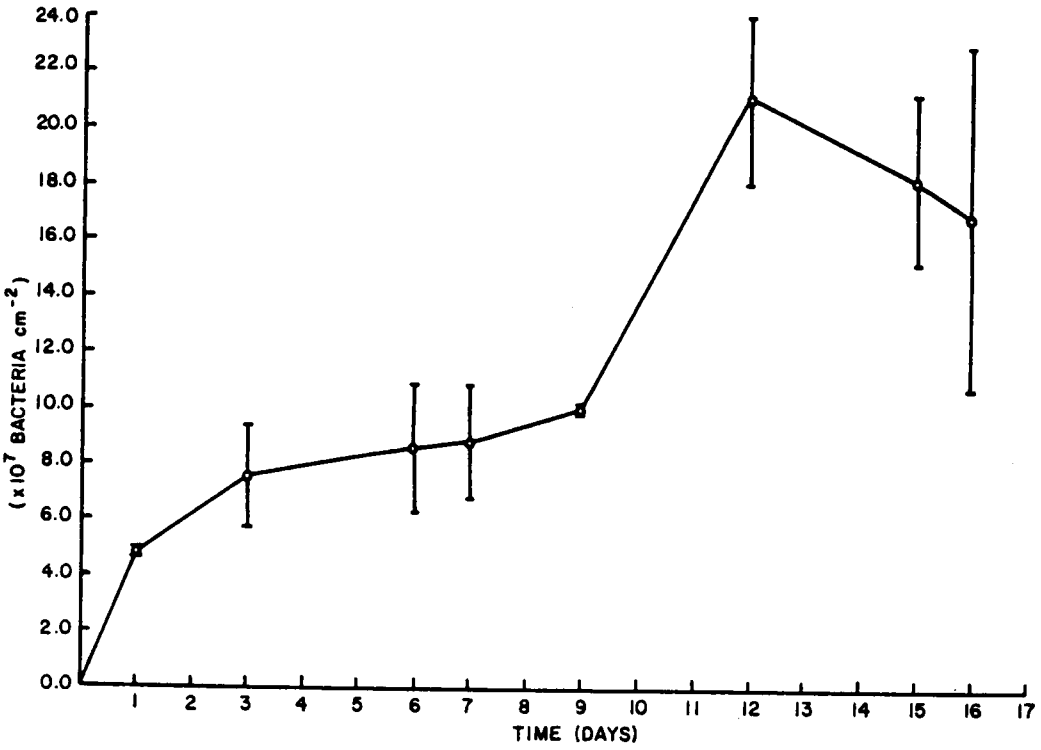

Figure 13. Acridine orange direct counts of the microorganism in the biofilm community. Each observation is the result of triplicate samples.

to ascertain the overall viability of the biofilm community during colonization and development. Figure 14 presents these results. The most rapid accumulation of ATP occurs within the first three days of colonization. After day 1 there was an average of 1.5 μg of ATP cm^{-2} increasing by over three-fold by day 3 to 4.75 μg of ATP cm^{-2}. After day 3 there occurs a gradual but steady increase in ATP until day 16 (5.7 μg of ATP cm^{-2}) although this represents only a 20% increase in ATP over a 13 day period (day 3 to day 16).

The biofilm community continually increased in both cell numbers and ATP content over the two week period when the community was grown on BMC slurry wastewaters. However, the microorganisms in the biofilm appeared to be more viable during the early stages of surface colonization (39). This was demonstrated by examining the average value of ATP per bacterial cell. During the earlier stages of colonization (one to three days) each bacterium contains approximately 6.30×10^{-8} μg of ATP. As indicated by Figure 13 the number of cells which remained attached increased over the next 13 days, but the ratio of ATP/bacterium steadily decreased until day 16. By day 16 there was a 47% reduction in this ratio to 3.37×10^{-8} μg of ATP/bacterium. Therefore, on a per cell basis, the biofilm community was more viable during the earlier stages of colonization of the substratum.

Biofilm Mineralization Capacity. AODC and ATP measurements are indirect assays for assessing the biological activity of the fixed film community. A more direct method is to examine the rate of substrate

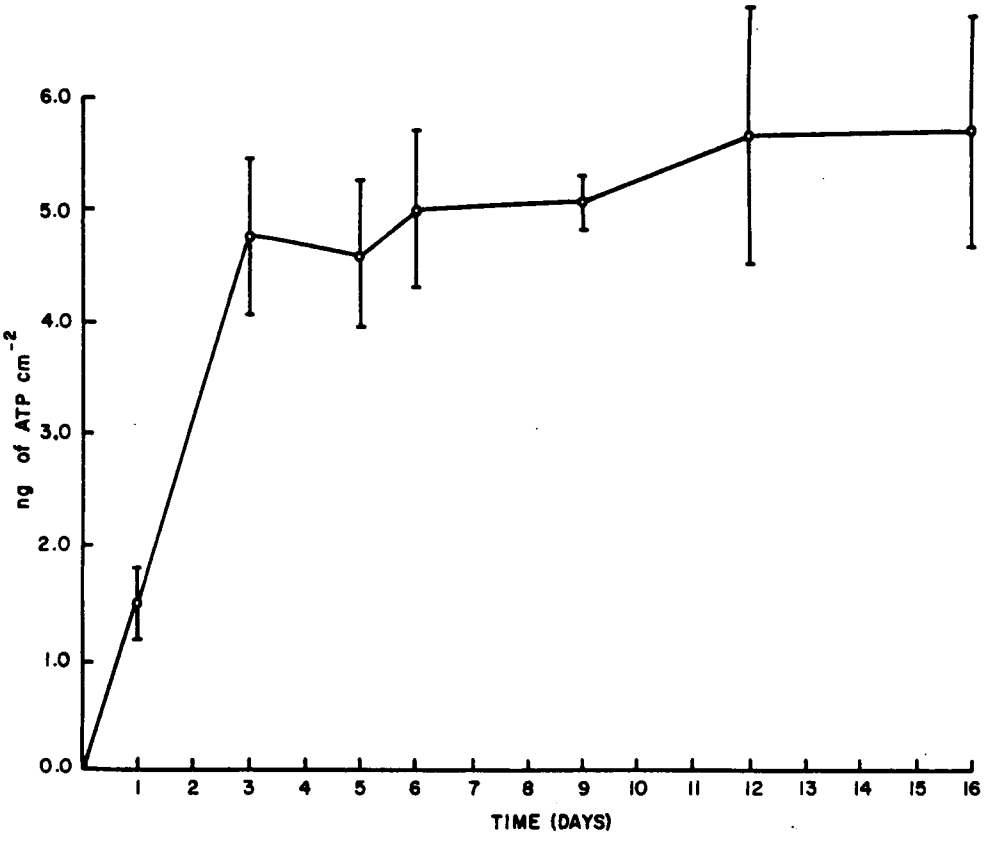

Figure 14. ATP determinations for the biofilm community. Each observation is the result of triplicate samples.

mineralization or substrate removal rates of organic compounds contained in the BMC slurry wastewater. To more closely examine the capacity of the biofilm community to assimilate and catabolize the organic constituents present in the slurry water, ^{14}C labeled organic compounds were added to the BMC slurry wastewater. Consequently, two separate radiolabeled compounds, glucose and para-hydroxybenzoic acid, were added separately to the slurry wastewater at final concentrations of 3.0×10^{-5} and 1.0×10^{-3} mg liter^{-1} respectively which represented less than 0.1% of the total DOC; thus the addition of the labeled organic compounds did not dramatically alter the total DOC concentration of the BMC slurry water.

Mineralization of glucose is commonly employed to evaluate heterotrophic activity of microbial populations. However, there was little evidence that glucose would be a chemical constituent of the BMC slurry wastewaters (152). A previous report by Davis and coworkers (55) documented that fulvic acids comprise a majority of the slurry water's DOC. The chemical structure of the fulvic acids has not been conclusively elucidated but various model organic chemicals have been suggested as compounds representing the phenolic polymer core of humic/fulvic acids. These compounds include 3,5-dihydroxybenzoic acids (23, 95) and hydroxybenzoic acids (106, 192). Thus, radiolabeled (^{14}C) para-hydroxybenzoic acid was chosen as a model compound to represent the core substituent of the fulvic acids present in BMC slurry wastewaters. The removal rate for both glucose and para-hydroxybenzoic acid from BMC slurry wastewater increased with time over the two week colonization period (Figure 15 and 16). The glucose

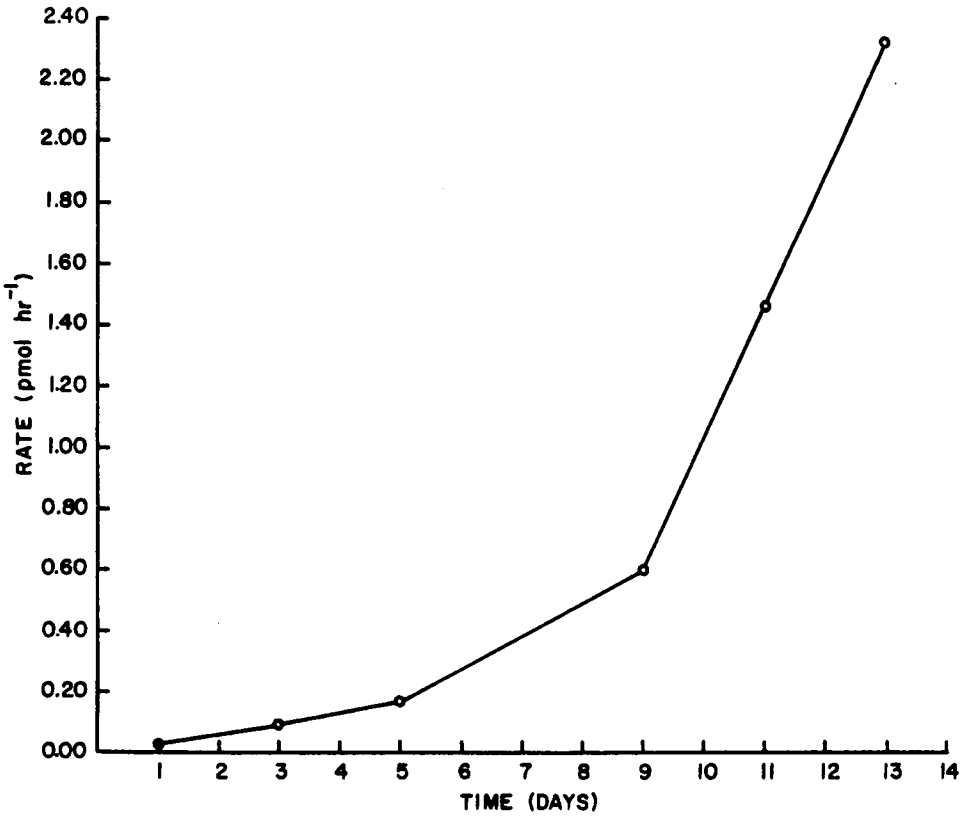

Figure 15. Rate of ^{14}C-glucose removal from slurry wastewater by biofilm community.

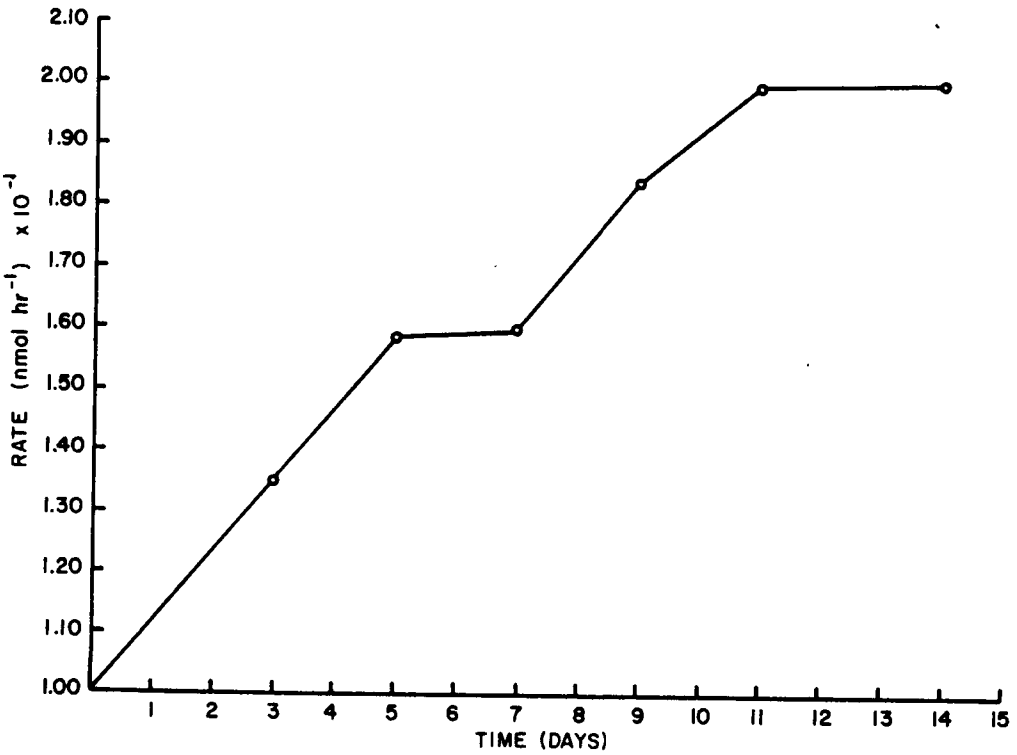

Figure 16. Rate of removal of ^{14}C-para-hydroxybenzoic acid from slurry wastewater by a biofilm community.

removal rate increased approximately twenty-four-fold from day 1 (9.72 x 10^{-2} pmol hr^{-1}) to day 13 (2.34 pmol hr^{-1}) (Figure 15). Since mineralization rates only increased by two to three-fold (6.80 x 10^{-2} to 1.61 x 10^{-1} pmol hr^{-1}) over the same time period, much of the glucose removed was accumulated into microbial biomass in the fixed film. Furthermore, approximately 70% of the labeled glucose removed on day 1 (6.80 x 10^{-2}/9.72 x 10^{-2} x 100%) was mineralized to carbon dioxide, while by day 13 only 7% (1.61 x 10^{-1}/2.34 x 100%) of the glucose removed was oxidized to carbon dioxide. These results suggest that during the early stages of biofilm development substrate utilization is primarily for energy production. This hypothesis is supported by the fact that most of the substrate removed during the early stages was completely mineralized. As the biofilm matures more substrate was diverted from energy production to biomass accumulation.

The mineralization assays utilizing para-hydroxybenzoic acid resulted in similar patterns exhibited by the glucose mineralization experiments. The rate of para-hydroxybenzoic acid removal from BMC slurry water only increased 51% (Figure 16, page 108) from day 3 (1.35 x 10^{-1} nmol hr^{-1}) until the termination of the study (2.04 x 10^{-1} nmol hr^{-1}). The para-hydroxybenzoic acid was more readily utilizable than the glucose since the rate of para-hydroxybenzoic acid removal was approximately two fold greater than the glucose removal rate even after the rates were normalized to substrate concentrations. This preference is not surprising since the biofilm was grown on slurry wastewater which contains numerous aromatic compounds (152) and may be

better acclimated to catabolizing aromatic acids rather than simple sugars like glucose.

The mineralization rates for para-hydroxybenzoic acid were examined in a separate series of experiments (Figure 17). There was a six to seven-fold increase in the mineralization rates of para-hydroxybenzoic acid over the course of the study, 4.75×10^{-2} nmol hr^{-1} at day 1, compared to 3.14×10^{-1} nmol hr^{-1} by day 10. A slight decrease in the mineralization rate was experienced at the termination of the study. As with the glucose, a greater percentage of the labeled para-hydroxybenzoic acid was directed into the biomass as the colonization time increased. Table 14 expresses this relationship quantitatively. This table represents the average value of two separate experiments and was based upon the ratio of the amount of para-hydroxybenzoic acid accumulated into the biomass over the total amount of labeled substrate removed. Initially (day 1) 17% of the labeled para-hydroxybenzoic acid removed was found in the biomass of the biofilm. By day 5 this ratio increased approximately 61% and now 27.5% of the label removed was found in the biomass fraction. For the next eight days there was a slight rise in this ratio but by day 13 the values were approximately the same as for day 5.

The results of both the para-hydroxybenzoic acid and glucose mineralization assays indicated that after colonization of the substratum was established by the activated sludge, more of the substrate removed from solution was diverted into the biofilm's biomass.

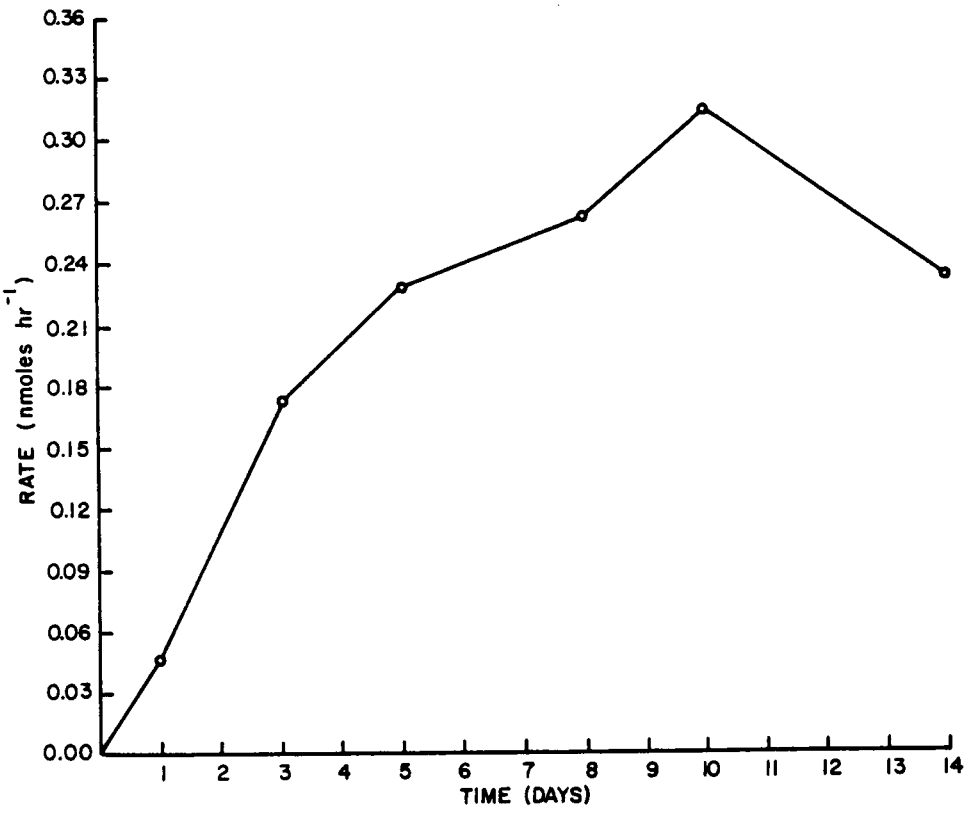

Figure 17. Rate of mineralization of ^{14}C-para-hydroxybenzoic acid in slurry wastewater by a biofilm community.

Table 14. Incorporation of ^{14}C-Para-Hydroxybenzoic Acid into the Biofilm

Day	$\frac{\text{Accumulated into Biomass}}{\text{Removal from Slurry Wastewater}} \times 100\%$
1	17.6 ± 1.0[b]
3	20.2
5	27.5 ± 3.4
7	30.5 ± 3.2
11	31.0 ± 7.1
13	28.6 ± 2.1

[a]All values are the average of two experiments except day 3 represents only the observation from one experiment.

[b]± one standard deviation.

Biofilm Removal of Wastewater DOC. The original purpose of this investigation was to examine the biologically mediated removal of slurry wastewater DOC by the fixed film community. However, over the time course examined there was a net increase in DOC in the raw BMC slurry water. Table 15 represents the average value of three separate experiments and the raw effluent DOC represents an approximate 29% increase (5.3 mg of carbon $liter^{-1}$) in the DOC concentration over the influent DOC levels. Approximately 70% of this increase (3.7 mg of carbon $liter^{-1}$) can be attributed to an increase in the DOC contained in the >5000 fraction.

Since a net increase in DOC occurred, an additional study was conducted to ascertain whether the biofilm community was capable of net DOC removal over extended time intervals. The growth chamber was inoculated once daily for one week. During this time period there occurred extensive colonization of the cellulose acetate strips. By the end of the seventh day, the level of DOC in the effluent was 30 ppm representing a 50% increase (Table 16). As the time after surface colonization increased there was a gradual reduction in the effluent DOC until day 29 when the effluent DOC was 17.4 mg of carbon $liter^{-1}$, representing a 13% reduction.

These fluctuations in effluent DOC represented an equilibration period for the biofilm community. Organic carbon compounds were leaching from the microorganisms present in the biofilm and there was no net reduction in the effluent DOC. After this equilibration period, there was a net removal of DOC by the biofilm microorganisms. However, these studies were terminated prematurely, therefore no kinetic

Table 15. Molecular Weight Characterization of Black Mesa Centrate After Contact with a Microbial Biofilm

	Influent DOC[a]	Effluent DOC
Greater than 5000	3.5 ± 2.7 (19%)[b]	7.2 ± 3.5 (31%)
5000-1000	5.3 ± 0.8 (29%)	4.0 ± 1.6 (17%)
Less than 1000	8.9 ± 2.3 (49%)	11.3 ± 3.5 (49%)
Raw BMC Slurry Water	18.0 ± 1.3	23.3 ± 3.2

[a]All values reported as mg of carbon liter^{-1}.

[b]Values in parentheses represent percentage of total DOC.

Table 16. Influent/Effluent DOC Levels in a Continuous Flow System in the Presence of a Biofilm Community

Day	Influent DOC	Effluent DOC
7	20[a]	30
11	20	28
18	20[b]	21
29	19	17

[a]All DOC values are recorded as mg of carbon $liter^{-1}$.

[b]After influent sample was taken a new batch of BMC slurry water was employed as influent.

parameters were determined for the fixed film community using the continuous flow system.

VI. DISCUSSION

Respirometer Studies and DOC Removal

Activated sludge treatment of municipal and industrial wastewaters is an accepted method for wastewater treatment in the United States (196). Since coal slurry wastewaters have been documented to contain elevated organic carbon levels, activated sludge was employed to reduce the organic load and improve the overall quality of the slurry wastewater. The organic constituents of the slurry wastewaters have been characterized as polar, water soluble compounds of variable but high molecular weight, and predominantly fulvic acid in nature originating from the organic compounds of the parent coal (152). Activated sludge contains a wide variety of microorganisms (58). These microorganisms may represent the source for the widest diversity of degradative enzymes capable of catabolizing the wide range of organic compounds present in the slurry wastewater.

DOC measurements conducted at the beginning and termination of each of the respirometer studies demonstrated that between 51% to 74% of the organic carbon was removed from the slurry wastewater (Table 4, page 68). These determinations did not reveal the mechanism(s) of DOC removal. Oxygen consumed during the course of respirometry studies provided evidence that the organic carbon removal was biologically mediated. The BOD is a measure of the amount of oxygen required by the microorganisms to biochemically oxidize the chemical constituents (both the organic and inorganic chemicals) present in an aqueous sample during a specific time interval. It is an indirect measurement of the pollution potential of a wastewater and the efficiency of wastewater

treatment. The BOD_{20} exerted by Wyodak slurry wastewater ranged from 52-216 mg $liter^{-1}$ (Table 3, page 63) indicating the organic constituents were oxidized by the activated sludge. However, there was not a direct relationship between the amount of organic carbon removed and the BOD exerted by the wastewater. The lack of correlation was demonstrated when comparing the results for different Wyodak wastewaters (Table 3, page 63 and Table 6, page 72). The net DOC removed from Wyodak samples 4 and 5 was similar (39.9 vs. 44.1 mg of carbon $liter^{-1}$--Table 6, page 72) but the BOD exhibited an approximate three-fold difference (152 vs. 52 mg $liter^{-1}$--Table 3, page 63). BOD determinations describe the oxygen demand for the slurry wastewater but do not provide a direct measurement of the overall organic load or carbon removal.

The lack of correlation between the BOD and the organic carbon concentration has been documented by others (14, 191). When the organic load of domestic sewage, septic tank effluent, and polluted groundwater was examined it failed to correlate with the BOD measurements made for the same wastewaters. Cooper and Young (45) warned against drawing spurious correlations between BOD measurements and nonbiological methods, such as DOC determinations, for assessing the organic carbon concentration of a wastewater for the following reasons:

1. DOC analysis does not differentiate between biodegradable and non-biodegradable organic carbon
2. Oxidation of reduced inorganic compounds can contribute to the increase in BOD

3. The seed inoculum employed in BOD studies can result in increase variability because of factors such as toxicity, lack of proper acclimation etc., which would not occur in the DOC analysis

Since BOD determinations were only an indirect measure of the potential for carbon removal, kinetic parameters were developed to provide predictive capabilities for the biologically mediated removal of DOC from slurry wastewaters. Factors controlling microbial transformation rates are poorly understood but are assumed to be enzymatically mediated and should follow Michaelis-Menten type kinetics (94, 111). Under non-saturation conditions, at substrate concentrations less than K_s, substrate removal is a first order process with respect to substrate concentration (107, 141, 142). In this investigation DOC removal was determined to be pseudo-first order with respect to the DOC concentration as predicted by the first order rate equation developed for batch reactors:

$$\frac{-d[DOC]}{dt} = k_1 [DOC]$$

When integrated the equation takes the form:

$$\log [DOC] = k_1 t + \log [DOC]_{initial}$$

Since substrate removal has also been shown to be a first order process with respect to biomass concentration (94, 107, 111, 141, 142), the first order rate constants were normalized to the dry weight of the activated sludge. The average value of the second order kinetic constants (k_2) for the batch culture (respirometer) studies was 1.93 x 10^{-4} liter day^{-1} (mg of sludge)$^{-1}$ (Table 5, page 70).

This difference was only 25% from that determined for the semi-continuous fill and draw reactors (1.43×10^{-4} liter day^{-1} [mg of sludge]$^{-1}$ Figure 6, page 83). In contrast, the first order kinetic constants (k_1) for the batch reactors varied from 0.022-0.049 day^{-1} (Table 5, page 70) and from 0.27-0.49 day^{-1} for the semi-continuous system. This difference is over twenty-fold for the two systems and renders direct comparison between the reactors difficult. When the results were normalized to the biomass concentration, the kinetic constants then became comparable between the two different reactor systems. Similar rate constants developed for the different reactors further supports the interpretation that the differences in the k_1 values were due to the total biomass. The relationship between biomass and organic carbon removal from Wyodak slurry wastewater is further demonstrated in Figure 7 (page 76). These results reinforce the importance of accounting for biomass concentration in the development of kinetic parameters for predicting biological treatability.

The differences in the reactor designs between the fill and draw and the batch reactor systems would be expected to affect the activity of the activated sludge. For the batch reactors, potentially toxic metabolic end products and nutrient depletion should occur reducing the overall metabolic activity of the microorganisms. In the fill and draw system there occurs, on a bidaily basis, a removal of a fraction of the clarified supernatant with a concomitant introduction of fresh nutrients (slurry wastewater). Microbial activity would be expected to be higher under these conditions. Despite the dissimilarities between

the reactor designs, comparable second order rate constants were determined for each system. Sayler et al. (162) reported similar observations for the biotransformation of aniline, phenol, and toluene in an activated sludge system. Whether batch or semi-continuous reactor systems were employed second order rate constants describing the mineralization of these aromatic compounds were nearly identical. The close agreement between the k_2 values developed for both systems imparts further credibility to the predictive capabilities of the kinetic constants for biological treatment under different environmental conditions.

The second order rate constants for the biological removal of the organic compounds from slurry wastewater compare favorably with those developed by other investigators (15, 140, 162). One problem with comparing rate constants with published literature values is the units for these constants are often different. This difference renders direct comparison impossible and is often the result of the varying procedures utilized for biomass estimations. To facilitate comparison between kinetic constants in the literature and those determined in this investigation, all values were converted to liter day^{-1} (mg of microorganism)$^{-1}$. The conversion was accomplished by multiplying by the appropriate conversion factor for biomass estimation.

Paris et al. (141) examined the transformation of phenols and substituted phenols by pure cultures of Pseudomonas putida. The k_2 values for these studies range from 2.25×10^{-5} to 8.00×10^{-4} liter day^{-1} (mg of bacteria)$^{-1}$. Since these values were determined for pure culture, they may not be directly applicable for

comparison with those developed for a mixed microbial community such as activated sludge. However, Sayler et al. (162) developed k_2 for the mineralization of aromatic compounds under batch culture conditions using acclimated activated sludge. Average k_2 values for phenol and toluene were 2.12×10^{-4} and 3.50×10^{-4} liter day^{-1} (mg of bacteria)$^{-1}$ which range very close to the average value determined for the removal of DOC from Wyodak slurry wastewater (1.93×10^{-4} liter day^{-1} [mg of sludge]$^{-1}$).

Rogers et al. (156) also examined the removal of DOC from shale retort wastewater by a soil inoculum. The compounds were identified by gas chromatography/mass spectrometry and were very similar to those reported for Wyodak slurry wastewater. Aromatic acids and aliphatic mono- and dicarboxyalic acids comprised the majority of the retort wastewater DOC. Rogers et al. (156) only listed raw values for DOC removal. When their data was fit to the second order model the average k_2 value was 3.5×10^{-5} liter day^{-1} (mg of bacteria)$^{-1}$. This value was lower than those recorded for DOC removal from Wyodak slurry wastewaters (Table 5, page 70) and could be attributed to the inoculum employed. For the shale retort wastewater an unacclimated soil inoculum was utilized as compared to the acclimated sludge inoculum used in the respirometer studies. The acclimation procedure rendered the activated sludge microorganisms more capable of metabolizing the DOC contained in the slurry wastewater.

The BOD studies and the rate kinetic parameters for DOC removal demonstrate that biological treatment with activated sludge was successful in reducing the organic load of the slurry wastewater. The

DOC removed was liberated as carbon dioxide or transformed into additional biomass by the activated sludge (Table 6, page 72). Total removal of the organic contaminants from the slurry wastewater was unsuccessful and a residual carbon concentration remained after treatment (an average of 33 mg of carbon $liter^{-1}$). The BOD_{20}/DOC ratios also corroborate this hypothesis. The average value for this investigation was 0.57 ± 0.16 (Table 3, page 63). Literature values in the range of 1.26 to 1.44 (14, 18) were reported for settled influent and domestic sewage. The BOD/TOC ratios for biologically treated industrial and domestic wastewaters routinely range from 0.2 to 0.7. These lower values were closer to those obtained in this investigation for untreated slurry wastewaters. Such reduced ratios (0.35 to 0.68--Table 3, page 63) indicate the slurry wastewater contains a high concentration of non-biodegradable (or slowly biodegradable) organic compounds.

The nature of this recalcitrant organic material was further elucidated using ultrafiltration studies. The molecular weight distribution profile of the Wyodak slurry wastewater (Figure 5, page 78) indicated that approximately 60% of the DOC was <1,000 MW. The microorganisms used in the respirometer studies preferentially removed the lower molecular weight compounds. This preferential utilization results in a shift in the molecular weight distribution after treatment to a wastewater dominated by higher molecular weight DOC. These results were in agreement with other investigators who found that higher molecular weight organic compounds comprised the major fraction of activated sludge treated effluent. For example, Manka and Rebhun

(119) reported 61% of the DOC from a sewage treatment plant was of higher molecular weight (>5,000 MW) and only 17% was <500 MW. Grady et al. (85) also examined the effluent from a synthetic waste, bench scale activated sludge reactors. They found the majority of the DOC disappearance was accomplished by the removal of the <1,000 MW fraction.

The unavailability of the higher molecular weight compounds to the microorganisms could explain the inability of the activated sludge to remove the higher molecular weight DOC. The increased size of these molecules may prohibit their transport and prevent their subsequent removal from solution. Alternately, the microorganisms present in the activated sludge may not possess the enzymatic machinery necessary for the metabolism of these larger organic compounds which were apparently humic polymers (152).

The DOC in the larger molecular weight fractions (> 5,000 MW and 1000-5000 MW) appeared to be more slowly transformed or were completely resistant to removal. The >1000 MW also comprised over 60% of the residual DOC. This residual fraction may not contain the same organic components that were present in the initial wastewater. The organic compounds present in the slurry wastewater prior to biological treatment may have been removed by the activated sludge and the residual DOC in these fractions may have originated through the metabolic activity of the activated sludge. There are numerous reports available which indicate the higher molecular weight compounds in biologically treated wastewater effluents are the direct byproducts of microbial metabolism (53, 57, 85). Ultrafiltration techniques employed

in this study to fractionate the DOC were unable to differentiate between these two possibilities. The failure of the higher molecular weight fraction (>5000 MW) to stimulate microbial activity in the form of oxygen consumption (Table 8, page 81) supports the original hypothesis. Thus, the higher molecular weight DOC compounds were recalcitrant under the conditions employed in this investigation.

Chlorination Studies

The chlorination of Wyodak and BMC slurry wastewaters resulted in an alteration in the DOC. Davis et al. (55) reported the production of elevated levels of trihalomethanes when these wastewaters were chlorinated. These volatile, halogenated, organic compounds originate primarily from the reaction of hypochlorous acid with the fulvic substances present in the slurry wastewater (157). The fulvic content for the Wyodak and BMC slurry wastewater was 56% and 66% of the total DOC (55). Because of the elevated fulvic content, other chlorinated organics will form when the slurry wastewater is chlorinated. This investigation did not provide direct evidence for the presence of nonvolatile chlorinated organic compounds in the chlorinated slurry wastewater. There is ample published evidence (23, 95, 126) which demonstrates the chlorination of fulvic substances result in the production of nonvolatile halogenated organic compounds. Thus, it is logical to expect these types of compounds would form in the chlorinated slurry wastewater as a result of the reaction of hypochlorous acid with the fulvic material.

Fulvic substances are complex high molecular weight compounds of uncertain molecular structure but are reported to have a high aromatic content. To facilitate identification of the end products resulting from the chlorination of fulvic substances, model phenolic compounds of fulvic substances such as dihydroxyaromatics, are often utilized in chlorination studies (23, 95, 157). After chlorination of these model fulvic organic compounds, mono-, di-, and trichlorinated aromatics as well as chlorinated aliphatic compounds were generated. Miller and Uden (126) examined the nonvolatile aqueous chlorination products after the chlorination of fulvic substances isolated from soil. They concluded the chlorination of these soil fulvic substances resulted in the production of chlorine containing polyfunctional, aromatic compounds as well as chloroaliphatic acids. Furthermore, fulvic substances originating from two distinctly different terrestrial habitats and one aqueous sample were also chlorinated. Fulvic substances from different sources were chlorinated to determine the variation which exist in the chlorination products. When the chlorine selective fingerprints (i.e. chromatograms) were compared, little differences were noted in the types of chlorination products. A significant quantitative difference did exist indicating that chlorination of fulvic material result in similar types of nonvolatile chlorinated byproducts regardless of the source of the fulvics. Similar types of compounds may be present in the chlorinated slurry wastewaters.

The chlorination of the slurry wastewater also altered the response of the microorganisms during biological treatment. A

comparison of the average second order rate constants (k_2) between the unchlorinated and chlorinated Wyodak slurry wastewater was made and over a 60% reduction was noted for the chlorinated wastewater (1.93×10^{-4} [Table 5, page 70] vs. 6.90×10^{-5} liter day^{-1} [mg of sludge]$^{-1}$ Table 10, page 90). This reduction can be attributed to the slower transformation of the chlorinated organic compounds which were formed as a result of the chlorination of the slurry wastewater. This reduction was not unexpected since increased chlorine substitution in organic molecules is known to inhibit their subsequent biodegradation (42, 118, 159). Banerjee et al. (15) determined the rates of biodegradation of phenols, aromatic acids, and chlorine substituted phenols and aromatic acids in pure cultures and natural freshwater systems. In every case examined the nonchlorinated parent compound had the highest rates of biodegradation and the rates became progressively lower as the number of chlorine substituents incorporated into the parent molecule increased. Therefore, an increase in the concentration of halogenated organics in the wastewater may have resulted in a lower efficiency of biological removal of the DOC, and was ultimately reflected in the lower k_2 values for the chlorinated slurry wastewater.

Increased chlorine substitution renders organic molecules recalcitrant, but an alternate explanation can be invoked to explain the diminished rate of DOC removal in the chlorinated slurry wastewaters. There may have been a decrease in the number of microorganisms capable of metabolizing the DOC contained in the chlorinated slurry wastewater. The lower number of metabolically

active microorganisms may have occurred without a concomitant decrease in the total biomass. Second order rate constants were developed by normalizing k_1 to the biomass concentration as determined by dry weight analysis. Dry weight determinations do not account for the overall viability of the activated sludge or the number of microorganisms capable of growth and/or utilization of the DOC in the chlorinated slurry wastewater. If a reduction in the number of actively catabolizing microorganisms occurred without a reduction in the dry weight of the activated sludge, the k_2 values obtained for the chlorinated studies may be an underestimation of the true rates. However, this explanation does not negate the observation that chlorination of the slurry wastewater reduces the efficiency of the activated sludge population rate of DOC removal.

One of the most significant variables affecting the biological transformation of the wastewater DOC was the acclimation process. Extended acclimation periods (greater than one month) of activated sludge proved to be an important parameter controlling the rate as well as the specificity of DOC removal. For the Wyodak and BMC slurry wastewaters, k_2 values (Table 10, page 90) were greater for the chlorinated as compared to the unchlorinated slurry wastewater when the inoculum was acclimated to the chlorinated slurry wastewater. For the Wyodak samples there was an approximate three-fold decrease in the k_2 (6.90×10^{-5} vs. 2.41×10^{-5} liter day^{-1} [mg of sludge]$^{-1}$--Table 10, page 90) while there was no net DOC removal from the unchlorinated BMC. These results demonstrate the chlorination of the slurry wastewater altered the original DOC to such an extent

the sludge acclimated to the chlorinated slurry wastewater could no longer metabolize the parent DOC or did so with a reduced efficiency. Crawford et al. (49) reported similar findings concerning the ability of a freshwater bacterial isolate to utilize chlorinated compounds but were unable to catabolize the non-halogenated parent substrate. They isolated a strain of Bacillus brevis which grew on 5-chlorsalicylate as the sole carbon and energy source. The organism catabolized this compound since it had evolved a novel enzyme, chlorosalicylate 1, 2-dioxygenase. This enzyme was capable of cleaving the aromatic ring. The unsubstituted parent compound, salicylate, was not cleaved by this enzyme and the bacterium could not grow on the parent substrate as a sole carbon source.

Extended acclimation of the activated sludge also enabled the microorganisms to transform the higher molecular weight compounds (>5000 MW). As seen in the results in Table 11 (page 94) an average of 42% and 32% of the total DOC removed was from the >5000 MW fraction. In contrast, the microorganisms employed for the unchlorinated respirometer studies (Table 7, page 76) displayed little activity in the removal of the >5000 MW fraction with the exception of one sample. Wyodak sample 5 exhibited a 28% reduction in the >5000 MW fraction. Again this reduction can be understood in terms of the acclimation period. For the other Wyodak samples (1 through 4) the acclimation period of the activated sludge was one week or less. This brief acclimation period allowed insufficient time for the activated sludge to acquire the capacity to attack the higher molecular weight compounds which were present in the residual slurry wastewater. The activated

sludge utilized for Wyodak sample 5 had been previously acclimated to Wyodak slurry wastewater for one month. During this time the activated sludge developed the capacity to transform the >5000 MW fraction as demonstrated by the net reduction in this fraction (Table 7, page 76).

The molecular weight fractionation studies of the slurry water DOC showed the importance of extended acclimation intervals for the successful removal of the higher molecular weight components. The microorganisms of the activated sludge, which had been previously exposed to the slurry wastewater for only brief periods (less than one week), preferentially removed only the simpler (<1000 MW) organic compounds with little net removal of the higher molecular weight fractions. In contrast, extended acclimation intervals (greater than one month) provides sufficient time for the activated sludge to develop the metabolic machinery enabling the microbial community the capabilities to transform the more complex organic constituents contained in the slurry wastewaters.

Biofilm Growth and Development

The primary goal of the final phase of this study was to develop kinetic parameters for DOC removal in a continuous flow system utilizing a microbial biofilm. This goal was not achieved because of the failure of the fixed film community to reduce the DOC of the effluent below that of the influent slurry wastewater. The increased DOC levels in the effluent indicated the biofilm was contributing to the overall concentration of the soluble organic carbon. Bacterial carbon storage products such as polyhydroxybutyrate (58) or the glycocalyx may have contributed towards the effluent DOC.

Since the biofilm failed to establish a reduction in the effluent DOC during the first month, this does not imply that the biofilm community was incapable of metabolizing the organic constituents present in the BMC slurry wastewater. On the contrary, the BMC slurry wastewater DOC provided sufficient carbon and energy for biofilm growth and development. The ability of the biofilm microorganisms to remove and mineralize model fulvic compounds provides further evidence of its heterotrophic potential during the early stages of development.

The increase in the effluent DOC suggests an equilibration period was required for the fixed film community before successful biological treatment. After approximately one month (29 days) the equilibration period for the biofilm community was complete and the net removal of slurry wastewater DOC was accomplished. Future studies would need to account for this equilibration period before developing kinetics parameters for wastewater treatment designs.

This investigation also help to elucidate the processes occurring within a biofilm community during attachment and growth. The early stages of biofilm growth (zero to three days) during the colonization of the substratum, the microorganisms involved required a larger expenditure of energy as compared to the latter stages (greater than three days). Substrate utilization during the first three days was primarily for the generation of cellular energy and only 17% to 20% of the para-hydroxybenzoic acid removed was incorporated into the biomass. The remainder of the substrate removed was mineralized for energy production. After day 3 a shift in the substrate metabolism occurred and more of the para-hydroxybenzoic acid was incorporated into the

biomass (28 to 31%). ATP measurements provided further support for this hypothesis since 83% of the maximum ATP produced by the fixed film was achieved by day 3 (Figure 14, page 105). These results taken together indicate the initial stages of colonization were more energy intensive. Up until day 3 nutrient utilization by the microorganisms was primarily for energy production with only a minor fraction used for increases in biomass. After the microorganisms had become more firmly established within the biofilm community, nutrient utilization shifted towards increases in biomass production.

The results for this stage of the investigation indicated the BMC slurry wastewater was capable of supporting microbial growth and community development of the microorganisms occurring within a fixed film community. The increases in biomass (AODC), viability (ATP), and heterotrophic potential (para-hydroxybenzoic acid removal and mineralization) demonstrated the soluble organic carbon material present in the slurry wastewater provided a readily utilizable carbon and energy source to sustain these functions. This preliminary evidence suggests the biofilm community can be applied as an adequate mechanism for reducing the pollution potential of the BMC slurry wastewater. However, this study was terminated prematurely and kinetic parameters describing the effectiveness of wastewater treatment were not determined.

VII. SUMMARY

This investigation was undertaken to determine if biological treatment could be successfully employed as a mechanism to reduce the pollution potential of coal slurry wastewaters. The major goal of these studies was to evaluate the potential for renovation of the slurry wastewater which would improve the water quality characteristics and reuse application. During the initial phase of this investigation, BOD studies provided evidence that the slurry wastewater's organic material was susceptible to biological oxidation. However, the BOD results do not directly correlate with the organic load or DOC removal for a particular wastewater. To assess the potential for the biological reduction of the soluble organic carbon, DOC removal was measured during the course of the BOD studies and a 54% to 71% reduction in the slurry wastewater's DOC was reported. Mass balance determinations for DOC removal (approaching 90%) indicated the DOC removed was transformed to carbon dioxide and additional sludge biomass.

A second order kinetic model and kinetic rate constants were developed to predict and compare wastewater treatability. The rate of DOC removal was found to be a function of the substrate concentration (i.e. DOC level) as well as the biomass concentration. First (k_1) and second (k_2) order kinetic rate constants for DOC removal were developed for batch and semi-continuous reactor designs. Comparison of the k_1 values between the two systems revealed over a twenty-fold difference (0.020 vs. 0.49 day^{-1}) and these differences could be attributed to the variation in the biomass concentration. When the

k_1 values were normalized to the biomass concentration these differences disappeared.

Although biological treatment of slurry wastewater was successful in reducing the pollution potential of the slurry wastewater, a component of the original organic material was resistant to biological attack. After treatment of Wyodak slurry wastewater with activated sludge there always remained a residual level of the DOC (an average of 33 mg of carbon $liter^{-1}$). In addition, low values obtained for the BOD_{20}/DOC ratios (0.35 to 0.72) further substantiated a non-biodegradable (or slowly biodegradable) organic fraction in the wastewater. Ultrafiltration studies were performed to characterize this recalcitrant DOC. Lower molecular weight compounds (<1000 MW) were preferentially removed during treatment and the higher molecular weight organic components (>1000 MW) predominated in the final treated wastewater. These higher molecular weight compounds were found to be more recalcitrant to biological attack and they failed to stimulate any oxygen uptake by the activated sludge.

Since the slurry wastewater may be subjected to chlorination prior to discharge, studies were undertaken to examine the affect of chlorination on biological treatment. Chlorination of the slurry wastewater resulted in an oxidation of the higher molecular weight DOC compounds to lower molecular weight (<1000 MW). Chlorination also altered the soluble organic carbon components to such an extent that a significant (>60%) reduction in the k_2 values occurred for DOC removal in Wyodak slurry wastewaters.

Despite the reduction in the kinetic constants for the chlorinated slurry wastewaters, activated sludge, which was acclimated to the chlorinated slurry wastewater, exhibited increased rates of removal from the chlorinated versus the unchlorinated slurry wastewater. Thus, chlorination of the slurry wastewater altered the parent DOC material to such an extent that the activated sludge acclimated to the chlorinated slurry wastewater could no longer metabolize the unchlorinated wastewater or did so with a marked reduction in efficiency. Nevertheless, extended acclimation of the activated sludge increased the efficiency of biological treatment. When the activated sludge was acclimated to the slurry wastewater for greater than one month the sludge acquired the ability to remove the higher molecular weight (>5000 MW) components from solution. When acclimation periods were short (less than one week) the maximum removal from the >5000 MW fraction was 4%. Acclimation periods greater than one month demonstrated removal efficiency ranging from 27% to 42% for the >5000 MW fraction.

In the final phase of the investigation studies were undertaken to assess the potential for using a microbial biofilm to mediate reduction in the slurry wastewater DOC. Because of the leaching of soluble organic material from the biofilm community, kinetic parameters were not developed. Biofilm microorganisms were capable of utilizing the organics within the slurry wastewater since the slurry wastewater was able to support the colonization and growth of the biofilm microorganisms. Further indication of the biofilm's heterotrophic

potential was demonstrated by its ability to remove and mineralize model fulvic acid compound such as para-hydroxybenzoic acid.

VIII. CONCLUSIONS

The coal slurry pipeline process results in significant leaching of organic material from the parent coal and causes a reduction in the quality of the transport water. Current wastewater treatment designs do not incorporate biological treatment as a method for reducing the organic content of the pipeline slurry wastewater. Preliminary evidence suggests laboratory and actual pipeline wastewaters can be potentially harmful to biological systems because of the presence of elevated levels of toxic inorganic compounds. A further detriment to biological treatment is the complex chemical nature of the slurry wastewater DOC. Evidence from this investigation provides important information on biological treatment as a successful method for removing the organic compounds contained in slurry wastewaters. This information would aid in the design of treatment processes which would improve the overall quality of slurry wastewater by rendering it more environmentally acceptable for discharge or for potential industrial or domestic reuse.

Although there is a paucity of information available concerning the chemical nature of the wastewater DOC, a second order kinetic model proved realistic in describing the biologically mediated DOC removal. The DOC and activated sludge concentration were identified as the controlling variables in this model. Determinations of these parameters proved essential for the accurate assessment of the efficiency of biological treatment.

Chlorination of pipeline slurry wastewaters to control biofouling causes significant alterations in the composition of the wastewater's

DOC. Chlorination of slurry wastewaters result in the production of potentially volatile mutagenic compounds (Davis et al., 1985) and decreases the effectiveness of biological treatment in reducing the DOC. To optimize the biologically mediated removal of slurry wastewater DOC, an alternative to water chlorination should be considered as a method to control biofouling in the cooling towers.

Despite the value of biological treatment as a mechanism for reducing the organic content of the slurry wastewater, this method was not successful in completely eliminating the DOC. The higher molecular weight DOC (>5000 MW) components are more resistant to biological attack and remain in the wastewater after treatment with activated sludge. The larger molecular weight compounds can represent a potential environmental threat since they would be present in the wastewater upon discharge. Future research should be directed towards assessing the human health hazards associated with these higher molecular weight DOC compounds.

Increased acclimation times can improve the activated sludge's utilization of the higher molecular weight organic compounds. Even with the increased acclimation times, biological removal of the higher molecular weight DOC was less efficient than the removal of the lower molecular weight DOC. Wastewater treatment designs must account for this extended acclimation and reduced efficiency in the removal of the higher molecular weight organic compounds. Parameters such as increased hydraulic retention time would facilitate the removal of this higher molecular weight DOC.

This investigation also provided evidence that the slurry wastewater was capable of sustaining a biofilm community. The potential exist for utilizing the biofilm microorganisms as a method for biological treatment of the slurry wastewater. However, these studies were preliminary in nature since the biofilm did not achieve a net reduction in the organic content of the slurry wastewater. The failure can be attributed to the inability of the biofilm to reach a steady state. Once a steady state was reached, the sloughing of soluble organic material from the biofilm community would be at a minimum. The successful application of a biofilm to wastewater treatment designs must account for the leaching of soluble organic material from the fixed-film microorganisms.

The following specific conclusions were based upon the results of this investigation:

1) Biological treatment improved the water quality of the slurry wastewater because of the reduction of the DOC.

2) A second order rate model proved successful in describing the biologically mediated DOC removal.

3) Activated sludge preferentially removed the lower molecular weight DOC (<1000 MW) and the higher molecular weight organic compounds (>1000 MW) were more recalcitrant to biological attack.

4) Chlorination of the slurry wastewater significantly affects the DOC and results in a reduction in the efficiency of the biologically mediated removal.

5) Extended acclimation times (greater than one month) can improve the efficiency of DOC removal from the chlorinated slurry wastewaters. Furthermore, these extended time intervals facilitated the removal of the higher (>5000) molecular weight DOC components from both chlorinated and unchlorinated slurry wastewaters.

6) Black Mesa Centrate slurry wastewater was capable of sustaining a biofilm community as characterized by microbial growth, community development, and the oxidation of model fulvic compounds.

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VITA

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