Deciduous Forest Biome
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ACTIVITY ESTIMATION OF AQUATIC FUNGAL AND BACTERIAL DECOMPOSERS

A final technical report for Union Carbide Subcontract No. 3567 for the Eastern Deciduous Forest Biome, IBP, Lake George Site

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A. Introduction

In order to study the dynamics of microbial activities in the aquatic ecosystem, suitable methods had to be tested, or developed and tested. The project year was spent critically evaluating the uptake of radioisotopically labelled amino acids as a method for the determination of microbial growth rates. From these rates and microbial yields, the associated turnover of elements in the process of decomposition can be estimated. In addition to evaluating the isotope method, a new method based on the kinetics of steady state cultivation was developed for growth rate determination.

It is obvious that measurements of microbial biomass per se do not reflect the dynamics of microbial productivity or the associated nutrient regeneration. Some indication of prior activity is given this way. However, the rates of removal of microbial biomass, in the aquatic system, are believed to approximately equal the rates of production resulting in a type of steady state. There is a cyclic nature to this over-all steady state in which the amplitude of each cycle is dependent on one or more substrates limiting the extent of microbial growth. The time factor for these cycles depends on the specific growth conditions. These growth conditions may be such that the time for a population to double is as short as 1/2 hour, however, more typically it seems to be about 6 hours in Lake George during the growing season as indicated in a later section.

B. Basic Amino Acid Uptake Method

1. Procedure. The basic amino acid uptake method used will be described below. Variations, such as the use of a labeled amino acid mixture, the addition of insulin to the assay, the addition or deletion of nutrient and carrier, are discussed in later sections.

In general four microcuries of labeled amino acid were used in a 10 ml assay containing the cells to be studied. The assay flasks, 50 ml Erlenmeyers, were stirred throughout the incubation period with magnetic stirrers. Unless otherwise specified, experiments were carried out at room temperature. At intervals, 1 ml samples were withdrawn and placed in test tubes into which 0.1 ml 1N KCN had been pipetted.

The cyanide killed cells were membrane filtered (sartorius 0.45μ filters) and washed three times with 2 ml 0.01 NHCl and three times with 2 ml 0.01 N NaOH. The filters were placed in precounted vials containing 10 ml scintillation fluid (700 ml Toluene, 300 ml Methanol, 4 g omnifluor) and counted for 1 min in a liquid scintillation counter. When possible, graphs were made on semilog paper of activity (in cpm) versus time. Graph # 1 is a characteristic plot, showing rapid uptake for 30 min. (perhaps to fill an amino acid pool), then a less rapid linear portion between about 30 and 120 min., followed by further leveling at points after about 120 min.

2. Washing Millipore Filters. The effectiveness of acid-base washing versus water washing of millipore filters (sartorius 0.45μ) was studied. A flask containing 0.5 ml C^{14}-leucine (3 μC), 4.5 ml distilled water and 5 ml 10% TCA was prepared to simulate an experimental situation (without cells). A somewhat different assay is now used, but this one serves the purpose for demonstrating washing problems. One ml aliquots were membrane filtered on we filters. Half were washed with six 2 ml washes of distilled water; half with two 2 ml washes of 0.01 N HCl, two 2 ml washes of 0.01 N KOH, and two 2 ml washes of distilled water. The filters were placed in scintillation vials containing 10 ml scintillation fluid and counted for 5 min. Vials with fluid had been counted previously and were all below 50 CPM. Results in CPM are as follows:

Sample	Water Wash	Acid-Base Wash
1	1333	178
2	879	187
3	594	109
4	511	82
5	390	75

An acid-base washed membrane filter (no radioactivity) gave 63 CPM.

Although the amount of leucine remaining on the filters was somewhat erratic, it is evident that acid-base washing is much more effective in removing leucine than water washing. The activity left on the filters is considerable in both cases, indicating the importance of washing cells before filtering.

The experiment was repeated with a solution of 0.7 ml glucose-C^{14} (170,520 CPM per 0.1 ml) and 9.3 ml water. Results were as follows:

Sample	Water Wash	Acid-Base Wash
1	176	329
2	709	212
3	169	230

In this case, overlooking the one high count, acid-base washing seems no more effective than water washing in removing glucose from membrane filters. Once again, however, the importance of washing cells is evident.

In a number of experiments with leucine uptake, even after a standard method had been devised, inexplicable erratic results were encountered. It was thought that this might be caused by very fast membrane filtering, since the wash is only in contact with the cells for a very shore time and may not

be effective. Also the possibility of adsorbed radioactivity from letting cells sit under hot supernatant for several days was considered.

To investigate this, a typical 15 ml assay flask with Escherichia coli was prepared. At three different times after C^{14}-leucine was added, a 4 ml aliquot was removed and 1 ml pipetted into each of 4 centrifuge tubes (containing KCN). The first portion was carried through the whole procedure immediately and washed regularly. The second was done immediately and washed with the vacuum pump off for 30 sec after each wash to allow that much contact. The third sat a week and was washed regularly; and the fourth sat a week, but received delayed washes. Both sat under hot supernatant. This procedure was done on three 4 ml aliquots. Results are shown below:

Worked straight through, regular wash	Worked straight through, delayed wash	Sat one week, regular wash	Sat one week delayed wash
1	2	3	4
1120	1115	961	717
1546	1488	1190	1263
1852	1855	1563	1270

Evidently delayed washes are no more effective. Also, there is apparently no adsorption of radioactivity from letting cells sit under hot supernatant one week. The fact that fewer counts were observed in portions 3 and 4 might be a sampling error; i.e., cells in the 4 ml aliquot may have settled toward the bottom of the pipet, thus being present in decreasing numbers as successive 1 ml samples were withdrawn.

In another washing study, triplicate 1 ml aliquots of an H^3-LEU solution (87,530 CPM per ml) was put through a membrane filter and given acid-base washing immediately. Another 1 ml aliquot was put through and the filter allowed to dry 5 min before being washed. This was repeated three times with the following results:

Sample	5 Min. Delay	Immediate
1	2169	414
2	2264	385
3	2089	384

Allowing the filter to dry increases the retained activity tremendously.

Ten ml of the same H^3-leucine solution when put through a membrane filter with immediate acid-base washing left 4110 CPM, indicating the increased retention problem with increased volume. Five ml of cold leucine solution (5 mg/ml) were put through a membrane filter, followed by 10 ml of the H^3-leucine solution. After acid-base washing, duplicates showed 3,555 and 4,503 CPM left on the filters. It was hoped that the cold leucine would occupy adsorption sites and reduce the activity remaining on the filter, but this was not the case.

It was suggested by other researchers that making our assay solution 5% in TCA and using another brand of filters might improve the washing problem. Two solutions were prepared; one with 10 ml H_2O and 1 ml stock H^3-leucine solution, one with 10 ml 5% TCA solution and 1 ml stock H^3-leucine solution. One ml aliquots were put through the filters and given regular acid-base washing. Results in CPM are as follows (all figures are averages of duplicate samples):

	H_2O	5% TCA
Millipore filter (HA 0.45μ)	275	225
Sartorius filter (SM 0.45μ)	296	238

The filters are comparable in retention properties, but there seems to be a small improvement in both cases with 5% TCA.

It was though that it might be a useful technique to allow cells to incorporate on a membrane filter. A filter was soaked in a regular assay solution (no cells) for one hour, placed on a filter apparatus and acid-base washed. Unfortunately, 11,016 CPM remained on the filter.

3. Thin Layer Chromatography for Residual Leucine. Some TLC was tried on stock solution of C^{14}-leucine with Gelman 1TLC-SG medium. The first solvent system used was 4:1:1 N-Butanol, glacial acetic acid, distilled H_2O. The R_f was approximately 50 for leucine, but the chromatogram took 2 hours to complete. This solvent was diluted 1:1 with N-Butanol (making it 10:1:1 N-Butanol; glacial acetic: distilled H_2O.) This gave an R_f of about 60 and only took about 45 minutes per chromatogram.

The chromatograms were stained with ninhydrin, which makes the leucine spots bright pink. Some diffuse pink and yellow streaking was evident from the location of the original spot to the final spot. The chromatograms were divided into small squares, and counted by liquid scintillation counting. Whereas much of the activity was in the final spot, a considerable amount was streaked rather evenly between the original and final spot.

A solvent system of MEK: puridine: distilled water: glacial acetic acid (70: 15: 15: 2) was tried. The chromatograms only took 20 min with this system with an R_f for leucine of about 85. Much more of the activity was concentrated in the final spots, but only about 25% of the original activity could be accounted for. It was thought that this could be due to several layers of medium preventing activity from expressing itself. 0.02 ml of a solution of C^{14}-leucine put directly into scintillation fluid gave 1600 CPM. Spotted on a single layer of silica gel medium placed in the bottom of a vial for counting, the same 0.02 ml gave 1402 CPM. However, when the 0.02 ml was divided on two pieces of medium, and the media placed one over the other in the bottom of a vial, only 987 CPM were noted. Thus it would seem important to have the final spot no bigger than will fit in a single layer in a scintillation vial.

TLC was used qualitatively in developing the C^{14}-leucine uptake method to show that leucine was still available in the decantate, and thus not a limiting factor for leucine incorporation.

C. Field Studies with the Amino Acid Method

1. Pond Water and Lake George Water. To see if the C^{14}-leucine uptake method is feasible for dilute systems, pond water (from Mt. Ida Cemetery in Troy, N.Y.) was sampled. Two assays were prepared as follows:

#1		#2	
19.4 ml	pond water	19.4 ml	distilled water
3.0 ml	H_2O	3.0 ml	broth*
0.8 ml	carrier-leucine (5 mg/ml)	0.8 ml	carrier
1.8 ml	C^{14}-leucine	1.8 ml	C^{14}
25.0		25.0	

* Tryptone-glucose-yeast extract broth (used throughout for added nutrient)

Tryptone - 10 g
Yeast extract - 1 g
NaCl - 8 g
glucose - 1 g
1 M $CaCl_2$ - 2 ml
Distilled water to 1 liter

To check the washing, 5 ml blanks of each assay were put through membrane filters and acid-base washed. The blank from #1 had 904 CPM, that from #2 had 448 CPM. Five ml aliquots were removed from the assay flasks at half hour intervals for 2 hours, killed with KCN, membrane filtered, washed and counted. There was no uptake pattern, each count being around 300-400 CPM which was probably undissolved C^{14}-leucine.

Because of the large blanks with these assays, it was decided to try regular 10 ml assays (1 ml aliquots) without carrier, in order to increase the specific activity. Pond water was sampled after a very hot, humid night (July 15), when there was an algal bloom on the pond. Two 25 ml aliquots were spun down for 15 min. The top 20 ml were withdrawn with a pipet and the bottom 5 ml in each centrifuge tube (which contained green pellets) were resuspended and combined. 0.8 ml C^{14}-leucine was added and 1 ml aliquots removed and killed every half hour for 6 hours. Assay flasks were stirred throughout the experiment. Graph #2 illustrates the results. After about 90-120 min, the CPM drop off from the established linearity.

Pond water was again sampled after a cool, dry night (July 21). Three assays were run following the same procedure, one with no carrier, one with 0.3 ml carrier, one with 0.03 ml carrier. No incorporation was observed in either of the carrier systems; however, the no carrier system showed linear uptake on log paper. The CPM reached a value of about 10% of that reached in the previous experiment in the same time period, possibly because of the change in weather. Once again, the dropping off of CPM was noticed at about the same time.

It was thought that substrate might be a limiting factor, so the pond water experiment was repeated after a record cold night on July 28. Two assays were run both with no carrier, but one with added nutrient (2 ml added in a 10 ml assay). The system without added nutrient showed linear uptake, although less than in the previous two experiments. This might be explained by record cold weather. The system with added nutrient had no measurable incorporation, probably because of the addition of nutrient had the effect of lowering the specific activity.

Since the no carrier, no added nutrient system worked with pond water, it was decided to try it on Lake George water. Water from Smith Bay was concentrated by centrifugation as in the pond water experiments. Two assays were run, one with and one without added nutrient, for a twenty hour period. The one with no added nutrient showed no incorporation. The one with added nutrient was cloudy after 20 hours and the 1 ml sample at that time had 5500 CPM. Cell multiplication had of course occurred during the long incubation so that the incorporated activity was without meaning in terms of actual activity in the lake system.

To help determine if nutrient limitation was responsible for the leveling off of uptake, two 6 hr assays were run on a 7 hour culture of E. coli, one with and one without nutrient added. After 6 hrs there was just beginning to

be some noticeable uptake in the system without added nutrient. The system with added nutrient showed a typic uptake pattern (see Graph #1). Evidently the E. coli, accustomed to growing on a nutrient-rich medium, was severely shocked by the low nutrient assay conditions.

It was found that the addition of insulin to an assay and the use of a C^{14}-glucose and C^{14}-amino acid mixture allowed the incorporation of more labeled material into E. coli. (See Section C.) This was tried on pond water after a cold spell, but no noticeable incorporation occurred in a 2 hr period indicating lower than threshold activity.

2. Sensitivity of Method. In order for the leucine uptake method to work for Lake George water, a large quantity of lake water must apparently be concentrated by centrifugation or filtration and the residue quantitatively recovered. Some preliminary studies on ultrafiltration indicate that some loss of activity occurs at the high pressures, but lower pressures and longer filtration times are possible. This will be pursued in the continuation of this project.

Another distinct possibility that is in the process of being tested is the use of teflon filters manufactured by the General Electric Corp. These filters prepared by neutron bombardment has a potential not possessed by the cellulose acetate membrane filters. Concentration and recovery from the teflon filters seems possible based upon the smooth surface as well as the self-lubricating quality of teflon. Todd et al. have prepared scanning electron micrographs of these filters illustrating this.

A study was done to determine the degree of concentration of Lake George water that is probably necessary before measurable leucine uptake could be detected in short incubation times. This was done with various dilutions of E. coli rather than with indigenous flora. In this experiment numbers of cells were counted at the beginning of each uptake assay.

Three assays were run, each with a different concentration of E. coli, using a C^{14}-glucose — C^{14}-amino acid mixture. Plate counts were done at each dilution immediately after the addition of isotope so that the number of cells at the beginning of the incubation period could be determined.

Five ml of a 22 hr culture of E. coli in broth (optical density 1.5 at 620 on Spectronic 20) was spun down and resuspended in 5 ml 0.1 M tris buffer (pH 7.0). Previous experiments had indicated that resuspension in water shocks the cells and delays incorporation. A one ml aliquot of this was serially diluted with 9.0 ml tris; this dilution was not used for an assay. Once again 1 ml of this was diluted with 9.0 ml tris; this was the first dilution used for an assay. The same dilution process was repeated two more times and these dilutions used for assays. The assay flasks contained 5 ml cells from each dilution, 0.02 ml broth, 4.2 ml H_2O and 0.8 ml C^{14}-glucose-C^{14} amino acid mix (2 μc). It was hoped that the small amount of broth would provide enough nutrient without drastically lowering the specific activity.

As soon as the isotope was added plate counts were run. The choice of dilutions proved fortunate: dilution #1 (4.6×10^7 cells/ml) gave 6863 CPM after the 155 min incubation; dilution #2 (7.6×10^6 cells/ml gave 402 CPM; dilution #3, 125 CPM. Graph #4 shows the results. There was no measurable incorporation at the third dilution, which contained 3.3×10^5 cells/ml.

Since Lake George water has been found to contain about 10^3 cells/ml in the open water, it is evident why no uptake of C^{14} leucine has been observed according to our assay procedure. Also the growth capacity of E. coli is considerably greater that the capacity of the indigenous species in the lake.

Since between 10^5 and 10^6 cells/ml are needed for this assay, at least a 100 fold concentration has to be made of open water samples from Lake George.

D. Increasing Activity

1. Insulin. Various methods were tried to increase net isotope uptake. According to Granitsas, the presence of insulin increases the incorporation of amino acids into proteins of E. coli. This possibility was investigated using E. coli in a no carrier system, with added nutrient. Two 10 ml assays were run, one without insulin, one with 0.7 ml prepared insulin solution. The insulin solution was Lilly Regular Iletin, 40 units per ml. Graph #3 illustrates the results. At the start of the incubation period, the effect of insulin was minimal, but its effect increased with time. Since it does increase the incorporation of C^{14}-leucine, it was decided to use it in further field work.

2. Glucose-Amino Acid Mixture. Instead of using a solution of C^{14}-leucine only, a mixture of C^{14}-amino acids and C^{14}-glucose containing the same activity as the C^{14}-leucine solution was tried. Three assays were run with added nutrient and no carrier; one with C^{14}-leucine, one with isotope mixture, one with isotope mixture plus insulin. The results are summarized below.

Time, Min.	CPM		
	Leucine	Isotope Mixture	Isotope Mixture + Insulin
60	6,492	17,742	17,870
80	7,811	24,771	26,509
95	10,707	30,332	35,187

There seems to be approximately a threefold increase with the isotope mixture over C^{14}-leucine alone. Once again, insulin increases the uptake and its effect increases with time. It was decided to use both the glucose-amino acid mixture and insulin to increase uptake in future experiments. The effect of added nutrient and carrier by incorporation is discussed in Section C on Field Studies.

E. Sediment Studies

1. Lysing Cells. Cell lysis after isotope uptake, followed by filtration and precipitation with TCA (trichloracetic acid), would be a useful technique for separating cellular proteins from debris. Therefore, several methods of cell lysis were attempted. Extent of lysis was determined both by phase microscopy and incorporation techniques. E. coli was incubated with C^{14}-leucine and 1 ml samples withdrawn periodically. Chloroform (0.2 ml) was added to each sample and the mixture vortexed and membrane filtered. There was no visible clearing on addition of $CHCl_3$. The filtrate was made 10% in TCA and membrane filtered. Results are as follows.

Pellet	TAC Precipitate
921	327
1,107	188
12,028	2,014

The activity in the pellet indicates incomplete lysis.

Sonication (for 5 min) was also tried as a method of lysis. In this case, the pellet had 1,407 CPM while the TCA precipitate had 287. There was no visible change in the optical density upon sonication.

Examination under the phase microscope showed no apparent change in E. coli as a result of either of the above treatments, Pseudomonas fluorescens was also subjected to both methods with surprisingly no noticeable change in cell motility.

Another method of lysis using lysozyme and BRIJ detergent was attempted. In this case there was a visible loss of optical density. Also, under the phase microscope the cells visibly changed. Instead of a uniform darkness, they appeared to have a dark outer rim and lighter center. This method of lysis is supposed to leave the cell wall fairly well intact. Unfortunately, if the

samples were allowed to sit until completely cleared, they became too viscous to be membrane filtered. An experiment was run in which samples were diluted with H_2O, then membrane filtered. Results are as follows:

Pellet	TCA Precipitate
356	63
279	56
234	69

Although this experiments indicates incomplete lysis, the method seems promising.

2. Sonication Prior to Plate Counts. In doing plate counts on slurry samples, the problem arises that a cluster of cells on a piece of debris produces a single colony; therefore, the cell count would be lower than it should be. Sonication was considered as a means of separating cells from debris. Slurry from Lake George was diluted just enough so that it could be pipetted (25 ml slurry diluted to 80 ml with distilled H_2O). An aliquot of 10 ml was placed in each of four 20 ml glass beakers and sonicated on a Biosonik sonicator at a high setting for 1, 2, 5 and 8 min. The beaker was surrounded by ice and the temperature of the slurry taken at intervals to ensure that no overheating was occurring. At no time did the temperature rise above 36° C. Plate counts were run after each sonication period. It was hoped that the cell could would rise continuously with time of sonication until a time was reached when all cells were separated from debris and accounted for as a discrete colony. However, the cell counts for unsonicated slurry and those taken after all periods of sonication were the same. This experiment was repeated with similar results.

In making plate counts, plates are routinely run on a membrane filtrate to correct for the spore distribution.

3. Uptake Method Applied to Slurry. To determine if the uptake method could be applied to slurry samples, Lake George sediment was diluted 1:1 with water and 1 ml of this dilute slurry used in a regular 10 ml assay with C^{14}-leucine. The incubation was carried out for three hours at 10°C, the approximate temperature of the lake at the depth where the sample was taken. There was no measurable incorporation during this period, the CPM remaining at about 200 throughout.

After finding that a C^{14}-glucose, C^{14}-amino acid mixture increased uptake, the above experiment was repeated on a fresh slurry sample using this mixture. This time two assays were run, one at 10° C and one at room temperature for two hours. Once again, the CPM remained relatively constant at 10° C, but there was a definite, although somewhat erratic, uptake at room temperature. Evidently, there was adsorption of isotope on the filter paper, probably encouraged by unusually slow membrane filtering due to the nature of the slurry. The problems associated with the filtration of slurries are being studied now.

Obviously microbial activity is largely curtailed by the low temperature of the deeper waters of Lake George. These studies were made at a time when the temperature was rapidly dropping. The data indicate the potential activity of the flora at higher temperatures. Studies are being made of the change in 30° C activity and 30° C plate counts as the winter approaches.

F. Continuous Flow Studies

A new approach to the estimation of microbial activity in natural systems was developed during the project year in an attempt to more accurately describe the in situ activity of the heterotrophic microbial population.

According to most investigators, a steady state exists in the aquatic system in which microbial productivity is, in general, balanced by the removal of microbial biomass. Therefore the steady state kinetics of Herbert et al. can be applied. The growth rate of these microbial populations can thus be expressed by the following equation:

$$dx/at = \mu x - \rho x$$

μ = growth constant
ρ = removal constant

The growth (μx) varies according to the specific yield coefficient since decreasing yield of biomass per quantity of substrate consumed is observed as substrate levels drop below the saturation level for that particular nutrient. The growth also reflects very closely the immediate aquatic environment with respect to type of organic material, inhibitors, activators, temperature, and oxygen.

Removal (ρx) depends on predation or transport of the organisms out of the locus by water currents or sedimentation.

In a laboratory continuous flow culture vessel, the rate at which medium is being introduced to the culture vessel dictates the growth rate of the population in the vessel. As the medium is introduced, it is completely mixed with the culture vessel contents and an equivalent volume of culture vessel contents is withdrawn. This dilution rate (rate of exchange of the medium) equals the flow divided by the volume of the vessel.

$$D = \frac{F}{V}$$

When v = 1, the dilution rate (reciprocal time units) is equal to F. And at steady state, dx/dt = 0. Thus the equation:

$$dx/at = \mu x - Dx$$

indicates that $\mu x = Dx$ or $\mu = D$, since x is constant.

In the natural system ρ can be equated with D. Thus laboratory studies can be run in which F is varied resulting in a range of values for D and consequently μ. The procedure is as follows:

1) One analyzes an aliquot of a lake sample for numbers of cells immediately upon collection from the field.
2) One allows another aliquot to grow in the continuous flow device.
3) One analyzes for numbers of cells as a function of time in the device. The number of cells will be observed to increase, decrease, or remain the same depending on whether the flow permitted a growth rate greater than, less than, or the same as that of the original environment.

The continuous flow device is very simple and consists only of a lake water reservoir (4 liters is convenient), a tube squeezing finger pump (Sigma micro flow), a series of 50 ml test tubes stirred with magnetic mixers and maintained at the temperature of the lake environment, and an effluent reservoir. The vessels are rubber stoppered and connections are glass and tygon tubing. The system is autoclaved before each run to prevent effects of laboratory flora. The lake water reservoir has been membrane filtered since only soluble organics have been thus far studied.

Application to lake samples taken from Smith Bay in Lake George during the growing season has shown growth rates corresponding to generation times of about six hours.

These studies are being continued throughout the year and will be applied to particulate organics.

G. Conclusion

One must differentiate between actual and potential activity in microbial activity estimations. This study has attempted to develop and apply two sets of methods for microbial activity estimation, isotope uptake for potential activity and continuous flow for actual activity.

The isotope uptake method appears to have sensitivity limitations under the short incubation times desired. It seems that the objective should be to determine the rate of uptake under initial reaction velocity conditions. It appears however that initial reaction velocity conditions are not actually apparent until after a thirty minute incubation period since an initial surge of incorporation masks the later uptake rate that seems to be correlated with growth. An unrealistic generation time is calculated based upon the initial surge bearing this out.

It is felt that the biotic and chemical changes occurring during long incubation times are too great for any extrapolation (potential or actual) to the ecosystem.

The growth rates determined by the continuous flow method appear to be realistic. The shock to the microbial flora during this assay is minimal since the environment mimics the natural environment so well.

Difficulties are encountered in applying the continuous flow technique to sediments but it is felt that these can be surmounted.

The point that should be made in conclusion is that these microbial activity studies are an indirect means of assessing turnover of the elements combined in decomposing organic material. The wide chemical variation of decomposing organic material makes the indirect approach attractive. The direct approach would, of course, measure the rate of decay of these organic compounds rather than the rate of growth of those organisms doing the decomposition.

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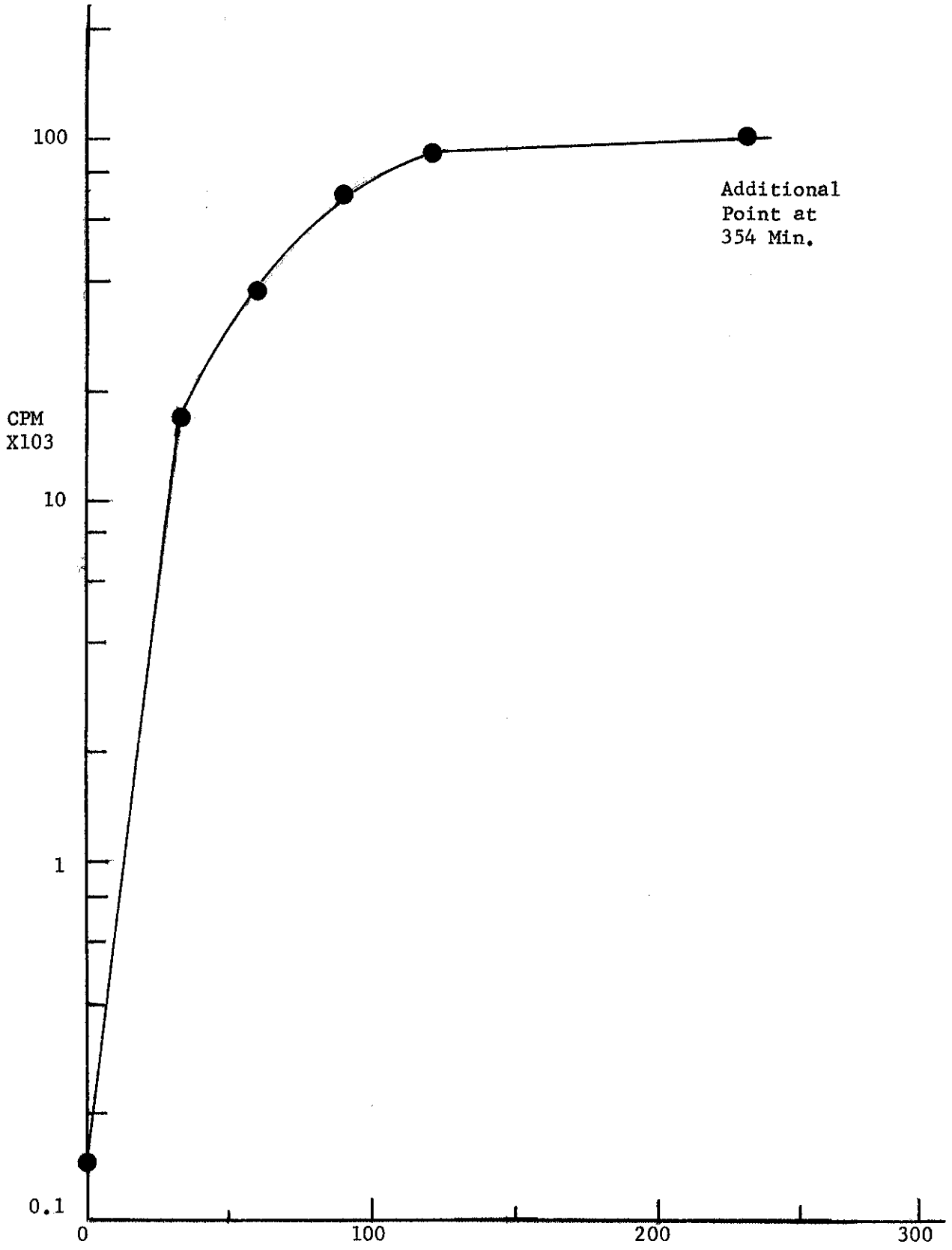
GRAPH #1

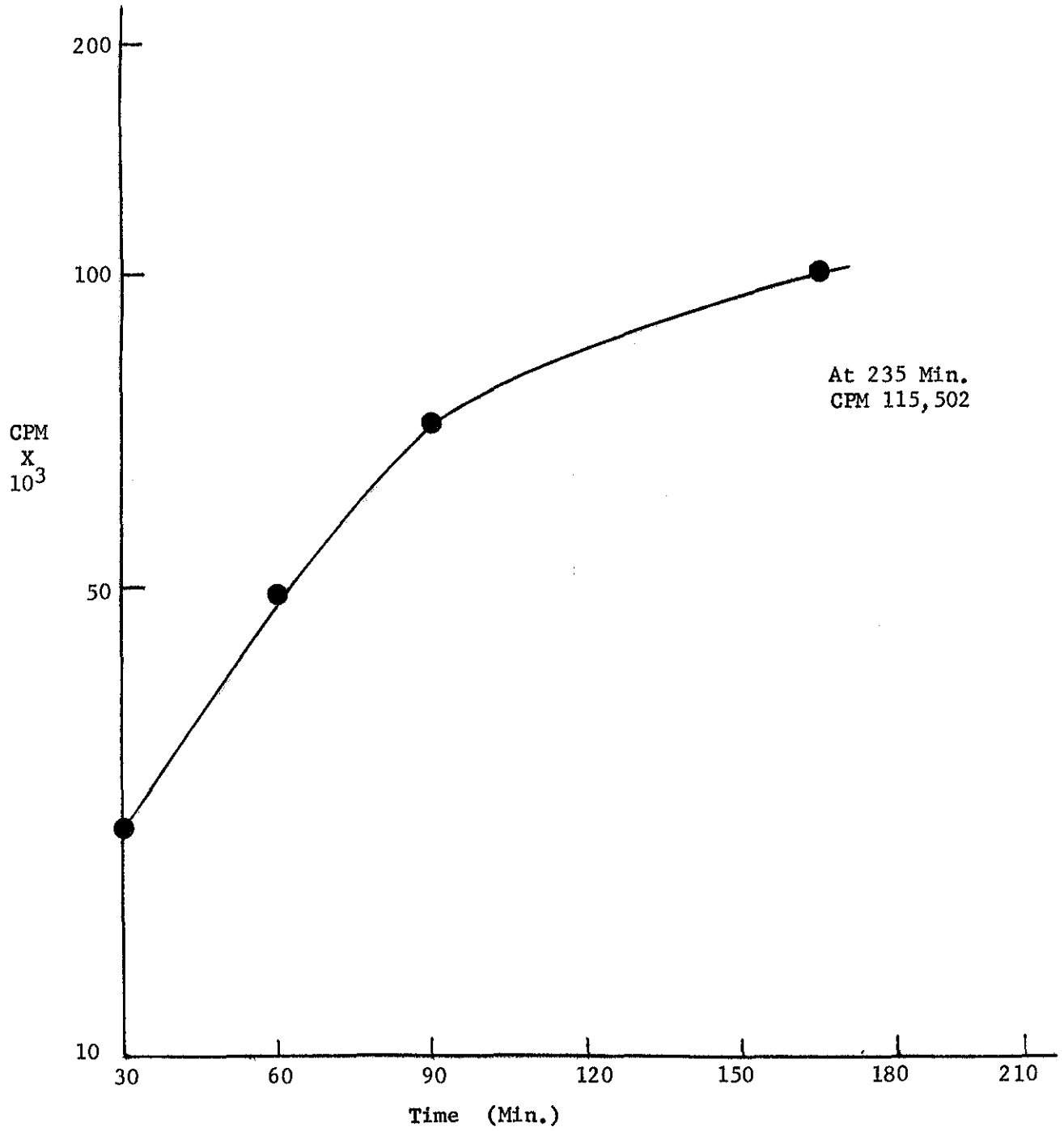
E. coli - 7 Hours
Nutrient and Carrier Added



GRAPH #2

Pond Water

10 ml Conc. Pond Water
0.8 ml Stock C^{14}-Leucine
No Carrier or Added Nutrient



GRAPH #3

E. Coli
22 Hrs.
No Carrier
Added Nutrient