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Effects Of Protozoa On The Fate Of Particulate Carbon

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EFFECTS OF PROTOZOA ON THE FATE OF PARTICULATE CARBON

by

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ABSTRACT

Laboratory studies were designed to define the role of protozoa in the fate of particulate (bacterial) organic carbon. Specific objectives were (1) to measure the effects of selected environmental parameters on protozoan growth rates, (2) to measure organic carbon in bacteria and protozoa, and (3) to quantitate carbon transformations in predator-prey experimental systems.

A growth system containing 2×10^8 Citrobacter/mℓ in 1×10^{-3} M phosphate of pH 7.5, incubated at 25°C at a shaking rate of 100 rpm, was found to be an optimal environment for protozoan growth.

The nutrient bacterium, Citrobacter, contained 8.6×10^{-11} mg C/cell, and Tetrahymena pyriformis contained 1.1×10^{-6} mg C/cell.

T. pyriformis altered the amount and form of carbon in the system while growing on bacteria. Of the total organic carbon present at the initiation of the predator-prey experiment (93 mg), 93% was in the bacterial fraction. Within 96 hours, 38% of the carbon was released as CO_2; 5% was present as inorganic carbon in the water and the remainder (57%) was present as organic carbon. The organic carbon in the bacterial fraction decreased from 86 to 2 mg within 96 hours, while the carbon in the protozoan biomass increased from 1 to 40 mg. In the bacterial control, 11% of the organic carbon was released as CO_2 within 96 hours while negligible amounts of inorganic carbon remained in the water.

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ACKNOWLEDGMENTS

Technical assistance was received from two individuals of the National Pollutants Fate Research Program during the carbon balance study. Mr. John Barnett set up the CO_2-free air-flow system for the carbon studies. Mrs. Donna Davis analyzed air samples for carbon dioxide content during certain phases of the research. The help from these individuals is gratefully acknowledged.

SECTION I

CONCLUSIONS

Protozoa have various effects on the fate of particulate carbon (bacteria) in aqueous environments:

- Total Organic Carbon (TOC) of bacterial-protozoan systems decreases as protozoa metabolize bacterial cells. Within 120 hours, 49-57% of the organic carbon was transformed to inorganic carbon in systems containing protozoa, whereas 4-17% of the organic carbon was similarly transformed in systems containing only bacteria.

- Within 120 hours, the bacterial-protozoan system released over three times as much CO_2 as the bacterial control.

- The Total Inorganic Carbon (TIC) concentration in the water of the bacterial-protozoan system increased from essentially zero to 4 mg carbon/ℓ during the experiment, about five times that of the bacterial control.

- According to calculations, soluble organic carbon may be produced in systems containing protozoa.

The protozoan Tetrahymena pyriformis requires 3×10^4 bacteria Citrobacter (2.6×10^{-6} mg carbon) for the production of each protozoal cell containing 1.1×10^{-6} mg carbon, representing a carbon assimilation efficiency of 42%.

Factors that affected the growth rate of T. pyriformis included phosphate concentration, pH, temperature, shaking rate, and bacterial concentration.

SECTION II

RECOMMENDATIONS

Protozoan populations have a significant role in determining the fate of pollutants in the environment. Further investigations should be directed toward clarifying the following points:

- This work shows that protozoa have an impact on the fate of carbon from one species of bacteria. To better predict the role of secondary heterotrophs on the fate of particulate carbon, several protozoa of distinct physiological and ecological types should be examined for abilities to utilize particulate carbon from several species of algae and bacteria.

- Influence of environmental factors, such as light and water flow, on protozoan ecology should be evaluated.

- The fate of other major nutrients (nitrogen and phosphorus) should be investigated in protozoan growth systems.

- The fate and effects of selected pollutants (e.g., heavy metals, pesticides, chlorine) should be examined in mixed ecosystems containing bacteria, algae, and protozoa to determine rate data that are meaningful for estimating the environmental impact of pollutants.

SECTION III

INTRODUCTION

'rotozoa are commonly occurring organisms in soils and water. Their importance in ecosystems is indicated by their ubiquitous nature and by their large biomass.

Since the majority of protozoa seen in polluted river systems[1], in ponds[2], in soils[3], and in sewage treatment facilities[4] are non-photosynthetic secondary heterotrophs, as a group they may be of significance in the fate of the ubiquitous nutrient, organic carbon. Indeed, some evidence implicates secondary heterotrophs with several roles in the fate of carbon.

First, protozoa need vast numbers of bacteria or other microorganisms as carbon sources[3]. This observation has led some[5,6] to believe that protozoa are harmful because they utilize functional bacteria. Others[7,8,9,10] note that a combination of bacteria and protozoa produces a higher quality sewage treatment than either population alone. Still others[11,12] believe protozoa are the primary agents in the sewage stabilization process.

Second, in addition to utilizing bacterial cells, certain protozoa can metabolize soluble organic compounds[13]. Wilson and Danforth[14] reported a non-photosynthetic strain of Euglena assimilated 58% of a soluble substrate, acetate, releasing the remainder as carbon dioxide.

Third, protozoa indirectly influence the fate of nutrients in the environment by stimulating the growth of certain bacteria[15,16].

Only a fraction of the bacterial carbon is thought to be assimilated by the protozoa; the remainder is released as carbon dioxide and soluble organics, which may be metabolized by other organisms. Few data are available that show the rate of carbon assimilation by protozoa with concomitant release of carbon dioxide. Indirect evidence from BOD tests presented by Busch[17] and confirmed by work of Curds, Cockburn, and Vandyke as presented by Sykes and Skinner[4] suggests that protozoa exhibit a significant oxygen demand, presumably for metabolizing the primary heterotrophs (bacteria). Heal[18] using data from Warburg manometry, calculated that Acanthamoeba respires 21% of the carbon taken up.

The present study was designed to measure the role of protozoa in the mineralization of carbon (organic → inorganic), and to define factors that might affect the protozoan growth rate and the mineralization process.

Specific objectives of the study were to determine (1) environmental conditions producing optimal growth rates of the protozoan Tetrahymena pyriformis; (2) the carbon content of T. pyriformis and the bacterium Citrobacter; and (3) the types and rate of carbon transformations in bacterial-protozoan ecosystems.

SECTION IV

MATERIALS AND METHODS

MATERIALS AND ANALYTICAL PROCEDURES

Organisms

A natural aquatic bacterium isolated from Shriner's Pond, Clarke County, Georgia, was used as the test organism. The bacterium was identified as a Citrobacter. In all experiments, the bacteria were grown in Payne and Feisal's[19] medium at 25°C for 48 hours, washed twice with dilution buffer[20], and resuspended in the appropriate medium for experiments.

An axenic species of protozoa commonly used for laboratory research[13], Tetrahymena pyriformis (ATCC 9357) was obtained from the culture collection of the Department of Microbiology, University of North Dakota, Grand Forks. The organism was grown in proteose peptone-glucose medium (1%, 1%)[21] for four days, and washed two times in dilution buffer by centrifugation at 100 × G for 45 seconds before use.

Reagents

Reagent-grade chemicals were used for the preparation of media.

Cylinder air (USP Compressed Air, Breathing Quality) was used for aeration in the carbon balance studies.

Growth Measurements

Bacteria were counted by two methods. A Coulter Counter equipped with a 30-μ aperture tube was used to determine initial bacterial concentrations of washed cell suspensions. With an aperture current of 1/2, amplification of 1/4, and a lower threshold of 12, counting agreed with pour plates. During the course of the experiments, bacteria were counted by plating serial dilutions, in duplicate, in Tryptone Glucose Extract Agar (TGE) pour plates, incubating at 25°C for 48 hours and counting the colonies.

Duplicate samples of protozoa, killed with Lugol's Iodine, were counted by direct microscopic counts at 100 × using a Levy counting chamber. The Coulter Counter, with an aperture current of 1 and an amplification of 1, equipped with the Model J plotter and a 200-μ aperture tube, was used for biomass determinations.

Analytical Procedures

The Beckman Model 915 Total Organic Carbon Analyzer was used for Total Organic Carbon (TOC), Total Inorganic Carbon (TIC), and carbon dioxide (CO_2) determinations. All analyses were made in duplicate.

Total Organic Carbon (TOC) was determined either on 5-mℓ samples frozen in Nalgene bottles, or on unstored samples. After appropriate dilutions, the sample was acidified to pH 4 with sulfuric acid and bubbled for five minutes with nitrogen gas to remove inorganic carbon.

Total Inorganic Carbon (TIC) was determined on samples collected with a 100-μℓ syringe and immediately injected into the inorganic channel of the Beckman Carbon Analyzer.

Carbon dioxide was measured by collecting gas samples from the effluent line of the flasks (1, 2, or 5 mℓ, depending on the CO_2 concentration) with a syringe and needle, and injecting them into the inorganic channel of the Beckman Carbon Analyzer.

Soluble organic carbon was not measured because the fragility of the protozoa made physical separation of cells from the medium unreliable. Measurements of pH were made with a Beckman Zeromatic pH meter equipped with a Beckman Combination electrode (#39183).

EXPERIMENTAL DESIGN

Phase I - Environmental Factors Affecting Protozoan Growth

Experiments were designed to determine the effects of certain chemical and physical factors on the growth rate of T. pyriformis in a simple batch culture, with bacteria, Citrobacter, as the carbon source.

Duplicate test systems consisted of 250-mℓ Erlenmeyer flasks containing known initial concentrations of washed Citrobacter and T. pyriformis brought to a volume of 100 mℓ with phosphate buffer of varying pH and concentration, depending on the experiment.

The growth rate of T. pyriformis was tested as a function of temperature, shaking rate, phosphate concentration, pH, and bacterial concentration.

Growth characteristics of T. pyriformis in various environments were expressed by growth rate constants, defined as the number of doublings per unit time and calculated using the following equation[22]:

$$k = \frac{\log_{10} N_t - \log_{10} N_o}{0.301\ t}$$

where N_o = population at a given time

N_t = population after a time lapse, t

t = time lapse between population measurements (in hours).

The data are tabulated as mean generation time (1/k).

Phase II - Carbon Content of Bacteria and Protozoa

The organic carbon content of bacterial and protozoal cells was measured by preparing washed cell suspensions of pure cultures of Citrobacter and T. pyriformis, counting each population, and measuring the TOC of each cell suspension.

Additionally, a size distribution of T. pyriformis was obtained and the carbon content of protozoa was calculated as a function of cell size using the TOC and size distribution data.

Phase III - Effect of Protozoa on Fate of Organic Carbon (Bacteria)

Two comprehensive duplicate experiments were completed to quantitate the role of growing populations of protozoa in carbon transformations.

Each of the Fernback flasks[23], equipped with ports for air bubbling, received one-liter volumes of 10^{-3} M phosphate, pH 7.5. Two flasks received washed Citrobacter to an initial concentration of 10^9/mℓ, and the two remaining flasks received bacteria (10^9/mℓ) plus washed T. pyriformis (initial concentration, 10^3/mℓ).

An incubation system[23] was used in which dry, CO_2-free compressed air was bubbled through the test flasks at a rate of 150 mℓ/minute. The flasks were incubated in a water bath at 23°C with gentle reciprocating shaking.

Carbon dioxide from the effluent line, TOC in the medium, and TIC in the medium were sampled at selected time periods and analyzed on the appropriate channel of the Beckman 915 Total Organic Carbon Analyzer.

Samples were collected from the medium for duplicate bacterial counts (TGE agar pour plates) and protozoan counts (direct microscopic counts using a Levy Counting chamber).

Samples from Experiment 1 were stored frozen for later analysis of total organic carbon; samples from Experiment 2 were analyzed at the time of collection.

SECTION V

RESULTS AND DISCUSSION

PHASE I - ENVIRONMENTAL FACTORS AFFECTING PROTOZOAL GROWTH

Preliminary results showed that T. pyriformis grew well on washed Citrobacter cells resuspended in dilution buffer[20]. A concentration of 2×10^8 bacteria/mℓ was arbitrarily chosen for initial experiments.

Growth rates of T. pyriformis were measured at 25°C and 30°C in both stationary and shaking (100 rpm) cultures (Table 1). Although the protozoan grew under each condition tested, the 25°C shaking system produced the best growth rate. In the following Phase I experiments, the system was incubated under these conditions.

The effects of phosphate concentration and pH on the growth of T. pyriformis were investigated in buffer solutions containing 2×10^8 bacteria/mℓ. The pH of each test environment (Table 2) was recorded before and immediately after the addition of the bacteria and protozoa, at 24 hours and at 48 hours. The pH of the 5×10^{-4}M phosphate system was significantly changed by addition of the organisms, whereas the pH of the 5×10^{-2}M phosphate systems was not.

The protozoan did not grow in 5×10^{-2}M phosphate, but did in 5×10^{-4}M phosphate (Table 3). The initial pH, within the range tested, did not greatly influence the growth rate.

To further determine optimal growth conditions, growth rates were measured over a large range of buffer concentrations at two pH values only.

Initial pH values were high because, in the previous experiment, addition of bacteria caused the pH (pH 7.5 buffer 5×10^{-4}M) to drop to pH 7.0 (Table 2), an ideal pH for many microbiological systems. Maximal growth rates (Table 4) occurred in 1×10^{-3}M phosphate, pH 7.5. Under these conditions, the pH of the system remained near 7.0 for the duration of the study (Table 5). T. pyriformis did not grow in either 5×10^{-3}M or 1×10^{-4}M phosphate.

The effect of bacterial concentration on the protozoan population was tested in a shaking system at 25°C containing 1×10^{-3}M phosphate at pH 7.5, 5×10^2 protozoa/mℓ, and varying concentrations of bacteria. Data (Table 6) show that the concentration used in all of the initial experiments (2×10^8/mℓ) does not limit the growth rate, and was therefore a suitable choice for this work. Concentrations as high as 10^9/mℓ did not adversely affect the growth kinetics of the protozoa.

Table 1. GENERATION TIMES OF Tetrahymena pyriformis AS A FUNCTION OF TEMPERATURE AND SHAKING.

Temperature	Generation time (hours)	
	Shaking (100 rpm)	Stationary
25°C	9.4	10.1
30°C	11.4	16.8

Table 2. pH STABILITY OF PROTOZOAN GROWTH SYSTEMS AS A FUNCTION OF INITIAL pH AND PHOSPHATE MOLARITY.

pH[a]	pH[b] of Medium					
	5×10^{-2} M phosphate hours			5×10^{-4} M phosphate hours		
	0	24	48	0	24	48
6.0	6.2	6.2	6.3	5.9	6.1	6.0
6.5	6.6	6.5	6.6	6.2	6.3	6.5
7.0	6.9	6.9	6.9	6.5	6.5	6.6
7.5	7.4	7.3	7.3	7.0	6.7	7.0
8.0	7.8	7.8	7.8	7.4	6.9	7.0

[a] pH of medium before addition of organisms

[b] pH after addition of Citrobacter and Tetrahymena pyriformis

Table 3. GENERATION TIMES (HOURS) OF *Tetrahymena pyriformis* AS A FUNCTION OF PHOSPHATE MOLARITY AND pH (EXPERIMENT 1).

pH	Phosphate concentration	
	5×10^{-2} M	5×10^{-4} M
6.0	No growth	5.6
6.5	No growth	6.9
7.0	No growth	6.6
7.5	No growth	5.4
8.0	No growth	6.6

Table 4. GENERATION TIMES (HOURS) OF Tetrahymena pyriformis AS A FUNCTION OF PHOSPHATE MOLARITY AND pH (EXPERIMENT 2).

Initial pH	Phosphate molarity			
	5×10^{-3} M	1×10^{-3} M	5×10^{-4} M	1×10^{-4} M
8.0	No growth	12.3	19.9	No growth
7.5	No growth	9.3	10.7	No growth

Table 5. pH STABILITY OF PROTOZOAN GROWTH SYSTEMS AS A FUNCTION OF INITIAL pH AND PHOSPHATE MOLARITY.

pH[a]	pH[b] of medium											
	5×10^{-3} M $PO_4 \equiv$ hours			1×10^{-3} M $PO_4 \equiv$ hours			5×10^{-4} M $PO_4 \equiv$ hours			1×10^{-4} M $PO_4 \equiv$ hours		
	0	24	48	0	24	48	0	24	48	0	24	48
7.5	7.4	7.4	7.5	7.0	7.0	6.9	6.7	6.8	6.9	6.4	6.7	6.6
8.0	7.6	7.4	7.3	7.2	7.3	7.1	6.9	7.0	6.9	6.4	6.8	6.5

[a] pH of medium before addition of organisms

[b] pH after addition of Citrobacter and Tetrahymena pyriformis

Table 6. GENERATION TIMES (HOURS) OF Tetrahymena pyriformis AS A FUNCTION OF BACTERIAL CONCENTRATION.

Bacteria/mℓ	Generation time
5×10^7	8.8
1×10^8	9.0
2×10^8	7.9
5×10^8	8.1
1×10^9	7.7

PHASE II - CARBON CONTENT OF BACTERIA AND PROTOZOA

Because of their fragility, the protozoa could not be separated from the mixed population culture for carbon content determination. Measurements were therefore made on axenic cultures of T. pyriformis grown in proteose peptone-glucose medium and Citrobacter grown in Payne and Feisal's medium[19].

Size distribution plots of protozoa after washing showed little evidence of cell breakage from the washing procedure (Figure 1). Windows 1, 2, and 3 represent debris in the system.

According to the measured data (Table 7), each Citrobacter contains 8.6×10^{-11}mg carbon, in agreement with a value calculated from literature data[24]. The mean carbon content of a protozoan is 1.1×10^{-6}mg.

Table 8 is a tabulation of the size distribution data obtained from Figure 1. Three points should be noted:

1. More protozoa are found in window 7 than any other (volume = 7,852 μ^3).

2. The largest biomass is associated with window 8 (831 protozoa with volumes of 9,060 μ^3, or a total biomass of $75.2 \times 10^6 \mu^3$).

3. The mean protozoan volume $\left(\frac{884.6 \times 10^5 \mu^3}{7,907 \text{ protozoa}} \right)$ is $1.1 \times 10^4 \mu^3$.

The weight of organic carbon per unit volume of protozoan biomass calculated from the mean organic carbon/protozoan (1.1×10^{-6}mg, Table 7) and the mean protozoan volume ($1.1 \times 10^4 \mu^3$, Table 8) is 1×10^{-7}µg carbon/μ^3. From this value, we can assign carbon content to protozoa of varying sizes.

PHASE III - EFFECT OF PROTOZOA ON FATE OF ORGANIC CARBON (BACTERIA)

Populations of T. pyriformis increased the amount of CO_2 released from the system over that of the control representing endogenous CO_2 released by substrate-limited bacteria (Figure 2). In the first 20 hours, the major portion of the CO_2 evolved was produced by washed bacterial cells; after the protozoa started growing, however, they produced significant amounts of CO_2. The CO_2 concentration curve is skewed to the right, probably reflecting a production of CO_2 by endogenous metabolism of the protozoan population. Under microscopic examination, protozoan cells were seen to be decreasing in size during this time.

Figure 3 shows the cumulative release of CO_2 from the test systems. Note the similarity between the CO_2 production in the bacterial control and the bacterial-protozoan test systems during the first 20 hours of the experiment Overall, the protozoan-bacterial system released three to four times as much as the bacteria alone. Between hours 24 and 96, 28.6 mg C as CO_2 were

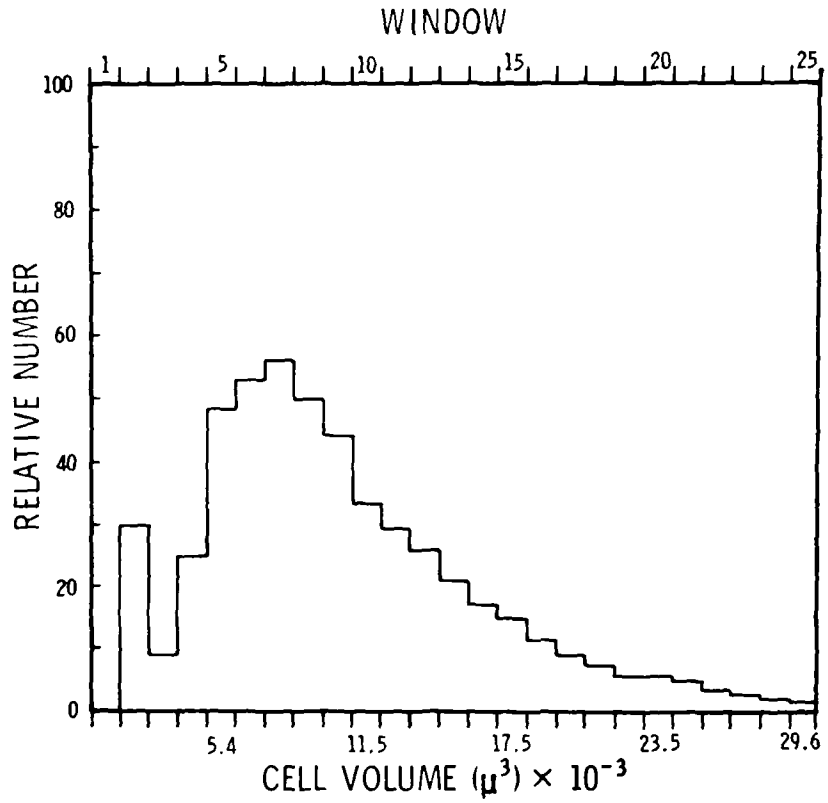

Figure 1. Size distribution of Tetrahymena pyriformis

Table 7. ORGANIC CARBON CONTENT OF *Tetrahymena pyriformis* AND *Citrobacter*.

Organism	Organisms/mℓ	TOC, mg/mℓ	mg organic carbon/organism
Citrobacter	1×10^{9}	0.086	8.6×10^{-11}
T. pyriformis	3.25×10^{6}	0.36	1.1×10^{-6}

Table 8. SIZE DISTRIBUTION OF Tetrahymena pyriformis.

Window	Average cell volume (μ^3)	Protozoa/window (0.73 mℓ)	Biomass/window (μ^3)
1	604	-	-
2	1,812	-	-
3	3,020	-	-
4	4,228	419	17.7×10^5
5	5,436	797	43.3×10^5
6	6,644	887	58.9×10^5
7	7,852	936	73.5×10^5
8	9,060	831	75.2×10^5
9	10,268	719	73.8×10^5
10	11,476	556	63.8×10^5
11	12,684	485	61.5×10^5
12	13,892	435	60.4×10^5
13	15,100	342	51.6×10^5
14	16,308	298	48.5×10^5
15	17,516	244	42.7×10^5
16	18,724	209	39.1×10^5
17	19,932	143	28.5×10^5
18	21,140	137	28.9×10^5
19	22,348	89	19.8×10^5
20	23,556	121	28.5×10^5
21	24,764	66	16.3×10^5
22	25,972	81	21.0×10^5
23	27,180	35	9.5×10^5
24	28,388	56	15.9×10^5
25	29,596	21	6.2×10^5
		7,907 protozoa/ 0.73 mℓ	884.6×10^5 μ^3/0.73 mℓ

$$\text{Mean protozoal volume} = \frac{884.6 \times 10^5 \ \mu^3}{7.9 \times 10^3 \text{ protozoa}} = 1.1 \times 10^4 \ \mu^3$$

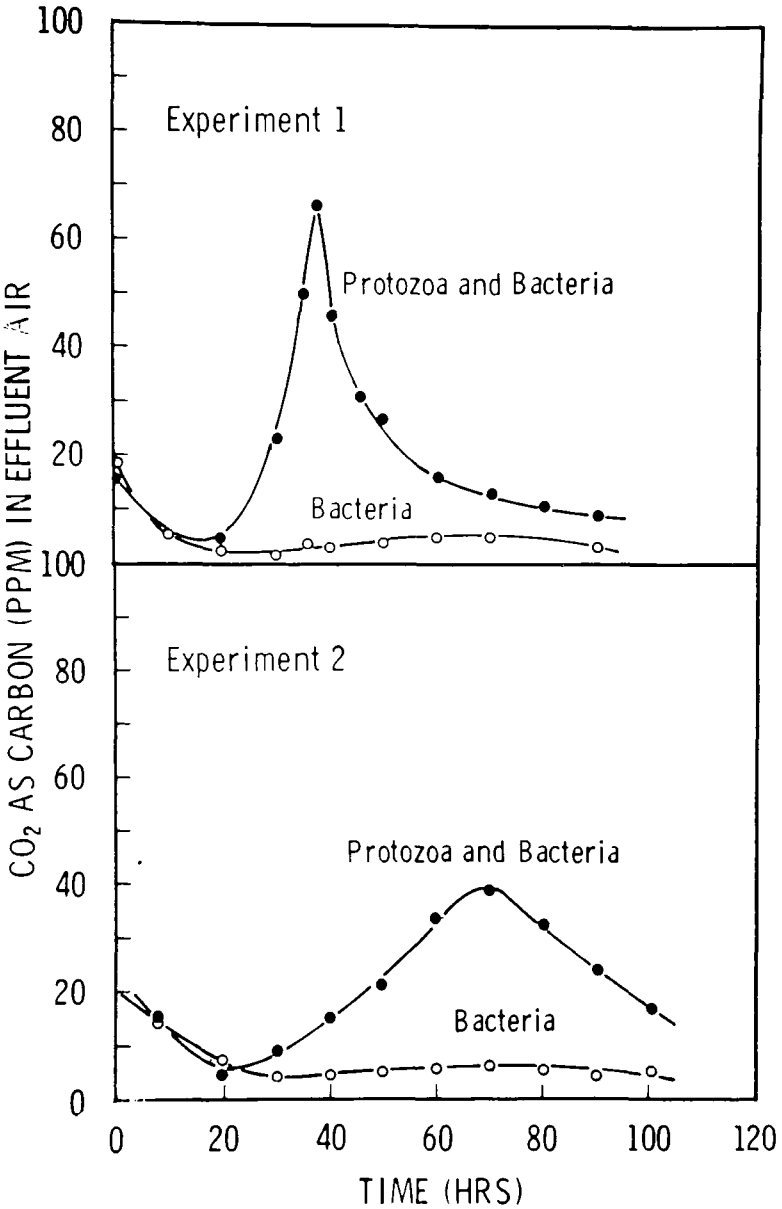

Figure 2. Concentration of CO_2 in effluent air (recorded as ppm)
Symbols: ● - protozoa and bacteria
o - bacteria

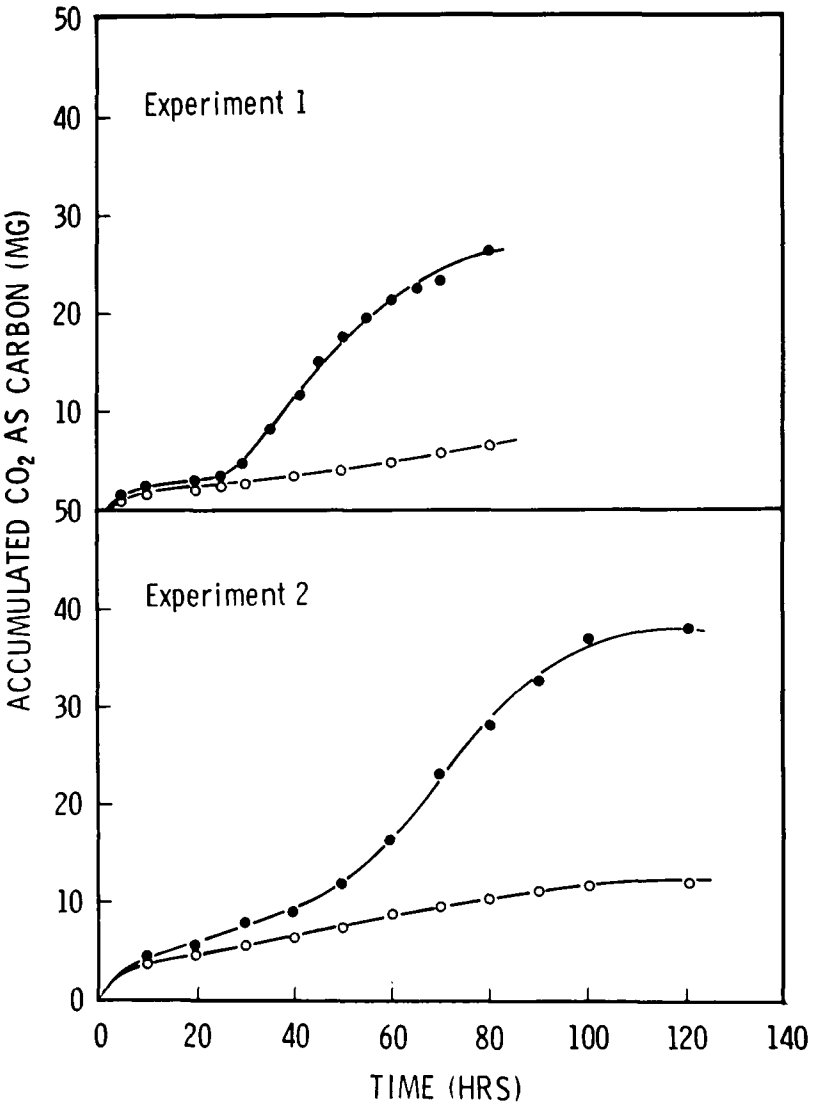

Figure 3. Accumulated carbon emitted as CO_2 (recorded as mg carbon)
Symbols: ● - bacteria and protozoa
o - bacteria

produced by the growth of 39×10^6 protozoa, a production of 0.7×10^{-6} mg C as CO_2 per protozoa.

Protozoa can also contribute inorganic carbon to water. The TIC concentration in the medium of the test system is illustrated as a function of time in Figure 4. When the protozoan population began growing (about 20 hours), inorganic carbon accumulated in the water. When the water became saturated, the excess was released into the atmosphere and measured as CO_2.

The amount of organic carbon in the system, as shown in Figure 5, decreased markedly as a function of time in systems containing protozoa. In experiment 1 (carbon values obtained from frozen samples), 83% of the initial organic carbon remained in the bacterial control flask, compared to 51% in systems containing protozoa. In experiment 2 (unstored samples), 96% and 43% of the initial organic carbon remained in the bacterial control and in the bacterial-protozoan system, respectively, at the termination of the experiment.

The protozoa utilized vast numbers of bacteria during their period of growth (Figures 6 and 7). According to calculations from Figures 6 and 7, each protozoan required 3×10^4 bacteria for growth. From the carbon values reported in Table 7, 2.6×10^{-6} mg bacterial carbon was required to produce protozoan biomass of 1.1×10^{-6} mg, representing a 42% efficiency of carbon assimilation.

As mentioned previously, the soluble organic carbon in the test system was impossible to measure. By use of formulation similar to that of Heal[18], the amount of carbon solubilized by protozoa was estimated:

carbon solubilized/protozoa = bacterial carbon ingested/protozoa

$$- (CO_2\text{-C produced/protozoa} + \text{organic carbon/protozoa})$$

$$= 2.6 \times 10^{-6}\text{mg} - (0.7 \times 10^{-6}\text{mg} + 1.1 \times 10^{-6}\text{mg})$$

$$= 0.8 \times 10^{-6}\text{mg/protozoan}$$

Autolysis of bacteria in this system was considered to be an insignificant factor in altering carbon form because the bacterial standing crop remained fairly constant in the bacterial control system (Figures 6 and 7). Also, after the period of maximal protozoan growth, the bacterial population increased, presumably using soluble carbon released by protozoa.

Data from experiment 2, used to construct carbon balances, are summarized in the following graphs. Figure 8 shows the partitioning of carbon in the bacterial control. By 120 hours, 12% of the total carbon was present as atmospheric CO_2, whereas less than 1% was in the form of TIC in the medium.

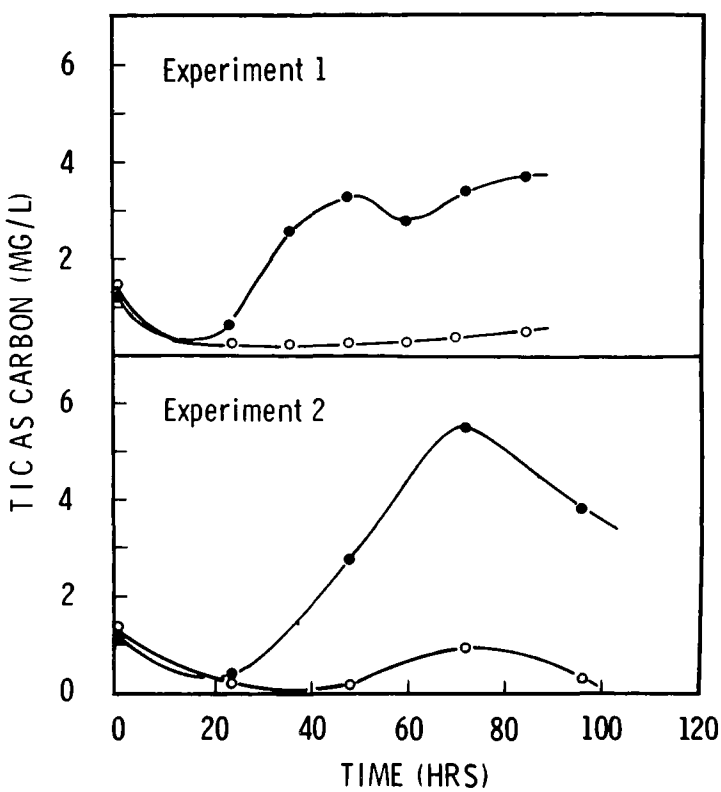

Figure 4. Total inorganic carbon (TIC) in the medium (recorded as mg carbon/ℓ)
Symbols: ● - bacteria and protozoa
o - bacteria

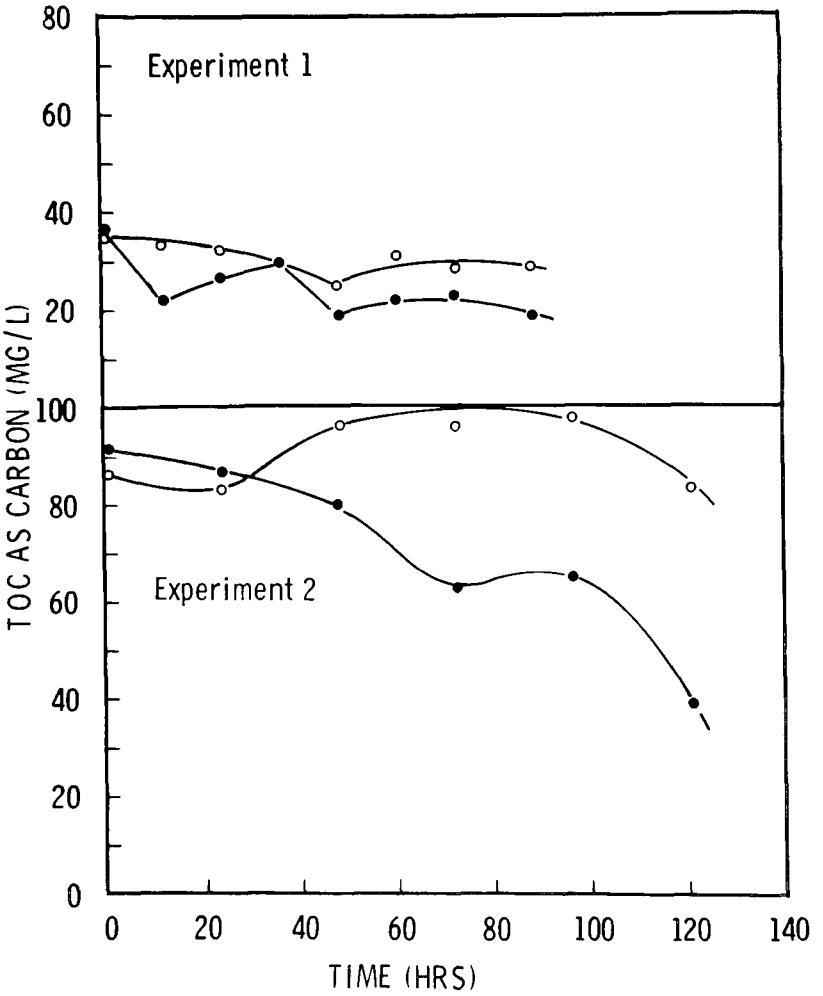

Figure 5. Total organic carbon (TOC) in the medium (recorded as mg carbon/ℓ)
Symbols: ● - bacteria and protozoa
o - bacteria

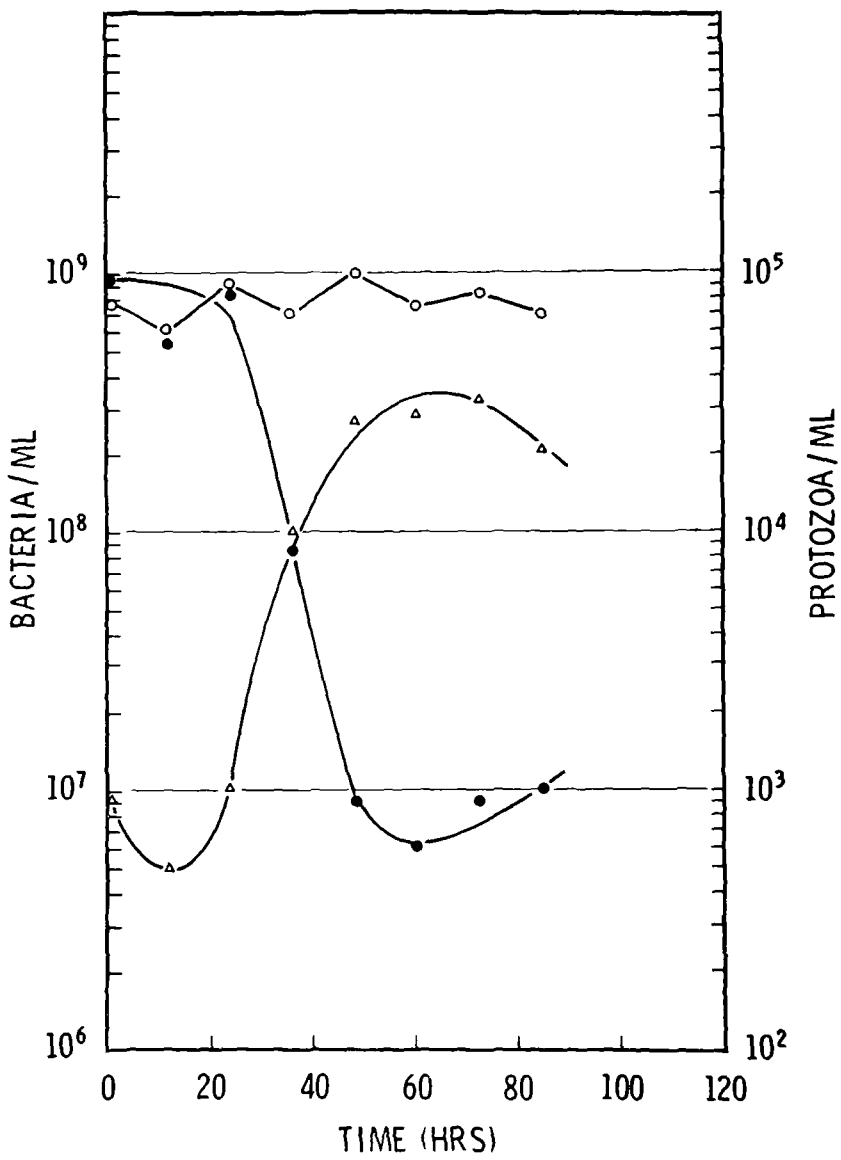

Figure 6. Growth of protozoa (Tetrahymena pyriformis) with bacteria as the carbon source (Experiment 1)
Symbols: o - bacterial population with no protozoa present
● - bacterial population with protozoa
Δ - protozoa growing on bacteria

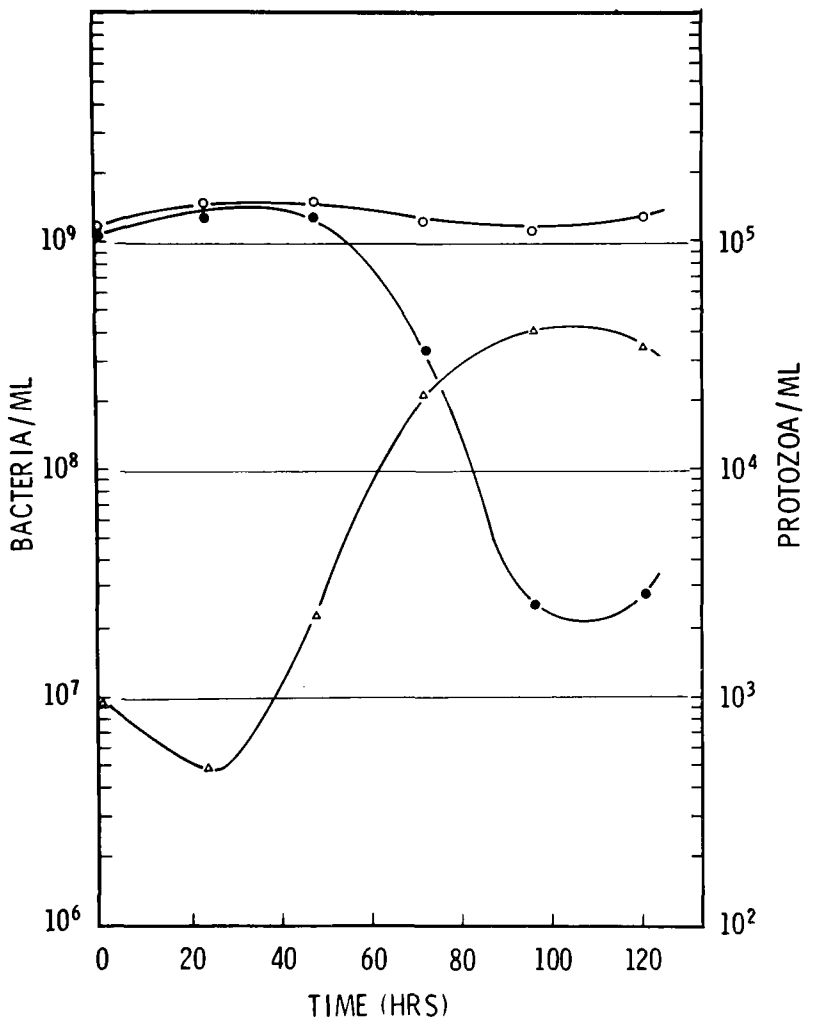

Figure 7. Growth of protozoa (Tetrahymena pyriformis) with bacteria as the carbon source (Experiment 2)
Symbols: o - bacterial population with no protozoa present
● - bacterial population with protozoa
Δ - protozoa growing on bacteria

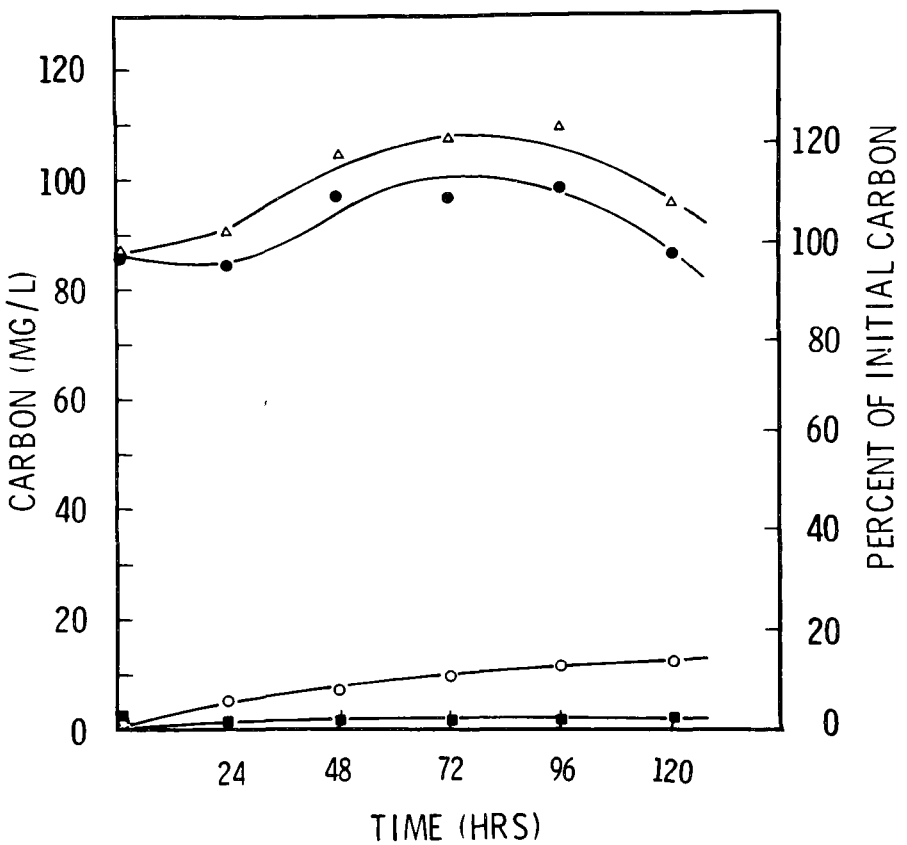

Figure 8. Carbon transformation -- bacterial control
Symbols: Δ - total carbon
● - total organic carbon
o - CO_2
■ - total inorganic carbon

Figure 9 summarizes the carbon conversions of the bacterial-protozoan system. By 120 hours, 42% of the organic carbon remained and 40% of the total carbon was converted to CO_2. Less than 5% of the organic carbon initially present was found in the medium as TIC.

Figure 10 shows the partitioning of organic carbon in the bacterial-protozoan system. Initially, 93% of the organic carbon was in the form of bacterial biomass. By 96 hours, about 3% of the initial carbon remained in viable bacterial cells. The protozoa accounted for 44% of the organic carbon at 96 hours. The remaining organic carbon, represented as soluble organic carbon, was calculated from TOC and organism carbon values. Up to 20% of the organic carbon may be in soluble form, as calculated from these data.

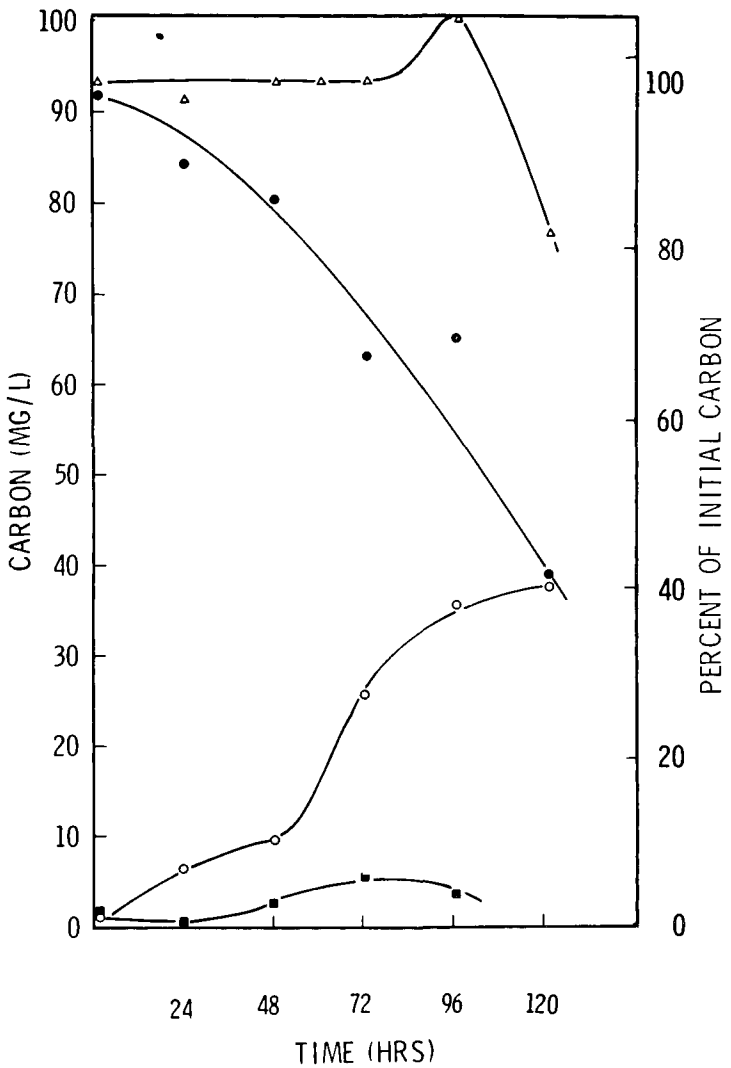

Figure 9. Carbon transformation -- bacterial-protozoan system
Symbols: Δ - total carbon
● - total organic carbon
o - CO_2
■ - total inorganic carbon

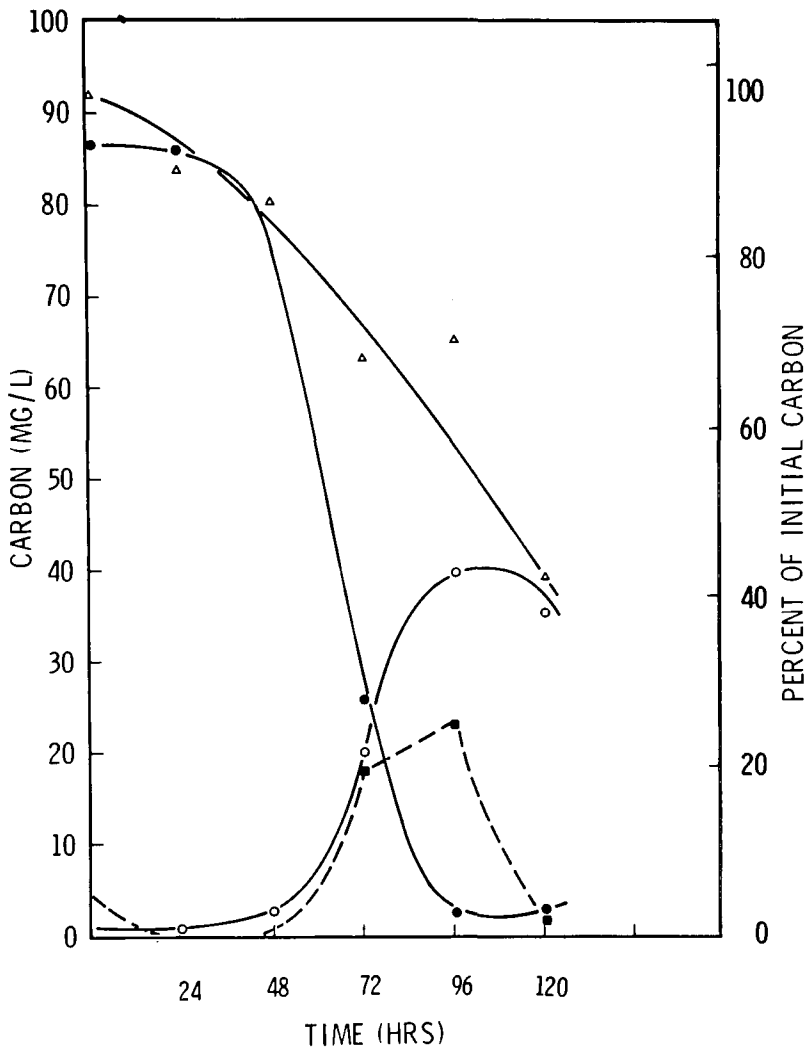

Figure 10. Partitioning of organic carbon in a bacterial-protozoan growth system
Symbols: Δ - total organic carbon
● - bacteria
o - protozoa
■ - soluble organic carbon

SECTION VI

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SECTION VII

APPENDICES

APPENDIX A

Data from which Generation Times of *Tetrahymena pyriformis* as a Function of Temperature and Aeration are Calculated.

X = hours; y = protozoa/ml; A,B = duplicate flasks

Experimental Condition	Flask		X (Hours) 0	24	48
23°C, Aerated	A	y =	1,250	47,500	51,200
	B	y =	2,500	42,500	71,800
23°C, Stationary	A	y =	750	26,000	29,600
	B	y =	2,000	22,500	39,000
30°C, Aerated	A	y =	3,000	49,000	48,100
	B	y =	2,750	49,600	58,700
30°C, Stationary	A	y =	4,250	28,000	32,500
	B	y =	5,730	35,000	39,000

APPENDIX B

Data from which Generation Times of Tetrahymena pyriformis as a Function of Phosphate Molarity and pH are Calculated (Experiment 1).

X = hours; y = protozoa/ml; A, B = duplicate flasks

Experimental Condition	pH	Flask		X (Hours) 0	24	36	48
5×10^{-2} M Phosphate	6.0	A & B	y =	----------No growth----------			
	6.5	A & B	y =	----------No growth----------			
	7.0	A & B	y =	----------No growth----------			
	7.5	A & B	y =	----------No growth----------			
	8.0	A & B	y =	----------No growth----------			
5×10^{-3} M Phosphate	6.0	A	y =	250	2,810	15,900	24,200
		B	y =	250	1,870	53,100	18,400
5×10^{-3} M Phosphate	6.5	A	y =	250	2,340	4,210	18,500
		B	y =	250	1,090	9,530	17,500
5×10^{-3} M Phosphate	7.0	A	y =	250	2,180	6,060	14,200
		B	y =	250	1,870	5,310	13,700
5×10^{-3} M Phosphate	7.5	A	y =	250	624	2,960	12,700
		B	y =	250	624	2,340	18,500
5×10^{-3} M Phosphate	8.0	A	y =	250	156	312	1,560
		B	y =	250	468	937	7,810
5×10^{-4} M Phosphate	6.0	A	y =	250	5,620	20,000	-
		B	y =	250	-	-	-
5×10^{-4} M Phosphate	6.5	A	y =	250	2,650	17,300	18,200
		B	y =	250	4,840	22,900	31,200
5×10^{-4} M Phosphate	7.0	A	y =	250	781	312	-
		B	y =	250	468	24,800	19,600
5×10^{-4} M Phosphate	7.5	A	y =	250	7,180	22,100	11,800
		B	y =	250	12,000	26,000	26,500
5×10^{-4} M Phosphate	8.0	A	y =	250	4,530	26,300	23,500
		B	y =	250	2,060	6,560	6,090

APPENDIX C

Data from which Generation Times of *Tetrahymena pyriformis* as a Function of Phosphate Molarity and pH are Calculated (Experiment 2).

X = hours; y = protozoa/ml; A, B = duplicate flasks

Experimental Condition	pH	Flask		X (Hours) 0	24	48
5×10^{-3} M Phosphate	7.5	A & B	y =	500	-	-
5×10^{-3} M Phosphate	8.0	A & B	y =	500	937	156
10^{-3} M Phosphate	7.5	A & B	y =	500	310	2,260
10^{-3} M Phosphate	8.0	A & B	y =	500	1,400	7,500
5×10^{-4} M Phosphate	7.5	A & B	y =	500	312	5,070
5×10^{-4} M Phosphate	8.0	A & B	y =	500	1,560	2,650
10^{-4} M Phosphate	7.5	A & B	y =	500	-	-
10^{-4} M Phosphate	8.0	A & B	y =	500	2,030	-

APPENDIX D

Data from which Generation Times of *Tetrahymena pyriformis* as a Function of Bacterial Concentration are Calculated.

X = hours; y = protozoa/ml; A, B = duplicate flasks

Experiment 1

Experimental Condition	Flask		X (Hours) 0	24	48
5×10^7 bacteria/ml	A	y =	500	10,750	-
	B	y =	500	8,750	750
10^8 bacteria/ml	A	y =	500	9,750	2,750
	B	y =	500	9,500	3,000
2×10^8 bacteria/ml	A	y =	500	10,750	-
	B	y =	500	9,250	1,250
5×10^8 bacteria/ml	A	y =	500	14,250	40,750
	B	y =	500	13,750	35,500
10^9 bacteria/ml	A	y =	500	15,250	67,000
	B	y =	500	14,500	67,750

Experiment 2

5×10^7 bacteria/ml	A & B	y =	500	2,187	9,687
10^8 bacteria/ml	A & B	y =	500	4,062	8,437
2×10^8 bacteria/ml	A & B	y =	500	6,406	14,531
5×10^8 bacteria/ml	A & B	y =	500	7,031	16,563
10^9 bacteria/ml	A & B	y =	500	8,400	19,062

Experiment 3

Experimental Condition			X (Hours) 0	24	36	48
5×10^7 bacteria/ml	A & B	y =	500	3,359	8,281	14,062
10^8 bacteria/ml	A & B	y =	500	3,437	10,156	14,453
2×10^8 bacteria/ml	A & B	y =	500	4,297	18,593	22,734
5×10^8 bacteria/ml	A & B	y =	500	3,281	29,687	38,906
10^9 bacteria/ml	A & B	y =	500	3,828	33,125	35,781

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EFFECTS OF PROTOZOA ON THE FATE OF PARTICULATE CARBON

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16. Abstract

Laboratory studies were designed to define the role of protozoa in the fate of particulate (bacterial) organic carbon. Specific objectives were (1) to measure the effects of selected environmental parameters on protozoan growth rates, (2) to measure organic carbon in bacteria and protozoa, and (3) to quantitate carbon transformations in predator-prey experimental systems.

A growth system containing 2×10^8 Citrobacter/mℓ in 1×10^{-3}M phosphate of pH 7.5, incubated at 25°C at a shaking rate of 100 rpm, was found to be an optimal environment for protozoan growth.

The nutrient bacterium, Citrobacter, contained 8.6×10^{-11} mg C/cell, and Tetrahymena pyriformis contained 1.1×10^{-6} mg C/cell.

T. pyriformis altered the amount and form of carbon in the system while growing on bacteria. Of the total organic carbon present at the initiation of the predator-prey experiment (93 mg), 93% was in the bacterial fraction. Within 96 hours, 38% of the carbon was released as CO_2; 5% was present as inorganic carbon in the water and the remainder (57%) was present as organic carbon. The organic carbon in the bacterial fraction decreased from 86 to 2 mg within 96 hours, while the carbon in the protozoan biomass increased from 1 to 40 mg. In the bacterial control, 11% of the organic carbon was released as CO_2 within 96 hours while negligible amounts of inorganic carbon remained in the water. (Holm - Southeast Environmental Research Laboratory)

17a. Descriptors: *Protozoa, *Aquatic microbiology, *Aquatic bacteria, *Carbon cycle, *Cycling nutrients, Carbon dioxide, Aquatic microorganisms, Food chain, Secondary productivity, Growth rates, Predation.

17b. Identifiers

*Carbon transformation, Carbon utilization, Population dynamics, Organic carbon, Citrobacter, Tetrahymena pyriformis.

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