Deciduous Forest Biome
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A MODEL FOR AQUATIC MICROBIAL DECOMPOSITION

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A twenty-two compartment decomposition process model was developed to represent the carbon transfers resulting from heterotrophic microbiological activity in the fresh water ecosystem.

The interactions occurring in the water column and surface sediments are illustrated in figure 1 whereas those occurring in the deep sediments are illustrated in figure 2.

The model accommodates anaerobic and aerobic interactions for the water column and surface sediments and only anaerobic interactions in the deep sediments. In addition thermal stratification is accounted for in the mathematical model by distinguishing between transfers occurring only in the epilimnion, in the hypolimnion, or both.

Preliminary laboratory and field investigations as well as the literature have dictated certain simplifications in the construction of the model.

One of these is that organic carbon has been divided into a compartment requiring prior hydrolysis before cellular assimulation (POC, particulate organic carbon), and into a compartment requiring no prior hydrolysis (DOC, dissolved organic carbon). This division, in fact, reduces a very complicated group of events to the bare minimum. These events involve microbial transformations, co-oxidations and related mechanisms, as well as various microbial permeases and extracellular hydrolases. A discussion of these, although significant, is beyond the purpose and scope of this report.

Another broad simplification is identifying the facultative populations of the aerobic system with those of the anaerobic system in the water and surface sediments model (figure 1). Being that these are functional groupings rather than taxonomic, this is warranted.

The system is assumed to be reduced carbon limited. This is reasonable for the large majority of aquatic systems and has been demonstrated for the Lake George system (unpublished data). However, carbon limitation for certain

Figure 1 Conceptual Model

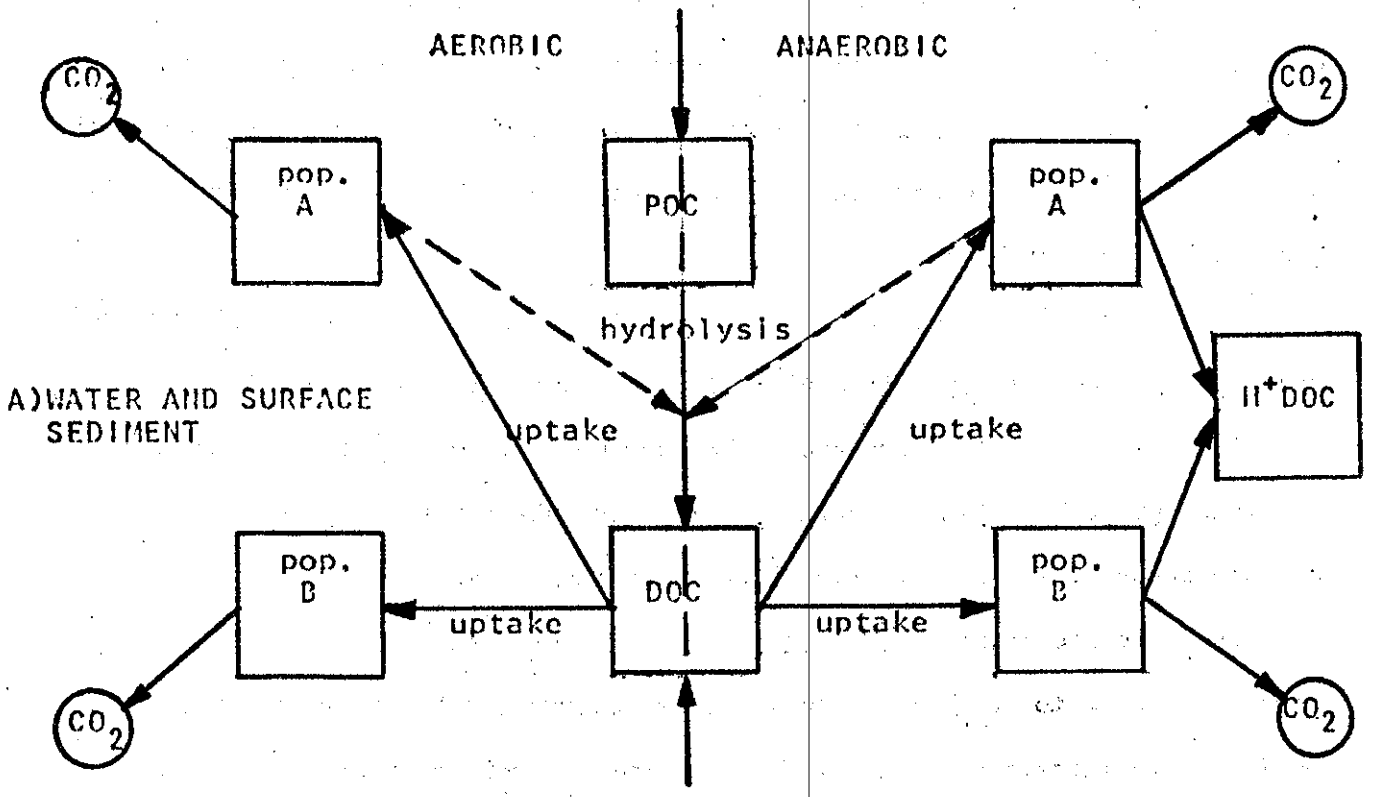
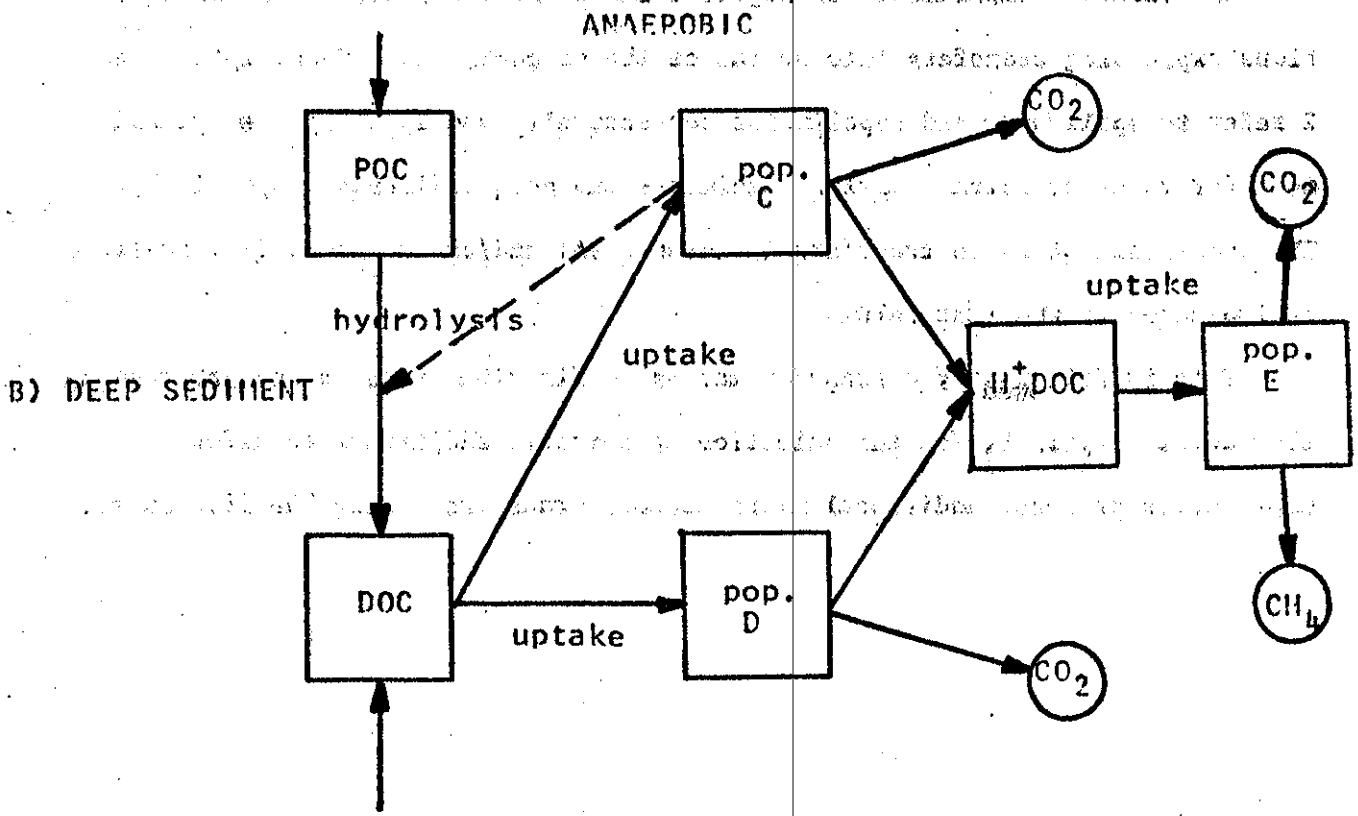


Figure 2 Conceptual Model



instances will have to be verified.

In addition, no mixing of dissolved organic carbon (DOC or H^+DOC), microbial populations (A,B,C,D,E), CO_2 or CH_4 is considered in this model.

Also it is assumed that the deep sediments serve as a carbon sink and that no carbon in any form is returned from the deep sediments.

The various compartments of figure 1 and 2 are identified below by equations expressing transfers into or out of the compartments. Subscripts 1 and 2 refer to epilimnion and hypolimnion respectively, and subscripts w, ss and ds refer to water column, surface sediments and deep sediments respectively. The occurrence of these transfers in aerobic (A) and/or anaerobic (An) environs is indicated in the last column.

This is followed by a transfer matrix, definition of terms and programming flow chart (figure 3) for the selection of suitable additional transfers. Explanation of these additional miscellaneous transfers follow the flow chart.

EQUATIONS ($Flux_{to,\ from}$)

1. $\dot{Pw}_1 = F_{1,2} + F_{1,3} - F_{2,1} - F_{3,1} - F_{5,1} + \sum$ (inputs-losses) A
2. $\dot{Pw}_2 = F_{2,1} - F_{1,2} - F_{3,2} - F_{6,2} + F_{2,3} + \sum$ (inputs-losses) A&An
3. $\dot{Pss} = F_{3,1} + F_{3,2} - F_{1,3} - F_{2,3} - F_{4,3} - F_{7,3} + \sum$ (inputs-losses) A&An
4. $\dot{Pds} = F_{4,3} - F_{8,4} + \sum$ (inputs-losses) An
5. $\dot{Dw}_1 = F_{5,1} - F_{12,5} - F_{15,5} + \sum$ (inputs-losses) A
6. $\dot{Dw}_2 = F_{6,2} - F_{13,6} - F_{16,6} + \sum$ (inputs-losses) An&A
7. $\dot{Dss} = F_{7,3} - F_{14,7} - F_{17,7} + \sum$ (inputs-losses) An&A
8. $\dot{Dds} = F_{8,4} - F_{18,8} - F_{19,8} + \sum$ (inputs-losses) An
9. $\dot{Hw}_2 = F_{9,13} + F_{9,16} + \sum$ (inputs-losses) An
10. $\dot{Hss} = F_{10,14} + F_{10,17} + \sum$ (inputs-losses) An
11. $\dot{Hds} = F_{11,18} + F_{11,19} - F_{20,11} + \sum$ (inputs-losses) An

12. $\dot{Aw}_1 = F_{12,5} - F_{21,12} + \sum$ (inputs-losses) A
13. $\dot{Aw}_2 = F_{13,6} - F_{9,13} - F_{21,13} + \sum$ (inputs-losses) A&An
14. $\dot{Ass} = F_{14,7} - F_{10,14} - F_{21,14} + \sum$ (inputs-losses) A
15. $\dot{Bw}_1 = F_{15,5} - F_{21,15} + \sum$ (inputs-losses) A
16. $\dot{Bw}_2 = F_{16,6} - F_{9,16} - F_{21,16} + \sum$ (inputs-losses) A&An
17. $\dot{Bss} = F_{17,7} - F_{10,17} - F_{21,17} + \sum$ (inputs-losses) A

18. $\dot{C} = F_{18,8} - F_{11,18} - F_{21,18} + \sum$ (inputs-losses) An
19. $\dot{D} = F_{19,8} - F_{11,19} - F_{21,19} + \sum$ (inputs-losses) An
20. $\dot{E} = F_{20,11} - F_{21,20} - F_{22,20} + \sum$ (inputs-losses) An

21. $\dot{CO}_2 = F_{21,12} + F_{21,13} + F_{21,14} + F_{21,15} + F_{21,16} + F_{21,17} + F_{21,18}$
$F_{21,19} + F_{21,20} + \sum$ (inputs-losses) A&An
22. $\dot{CH}_4 = F_{22,20} + \sum$ (inputs-losses) An

TRANSFER MATRIX

To / From	PW_1 1	PW_2 2	PSS 3	PDS 4	DW_1 5	DW_2 6	DSS 7	DDS 8	HW_2 9	HSS 10	HDS 11	AW_1 12	AW_2 13	ASS 14	BW_1 15	BW_2 16	BSS 17	C 18	D 19	E 20	CO_2 21	CH_4 22
1 PW_1	-	S	NS		+																	
2 PW_2	S	-	S			S																
3 PSS	NS	S	-	+			+															
4 PDS				-				+														
5 DW_1					-							+			+							
6 DW_2						-							S			S						
7 DSS							-							+			+					
8 DDS								-										+ AN	+ AN			
9 HW_2									-													
10 HSS										-										+ AN		
11 HDS											-											
12 AW_1												-									+	
13 AW_2									S AN				-								S	
14 ASS										AN				-							+	
15 BW_1															-						+	
16 BW_2									S AN							-					S	
17 BSS										AN							-				+	
18 C											+ AN							-			+ AN	
19 D											+ AN								-		+ AN	
20 E																				-	+ AN	+ AN
21 CO_2																					-	
22 CH_4																						-

S - Stratification Only, NS - No Stratification Only, AN - Anaerobic Only, + - Positive Transfer

TERMS

A. Terms from the sedimentation model:

1. of form, ΨP where Ψ = sedimentation factor
   P = particulate matter

2. for fluxes,

$$F_{2,1};\ F_{3,1};\ F_{3,2};\ F_{4,3}$$

B. Terms from the mixing model and/or resuspension by detritus feeders:

1. of form, α where α = mixing factor

2. for fluxes,

$$F_{1,2};\ F_{1,3};\ F_{2,3}$$

C. Terms due to microbial hydrolysis of POC:

1. of form,

$$F = \beta max\ (1 - e^{-\lambda_1 (AorC)})\ (1 - e^{-\lambda_2 (T-Tmin)})\ (P)$$

$$F = 0 \text{ when } T \leq Tmin$$

where λ_1 = hydrolysis coefficient

λ_2 = temperature coefficient

A = hydrolyzing facultative microbial population

C = hydrolyzing anaerobic microbial population

2. for fluxes,

$$F_{5,1};\ F_{6,2};\ F_{7,3};\ F_{8,4}$$

D. Terms due to microbial uptake of DOC:

1. of form,

$$F = \beta max\ \left(\frac{D-Dmin}{D-Dmin + K_D}\right)\ (1 - e^{-\lambda_2\ (T-Tmin)})\ (A\&B \text{ or } C\&D)$$

$$F = 0 \quad \text{when } T \leq Tmin \text{ or } D \leq Dmin$$

where D = amount of DOC

K_D = saturation constant

B = non-hydrolyzing facultative microbial population

D = non-hydrolyzing anaerobic microbial population

2. Rate of uptake is a function of substrate concentration when substrate concentration is above a certain minimum.

3. for fluxes,

$F_{12,5}$; $F_{13,6}$; $F_{14,7}$; $F_{15,5}$; $F_{16,6}$; $F_{17,7}$; $F_{18,8}$; $F_{19,8}$

E. Terms due to the microbial production of H^+DOC:

1. of form,

$F = \beta max\ (1 - e^{-\lambda_2(T-Tmin)})$ (A&B or C&D)

$F = 0$ when $T \leq Tmin$

2. for fluxes,

$F_{9,13}$; $F_{9,16}$; $F_{10,14}$; $F_{10,17}$; $F_{11,19}$; $F_{11,18}$

F. Terms due to microbial production of CO_2:

1. of form,

$F = \beta max\ (1 - e^{-\lambda_2(T-Tmin)})$ (A&B or C,D&E)

$F = 0$ when $T < Tmin$

2. for fluxes,

$F_{21,20}$; $F_{21,12}$; $F_{21,13}$; $F_{21,14}$; $F_{21,15}$; $F_{21,16}$; $F_{21,17}$; $F_{21,18}$; $F_{21,19}$

G. Terms due to microbial production of CH_4:

1. of form,

$$F = \beta max\ (1 - e^{-\lambda(T-Tmin)})\ (E)$$

$F = 0$ when $T < Tmin$

2. for flux,

$F_{22,20}$

H. Terms due to microbial uptake of H^+DOC:

1. of form,

$$F = \beta max\ \left(\frac{H-Hmin}{H-Hmin + K_H}\right)\ (1 - e^{-\lambda\ (T-Tmin)})\ (E)$$

$F = 0$ when $T < Tmin$

2. for flux,

$F_{20,11}$

MISCELLANEOUS TRANSFERS

When stratification breaks down the following additional transfers occur:

Hw_2 into $Dw_1 = F_{5,9}$

Dw_2 into $Dw_1 = F_{5,6}$

Pw_2 into $Pw_1 = F_{1,2}$*

Aw_2 into $Aw_1 = F_{12,13}$

Bw_2 into $Bw_2 = F_{15,16}$

When stratification occurs:

Dw_1 into Dw_1 and $Dw_2 = F_{6,5}$

Pw_1 into Pw_1 and $Pw_2 = F_{2,1}$*

Aw_1 into Aw_1 and $Aw_2 = F_{13,12}$

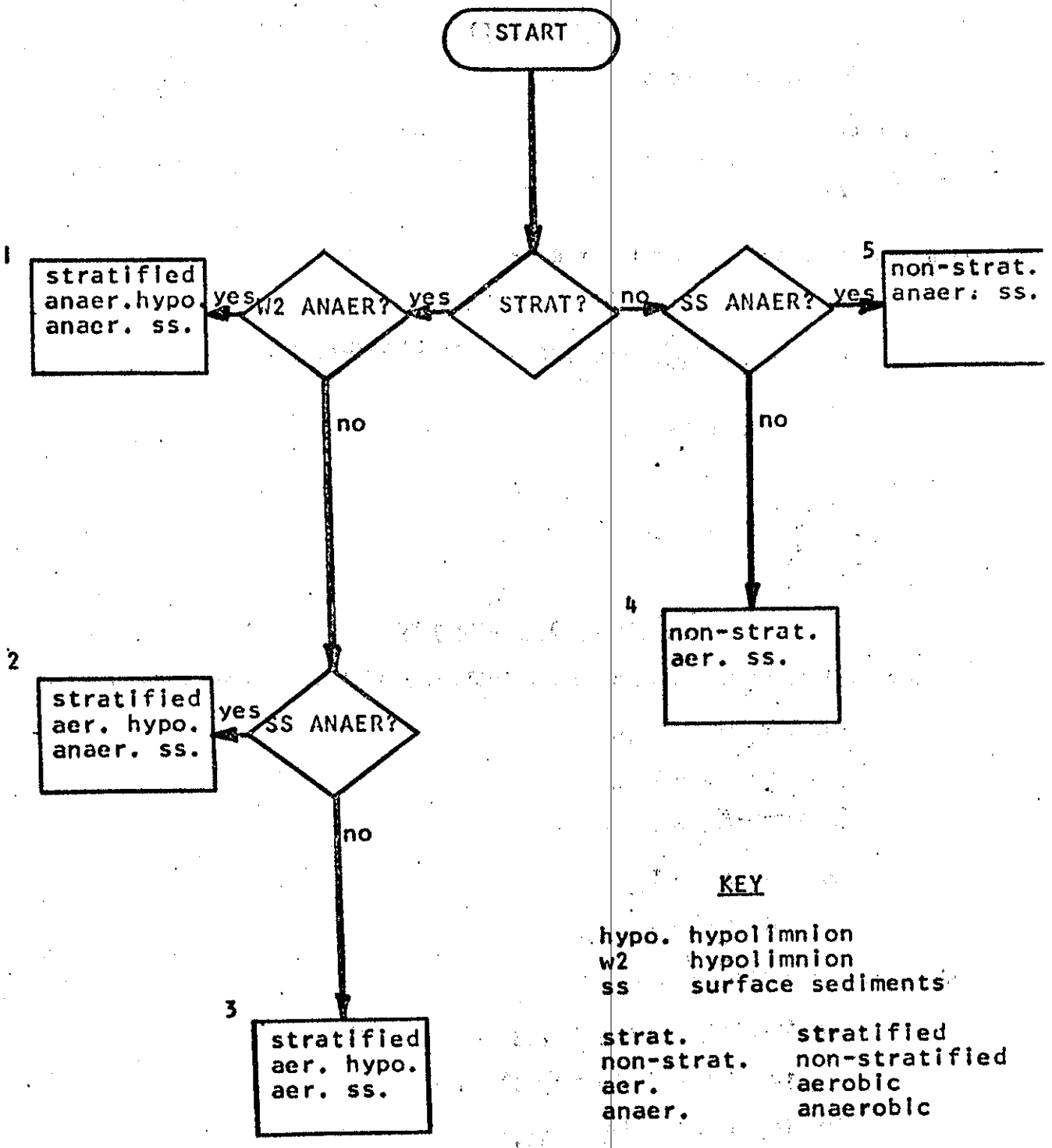
Bw_1 into Bw_1 and $Bw_2 = F_{16,15}$

Under conditions whereby the surface sediments become aerobic

Hss into $Dss = F_{7,10}$

*different from previous fluxes

Figure 3 Programming Flow Chart



KEY

hypo.	hypolimnion
w2	hypolimnion
ss	surface sediments
strat.	stratified
non-strat.	non-stratified
aer.	aerobic
anaer.	anaerobic

IMPLEMENTATION OF THE MODEL

Each of the transfers of figure 1 and 2 occurs as a result of microbial growth. Implementation of the model thus depends upon the ability to measure microbial growth rates. This can be done in a variety of ways (1). According to many investigators, the aquatic ecosystem can be considered to be in a steady state with respect to the microbial population i.e. microbial biomass production equals microbial biomass removal. Removal may be the result of any one of a variety of mechanisms such as predation, sedimentation, transport out of a locale via currents etc. The steady state in pure culture has been expressed as:

$$dx/dt = \mu x - DX$$

or growth rate = (growth rate constant) (concentration of cells) —
(dilution rate constant) (concentration of cells)

For mixed culture it seems better to express this condition as:

$$dx/dt = \mu x - \rho X$$

where ρ incorporates all of the removal mechanism found in the natural system and can be called a removal rate constant.

This steady state condition is most likely one in which short range fluctuations in population size occur, but over the longer range these fluctuations are negligible. In oligotrophic or mesotrophic lakes, the microbial decomposers seem to be present at levels of 10^3-10^4 cells/ml. The accuracy with which one can determine this amount of biomass is severly limited if one considers 10^{-10} to 10^{-12} gm to be the weight of an average bacterial cell. Estimation of microbial biomass via ATP concentration seems to possess the sensitivity required, but the fact that ATP is not limited to decomposers and the variation in ATP concentration among different cells are problems. If, however, steady state populations are being studied such that changes in population type are minimal, this method can be extremely valuable. Alternatively, cell counts by

various methods can be used (Coulter counting, plate counts, direct microscopic counts).

It must be clearly recognized that in the steady state, biomass measurements give no indication of turnover of organic material since there is no change in population size even though growth may be occurring at a rapid rate. One can theorize about what dictates the maintenance level of the cells in the lake system.

The open system character of the natural system can be best approximated, for the purposes of analysis, with a chemostat (2). The use of a chemostat permits the measurement of the growth rate of a mixed population at steady state from a knowledge of the volume of the system (v) and the flow rate through the system (f). The growth rate constant (μ) is f/v and the doubling time or generation time of the population is v/f. When biomass measurements accompany chemostat operation then heterotrophic microbial productivity is obtained. Chemical analyses of influent and effluent allow estimation of C, N & P turnover.

A simple chemostat has been used for this work (3) at Lake George and is diagrammed in figure 4. Membrane filtered lake water is the substrate. Temperature and dissolved oxygen level (if less than 1 ppm) are maintained at that of the environment. Simple aerobic organisms respire at rates that are independent of oxygen tension until the level is less than 1 ppm.

In operation, a lake water sample is placed in the culture vessel. An initial determination of cell concentration is made as the flow of substrate into the culture vessel is begun. At least two vessels are used in the determination of a single growth rate constant. One flow is chosen that is estimated to produce a dilution rate (D_1) greater than the anticipated growth rate constant. This system is expected to lose cells since the substrate is replacing the culture vessel volume faster than the cells are able to grow therefore $\mu < D_1$.

Another flow is chosen that is estimated to produce a dilution rate (D_2) less than the anticipated growth rate constant. This system will accumulate cells since the substrate is replacing the culture vessel volume at a rate less than that of the growth rate constant. Therefore the cells are in contact with the substrate for some time after cell division permitting an additional cell division by some of the cells. Thus the cells increase in concentration with time and the effluent is more depleted in substrate than in the first system ($\mu > D_2$).

When the rate of loss or accumulation of cells is plotted against dilution rate, the line drawn between these points intersects the abscissa at a point corresponding to the dilution rate producing the in situ steady state (figure 5).

If one is to convert values for growth rate into substrate turnover numbers one must either know the yield coefficient (grams cells/grams substrate) for the system or measure the per cent utilization of substrate at steady state. An extensive listing of yield coefficients can be found in the literature (4). In figure 6, one can see the relation between yield coefficient and growth rate constant for a pure culture (5). Yield coefficients vary much more between species.

Chances of choosing a dilution rate that exactly maintains the steady state of the aquatic environs are quite slim. When it is determined by the "above and below" method, then of course the sample is no longer available for experimental observation at the extrapolated dilution rate.

Since species composition is the greater consideration with respect to yield, one can with reasonable accuracy, determine the specific yield coefficient for a particular system growing at a rate other than that of the natural system.

For soluble systems, this can be readily done by analyzing the membrane filtered influent and effluent for total carbon via an infra-red total carbon analyzer or other suitable technique. In addition nitrogen and phosphorus

Figure 5 Relation between Cell Accumulation and Dilution Rate

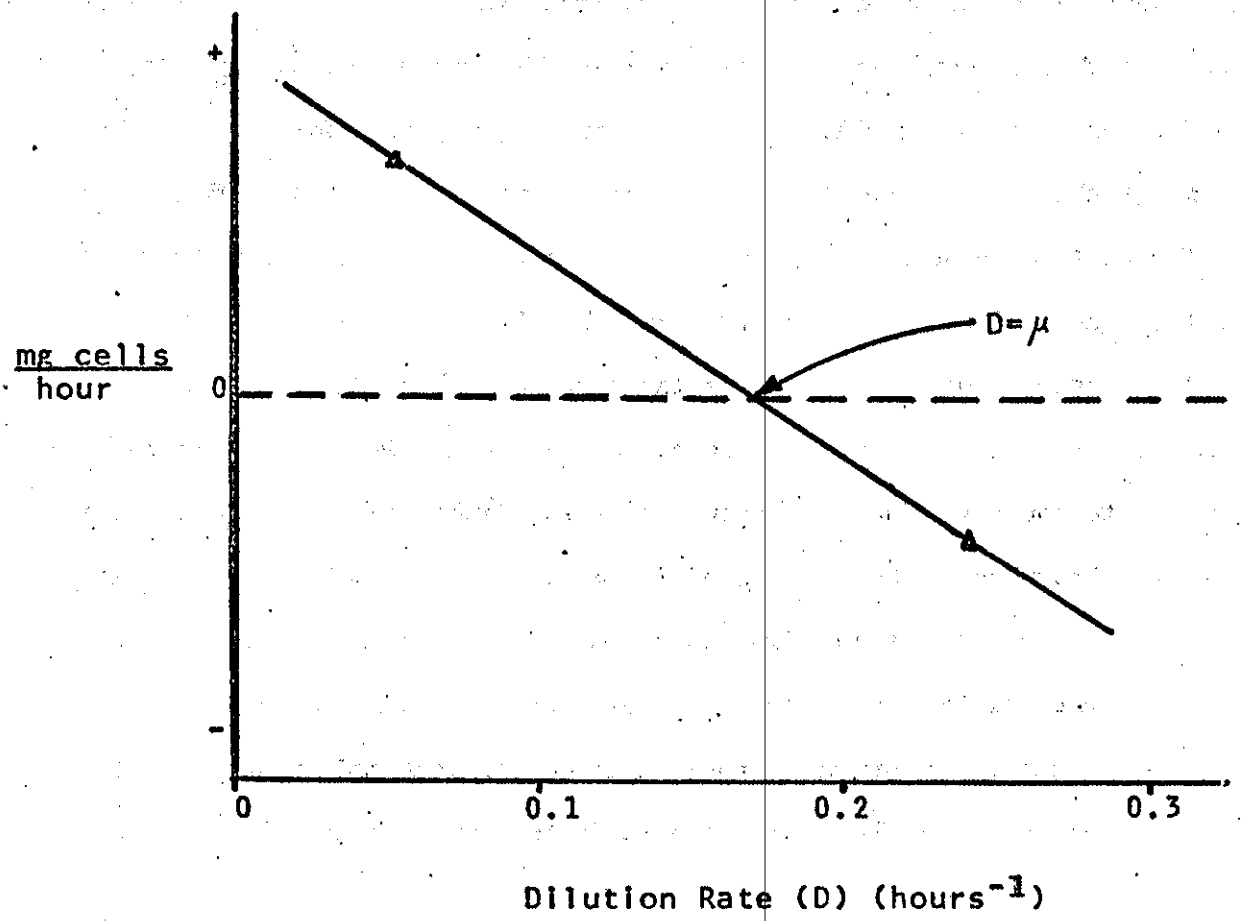
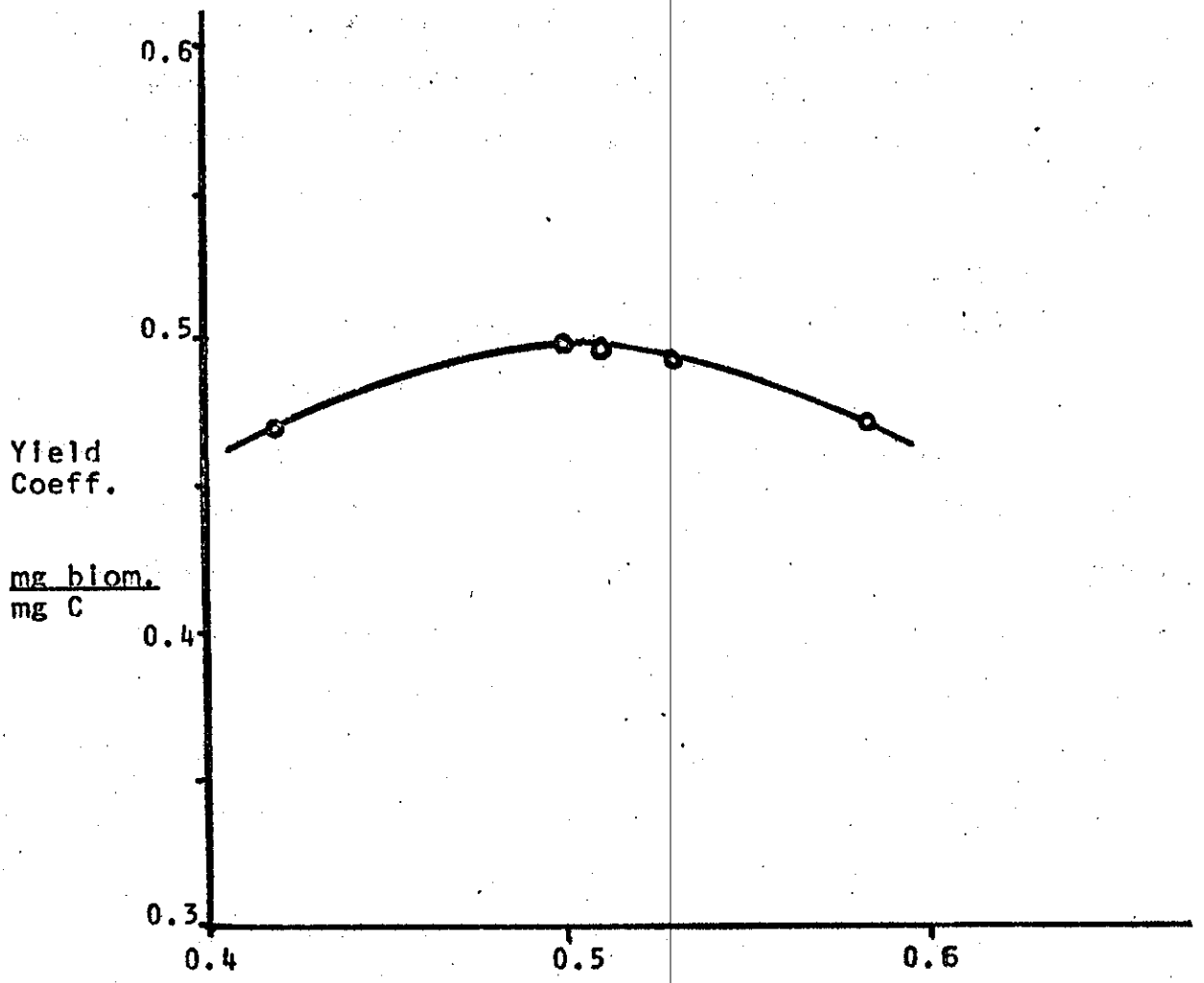


Figure 6 Effect of Dilution Rate on Yield Coefficient



Psuedomonas fluorescens

T = 25° C

μ_{max} = 0.5

turnover can be determined by the analysis of influent and effluent of the chemostat and making the same assumption with respect to yield coefficient.

For systems containing particulates, these turnover values can only be estimated since there is no way to chemically differentiate between cellular carbon, nitrogen and phosphorus and particulate non-cellular carbon, nitrogen and phosphorus. Estimation can therefore be based upon microbial productivity and approximated yield coefficients.

Production of CO_2 and CH_4 from both soluble and particulate systems can be readily measured by monitoring the off-gas of the chemostat.

For modeling purposes a wide range of growth rates and turnover values should be obtained. The nutrient conditions of the natural aquatic system do not approach conditions that would produce the maximum reaction velocities observed in saturation growth kinetics. Therefore lake water can be concentrated by means of lyophilization or ultra-filtration in order to obtain a wide range of growth rates for particular samples. Some disadvantages can be pointed out for both of these methods. In lyophilization certain organics may volatilize producing a change in relative composition of the concentrated sample. Also salts may be concentrated to a point at which they exert an additional influence on microbial growth. Dialysis of the concentrated material avoids this but also may result in the loss of low molecular weight organics. Ultrafiltration (pressurized selective filtration through polymeric membranes) results in a concentrate that is partially depleted in low molecular weight organics and is essentially equivalent to the dialyzed product of lyophilization.

However, for the development of the mathematical model for decomposition, these concentration effects must be measured in the best available manner.

The chemostats are run until there is positive evidence of washout or accumulation. The time naturally depends upon the inherent growth rate of the organisms and may range from 12 hours to 7 days.

Obviously there is concern about population changes during chemostat operation. Maintaining the temperature, dissolved oxygen, and substrate quality of the natural habitat produces an environment very close to that of the lake. However the operation at washout or accumulation does, in fact, produce different cellular interactions than those occurring in nature. One can minimize this problem by operating as close as possible to the dilution rate that produces the *in situ* growth rate.

Thus far nothing has been said about distinguishing population A from population B (figure 1) or C from D (figure 2). A relatively arbitrary distinction will be made between hydrolyzing and non-hydrolyzing microorganisms by filtration through a 1 micron pore size membrane filter. Analysis of a sample directly for population A plus B or C plus D and again after membrane filtration of the sample for population B or C will permit this distinction. In order for this to be valid, the assumption that the hydrolyzing populations are associated with particulate organic carbon, must hold true.

Techniques for distinguishing between (H^+DOC) and other dissolved organic carbon are available. Thin layer chromatography on silica gel would seem to be the method of choice to quantify the organic acid contribution to the total dissolved organic carbon.

A mathematical model for microbial decomposition along with the methodology for implementation of the model has been presented. Validation of the model awaits further data accumulation.

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