TECHNICAL REPORT NUMBER 191

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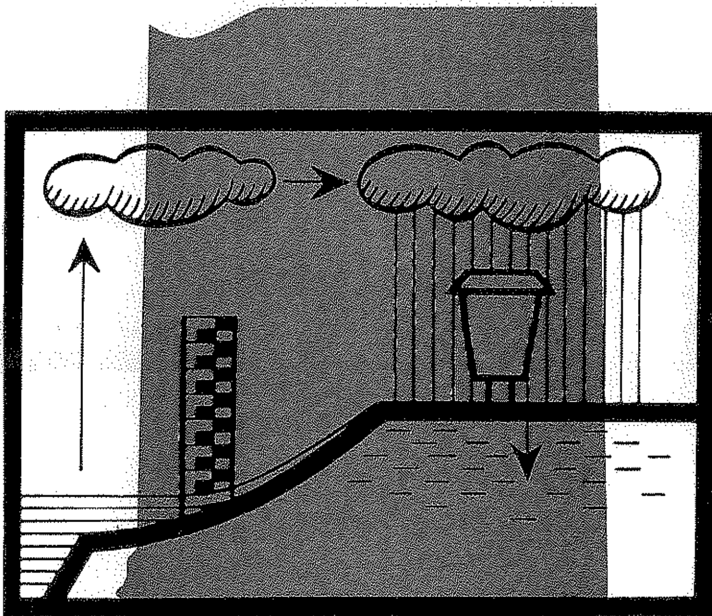
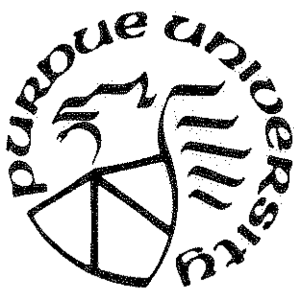


by

Ronald F. Turco

October 1990



PURDUE UNIVERSITY
WATER RESOURCES RESEARCH CENTER
WEST LAFAYETTE, INDIANA

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CHANGES IN STRUCTURE AND ADSORPTIVE BEHAVIOR OF DISSOLVED FULVIC ACID MEDIATED BY GROUND WATER MICROORGANISMS

by

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ABSTRACT

Degradation of a ground water supply can follow the introduction of xenobiotics onto the soil surface overlying the aquifer. It is clear that dissolved organic matter (DOM) or fulvic acid (FA), are present in aquifers and surface waters. The degree to which the DOM can enhance the transport of either organic chemicals or metals is unclear. This altered solubility may be significant enough to allow rapid movement of otherwise low solubility chemicals through soil to ground water or within an aquifer.

The microbial population is a remedial force in controlling the fate of pesticides. The size and status of the microbial biomass, or population, in either soil or ground water is a direct reflection of the supply of available nutrients. Of the necessary nutrients, carbon is often most limiting. Fulvic acid may serve as one carbon source for ground water resident bacteria. How the microbial populations use and change the available carbon (FA) impacts both the population size and reactivity of the carbon macro-molecule. The reactivity of the fulvic acid is a reflection of the degree of utilization by the population.

Our efforts are aimed at understanding the significance of the DOM in controlling both the size of the microbial biomass and the type of bacteria making up the biomass.

The objectives of the research conducted during the year were the following:

1. Isolate a population of bacteria capable of utilizing fulvic acid and measure microbial growth.
2. Define the structural changes in fulvic acid when it serves as a carbon and energy source for microbial growth in flow-through culture systems.

Organic materials were recovered from either soil or ground water. Fulvic acid was collected by passing ground water over a XAD-8 resin. In order to recover 760 mg of FA, 152 liters of ground water were extracted. Water was collected from a monitoring well located at the Purdue Water Quality Field Station.

A continuous flow culture system was established and fed a low concentration of the recovered FA. The system was inoculated with sub-surface sediments collected when the monitoring well was installed. The fulvic acids were characterized using Fourier Transformed Infrared spectroscopy (FTIR). The size of the fractions were determined using gel permeation chromatography.

A microbial population (> 1×10^6 cells ml^{-1}) capable of using FA as a sole carbon source developed following inoculation. The population appears to be made up of about seven morphological types and is capable of using a wide array of other carbon sources including phenol, glucose and gelatin. Gel permeation chromatography indicates the population reduces the size of the polymeric FA to that of a monomeric compound. FTIR indicated a configuration of FA typical of other reported FA structures. Utilization by the microbial population reduced the amount of carboxyl materials present.

INTRODUCTION

When biological samples are taken from soils and sediments at increasing depth, microbial activity is lower when compared to the surface. The exception is when the sampling hole intersects a saturated, water bearing zone (Beloin et al., 1988). Total number of bacteria in deeper sediments and aquifers has been shown to vary from zero to $1 * 10^9$ cells ml^{-1} (or gr solid) Ghiorse and Wilson, 1988). Water bearing zones compose the most microbially active regions within the deeper parts of the profile.

The majority of ground water bacteria are non-mobile in that they are attached to particle surfaces (Ghiorse and Wilson, 1988). Estimations of ground water microbial activity based solely on free swimming organisms will underestimate total activity (Harvey et al., 1984). The microbial activity in an aquifer is an integrated function of oxygen content and concentration of dissolved organic matter (DOM).

The origin of DOM in ground water is thought to be by one of two pathways. One hypothesis contends that soil overlying the aquifer's recharge area serves as the primary source. A second hypothesis maintains that materials are leached from kerogen, polymerized organic matter found in

sedimentary materials of the aquifer (Thurman, 1985). Data presented by Nissenbaum (1973) showed that ground water carbon is generally from terrestrial, not aquatic sources. The content of carbon in ground water is variable. Correlation of carbon content with physical factors such as depth to ground water have been poor (Leenheer et al., 1974). The median concentration of organic carbon is thought to be less than 1 mg C l^{-1} for most aquifers (Leenheer et al., 1974; Feder and Lee, 1981) and in the range of 0.7 mg C l^{-1} for sand and gravel aquifers typical of Indiana. This contrasts with the 20 mg l^{-1} carbon content common to soil water in the A horizon.

Ground water carbon like surface water carbon is apportioned primarily between humic acid (HA) and fulvic acid (FA). The relative distribution of fulvic or humic materials reflects the parent source, resident time and biologic activity of the aquifer (Ghiorse and Wilson, 1988). Wallis and Ladd (1983) showed the DOM of a aquifer to consist of 20% humic, 6% carbohydrate, 4% tannins and 68% fulvic acid. Humics found in ground water appears to contain greater amounts of carbon and hydrogen but less oxygen then FA or HA from surface waters. Most of the oxygen in ground water organics is found in carboxyl groups (Thurman and Malcolm, 1981). Thurman et al. (1982) concluded that ground water humic acids are larger, older and more humified (reacted), than fulvic acids. However, little work on the physical makeup of ground water HA or FA as affected by microbial transformation has been conducted.

Evidence indicates that ground water humic substances provide the primary source of carbon and energy for resident microbial population. This is supported by findings that the carbohydrate content of ground water is lower than surface waters, implying utilization by the microbial population during transport in the aquifer (Thurman, 1985). Work by Sederholm et al. (1973) demonstrated that total growth of bacteria in lake water was correlated to concentration of DOM. Other studies have shown a positive correlation between DOM and microbial biomass in ground water (Wallis and Ladd, 1983; Tranvik, 1988). Using dilution culture, Tranvik (1988), concluded that about 9.5% of a DOM pool was consumed by ground water bacteria in a 160 h period. Growth efficiency was placed at 0.26 g biomass g^{-1} DOC. Bacterial isolates taken from lake waters have been shown to directly metabolize

fulvic acid (de Haan, 1974). It is suggested from this study that FA serves as a source of chelated metals as well as carbon.

Numerous studies have indicated that low concentrations of DOM can significantly increase the solubility of organic materials, particularly DDT and PCB (Chiou et al. 1986; Caron et al. 1985; Gschwend and Wu, 1985; Carter et al. 1982). Means and Wiyajaratne (1982) evaluated the adsorption of atrazine to DOM and concluded that a significant increase in the apparent solubility occurred following binding. Few attempts to evaluate functional structure of FA and adsorption have been made.

Chiou et al. (1986) reported that the solubility of DDT was enhanced in the presence of both HA and FA. They reported the most significant effects of a variety of FA material were on very low solubility materials-DDT and 2,4,5,2',5'-PCB. Moreover, enhanced water solubility was correlated with size as well as chemical makeup of the FA. FA that contained larger, less polar materials was better able to enhance solubility. The polar nature of the materials was directly correlated with a decreased oxygen content in the FA. No efforts to equate changes in structure related to microbial utilization of FA and adsorption were conducted.

It is clear that few efforts to understand the relationship between resident aquifer microorganisms and turnover of carbon have been undertaken. Concurrent to this is a lack of information on the function of DOM-fulvic acid in the ground water transport of environmental pollutants. A fundamental investigation of factors that mediate the reactivity of dissolved organic matter will define these relationships. It is our intention to address how the reactivity of FA is regulated in the aquifer environment.

MATERIALS AND METHODS

Fulvic Acid Recovery from Ground Water

Ground water used in all studies was collected from a 26 m monitoring well located east of Purdue Agronomy Research Center. The well was placed in an unconfined sand and gravel aquifer in November of 1988. The well is screened over the bottom 5 m. Water was recovered using a tyfleon

hand pump. The collected water was passed through a silver membrane filter (0.45 mm) and acidified to pH 2. The acidified water was passed over a XAD-8 resin. The resin was back eluted with 0.1 N NaOH and the recovered sample again acidified. Humic acid was removed by acidifying to a pH of 1 and centrifuging. The pH of the supernate was adjusted to 2 and fulvic acid refined.

Fulvic acid was refined by passing it over a second XAD-8 resin column. The trapped fulvic acid was desalted with distilled D-H_20 and recovered by back elution with 0.1 N NaOH. The fulvic acid was concentrated by freeze drying. A total of 152 l of well water was extracted. Yield of fulvic acid was 760 mg.

Soil fulvic and humic acid was recovered from a site located adjacent to the wellhead. Soil (10g) was placed into 200 ml propylene bottles and 100 ml of 0.5 N NaOH added. The bottle was purged with N_2 gas and shaken overnight. The humic and fulvic acids were separated from the humic by centrifugation. Humic acid was separated from fulvic acid following acidification (pH 2) of the alkaline extract. The coagulate humic acid was recovered while fulvic acid remained in solution. The fulvic acid was purified as before; humic acid was purified by repeatedly washing in a HCl-HF mixture and freeze drying. Recovery of fulvic acid and humic acid was 0.55 and 125 mg g^{-1} soil, respectively. Purified fulvic or humic acid was used in all studies.

Pure Culture Selection and Enumeration

A microbial population capable of using fulvic acid as a soil carbon source was isolated from aquifer sediments collected from the monitoring well installation. Because of limited quantities of ground water FA, surface soil FA was used to develop the population. Semi-continuous cultures of filter-sterilized dilute XBM were supplemented with either 50 or 100 mg fulvic acid per liter and maintained at turnover rate of 15 or 60 days. Sediments were added to the vessels and the system incubated at 23#C. Starting at day 42, samples were collected and accumulated for one week intervals. With these pooled samples, the populations were enumerated and the size distribution of the carbon fractions determined.

Populations were enumerated by either acridine direct counts (AODC) or viable plating. For AODC, 4 ml of dilute cell suspension was passed through a black, 0.2 mm filter. The filter was washed with sterile water and 0.3 ml of 4% acridine organic solution passed through. Several fields were then counted. Viable plating was conducted on four carbon sources: Phenol 1.5 mM, Glucose 8 mM, Lactate 16 mM and Gelatin 0.5% added to dilute XBM. A complex medium (API) was also tested.

Changes in the size fractions of the FA were followed using sephadex G-50. The columns were prepared by first swelling the beads in 100 mM phosphate buffer in the cold for 2 days. The column was poured and maintained with a 25 mM sodium borate buffer (pH 7.5). A total volume of 23.5 ml with a void volume of 8.4 ml was established. Void volume was determined with blue dextran. The recovered chemostat material was lypholized and resuspended in a small volume of running buffer. To load the column, the phosphate buffer was eluted to expose the beads and 0.2 mls of the concentrated materials applied. Approximately 1 ml fractions were collected. The concentration of organic materials was estimated by measuring absorbance at 200 nm. Changes in FA structure were estimated by comparing metabolized to non-metabolized (stock) materials. The materials were freeze dried and the residue was used to form KBr pellets, for FTIR spectrometric analysis.

Subsequent to the chemostat studies, batch cultures were established using the chemostat as the inoculum source. The batch culture was supplied with 100 mg l^{-1} FA. Growth from the starting point was monitored with AODC. At the end of 7 days the material was concentrated as before and applied to a G-50 sephadex column. The fractions were collected and those showing similar absorbance bulked together.

Approximately 300 mg of stabilized KBr was mixed with 1 mg of the fulvic acid fraction. The materials were mixed and transferred to a pellet die. Pressure (7 tons) was applied and after 20 mins the resulting pellet recovered. Spectra were recorded on a Perkin and Elmer 1600 infrared spectrophotometer at a resolution of 2 cm^{-1}. In most cases 16 scans were averaged for each spectrum. Build up of CO_2 (2350 cm^{-1}) was evident in most samples and was noncompensated. Spectrum were also recorded for soil and ground water FA using a similar approach.

RESULTS

Characterization of FA

Figure 1 shows the 2000-800 cm^{-1} region of the FTIR spectrum of FA recovered from either surface soil or ground water. While the overall intensity of absorbance differs for the two FA materials, general absorbance patterns are similar. In both samples, the region from 3000 to 1800 cm^{-1} provides little information. However, absorbance at 3400 shows O-H stretching and small amounts of absorbance at 2900 cm^{-1} indicate some aliphatic C-H stretching. Both samples have bands at 1725 cm^{-1} indicating C=0 stretching of COOH. However, subsurface FA had a more defined band. A band at 1620-1625 cm^{-1} is seen in the surface FA and indicates aromatic C=C or H bonded C=0. This band is missing in the subsurface FA. Both samples showed a band at about 1400 cm^{-1} and indicates the presence of phenolic OH. In the subsurface FA many small bands appear between 1625 and 1500 cm^{-1}. This is not the case in the surface FA. The surface FA spectrum tends to imply a stronger aromatic nature than the subsurface FA. The band at 1200 cm^{-1} on the surface FA indicates C-0 stretching. This band is absent in the subsurface material. A strong band at 1100 cm^{-1} in the subsurface FA implies some polysaccharite nature in the material. A small band in the surface FA is also indicated at 1200 cm^{-1}. Bands below 800 cm were not considered.

Microbial Growth on FA

Introduction of subsurface materials provided a population of bacteria capable of using dissolved FA as a carbon and energy source. Chemostats at a dilution rate of either 15 or 60 days developed total populations (AODC) of about 1 x 10^6 cell ml^{-1}. The total population was constant over the length of the experiment. Changes in the population of gelatin degraders were similar magnitude to change in the total population. However, populations of glucose or phenol degraders fluctuated radically. Declines or increases in population for the glucose and phenol degraders were typically in excess of one log unit. Changes in AODC or gelatin degraders were typically less than 0.2 log units.

These changes may reflect the build-up and use of glucose or phenolic materials. Gelatin has a more complex structure-possibly resembling the complex nature of the FA. Hence, as part A of the

population utilizes the FA, a release of phenol or glucose-like materials utilizable by part B of the population occurs. Part A and B of the population are tied together until after day 48. Part B declines when part A stabilized but increases with part A. This trend occurs in the 15-day system. But is not as apparent. What is also apparent in the 15-day systems, is that the build-up of phenol degraders is limited. Perhaps the phenol or phenol-like material does not build-up under this faster turnover and limits this portion of the population.

When a batch culture containing FA was inoculated with a portion of the population from the chemostat, a population in excess of 1×10^7 cells ml^{-1} developed. The doubling time of the population was about 4 hours.

Fractionation of Chemostat Products

Initial fractionation of the FA in either the 15 or 60 day continuous culture revealed one major peak atabout fraction 15 (Figure 2). After 40 days in either the 15 or 60 day system, no differences from the stock were seen. However by day 42 a slight increase at fraction 22 had occurred. By day 100 a major shift to fractions 22-28 had occurred. By day 160, this fraction made up a majority of the recovered materials.

When the batch culture inoculated with materials from the chemostat was fractionated, the major new fraction was formed after 7 days.

Characterization of Fractions

FTIR patterns for the surface FA, fraction 17-19 and fraction 22-26 are shown in Figure 6. These materials were from 150 day old incubation. Differences between the fractions and the starting material exist. Utilization of the FA resulted in reduction loss of the band at 3300-3400. This region has been associated with O-H stretching. These differences may reflect clean-up given the materials by the sephadex column as well as microbial utilization. A major loss in absorbance occurs at bands 1725-1720 cm^{-1} and implies a loss of C=0 from COOH groups. Below 1600 cm^{-1} absorbance in both fractions is enhanced as compared to the starting material. The presence of aliphatic C-H is indicated by bands at 1460-1450. Bands at 1390 cm^{-1} imply the presence of C-OH groups. Bands at 1170-950

imply C-O stretching common with a polysaccharite like substance. Clearly, microbial utilization has changed the macromolecule.

DISCUSSION

When supplied with concentrations of FA, microorganism typical to subsurface sediments can utilize that material for carbon and energy. FA isolated from surface soils appears to be fairly similar to subsurface materials. It is apparent that the process of microbial utilization starts with the large macromolecule and breaks it into smaller, but similar subsections. A basal portion of the population appears to carry out this conversion, with glucose and phenol like materials possibly released. Populations capable of utilizing the secondary materials may be responding to their build-up.

The breakdown of FA results in one major new structure being formed. This is the case at slow or high turnover rates. The two structures or fractions appear to be similar in structure but have a reduced carboxly content as compared to surface FA. The structure of the reacted materials appear to be more FTIR active as a more complex pattern was observed. Schnitzer and Khan (1972) showed FA to consist of phenolic and benezenecarboxylic acids held together by H-bonding. Our work shows the microbial utilization of FA reduces the COOH and OH content of the structure. It is apparent that the bacteria feed on the material by breaking the H-bond and feeding on the COOH group. Moreover, the batch culture work shows the population to reach a maximum level after only 90 hours. This implies that product formation is or can be rapid in both the batch and chemostat conditions. The utilization is not extensive as a major portion of the structure remains.

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FIGURE CAPTIONS

Figure 1. FTIR spectra (upper) of surface soil FA and (lower) groundwater FA.

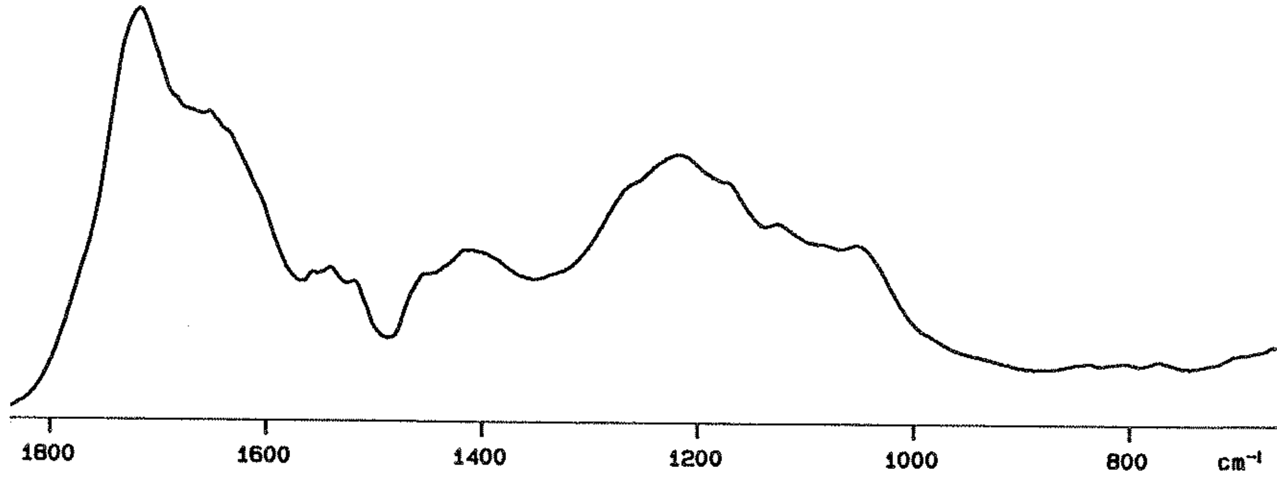
Figure 2. Growth of a mixed microbial population or FA as a sole carbon source complete turnover of the system occurred every 15 days.

Figure 3. Growth of a mixed microbial population or FA as a sole carbon source complete turnover of the system occurred every 60 days.

Figure 4. Fractionation of FA growth solution before and after growth of population complete turnover of the system in 15 days.

Figure 5. Fractionation of FA growth solution before and after growth of population complete turnover of the system in 60 days.

Figure 6. FTIR spectra of (upper) surface FA, (middle) fractions 10-20 and lower fraction 27-27 from a 150-day chemostat.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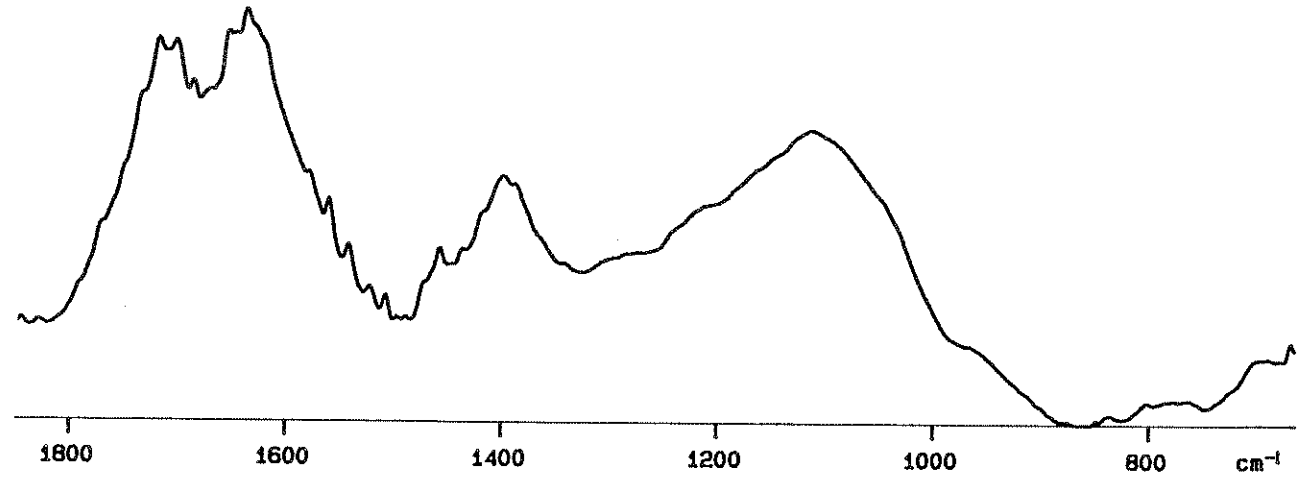
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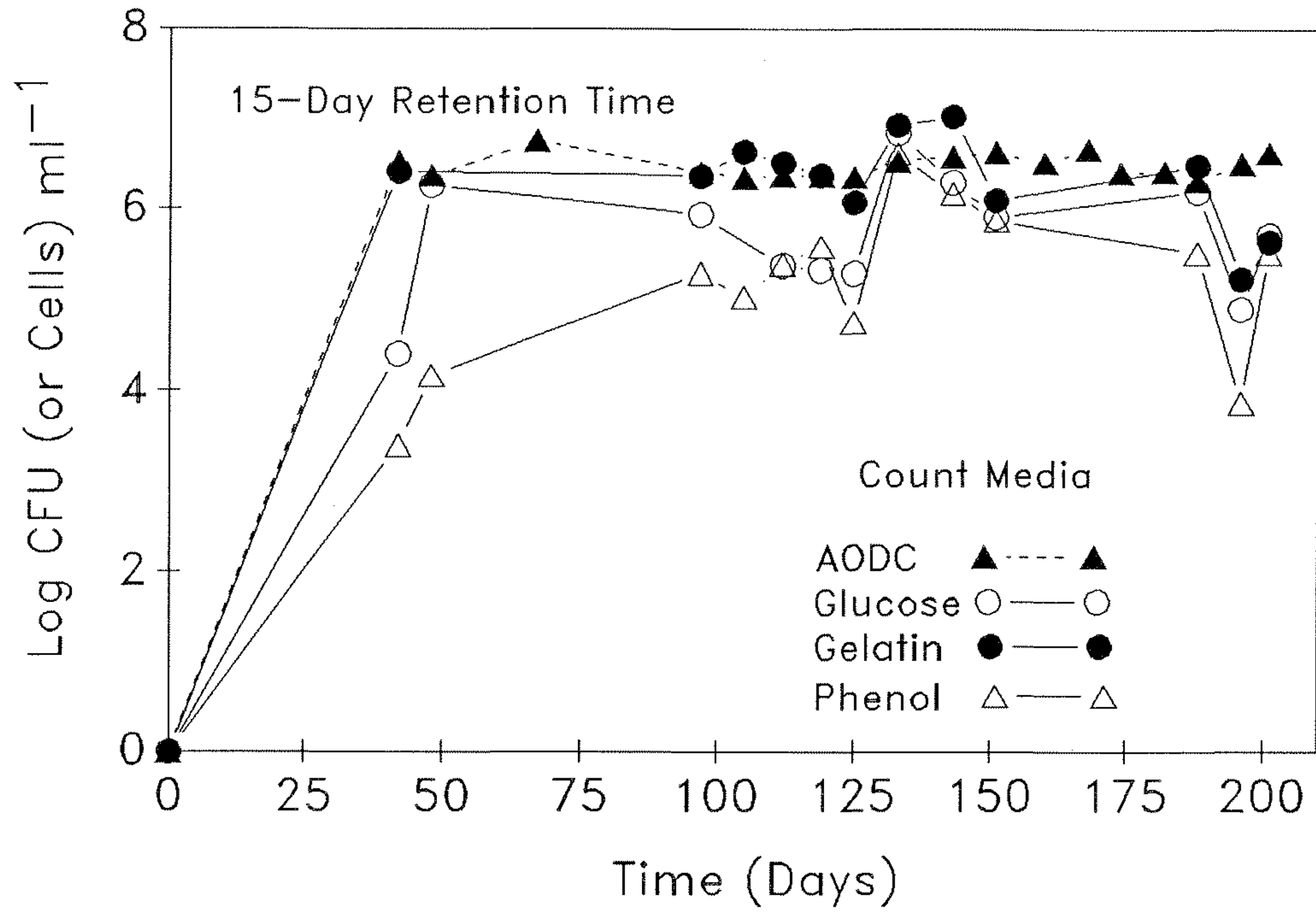
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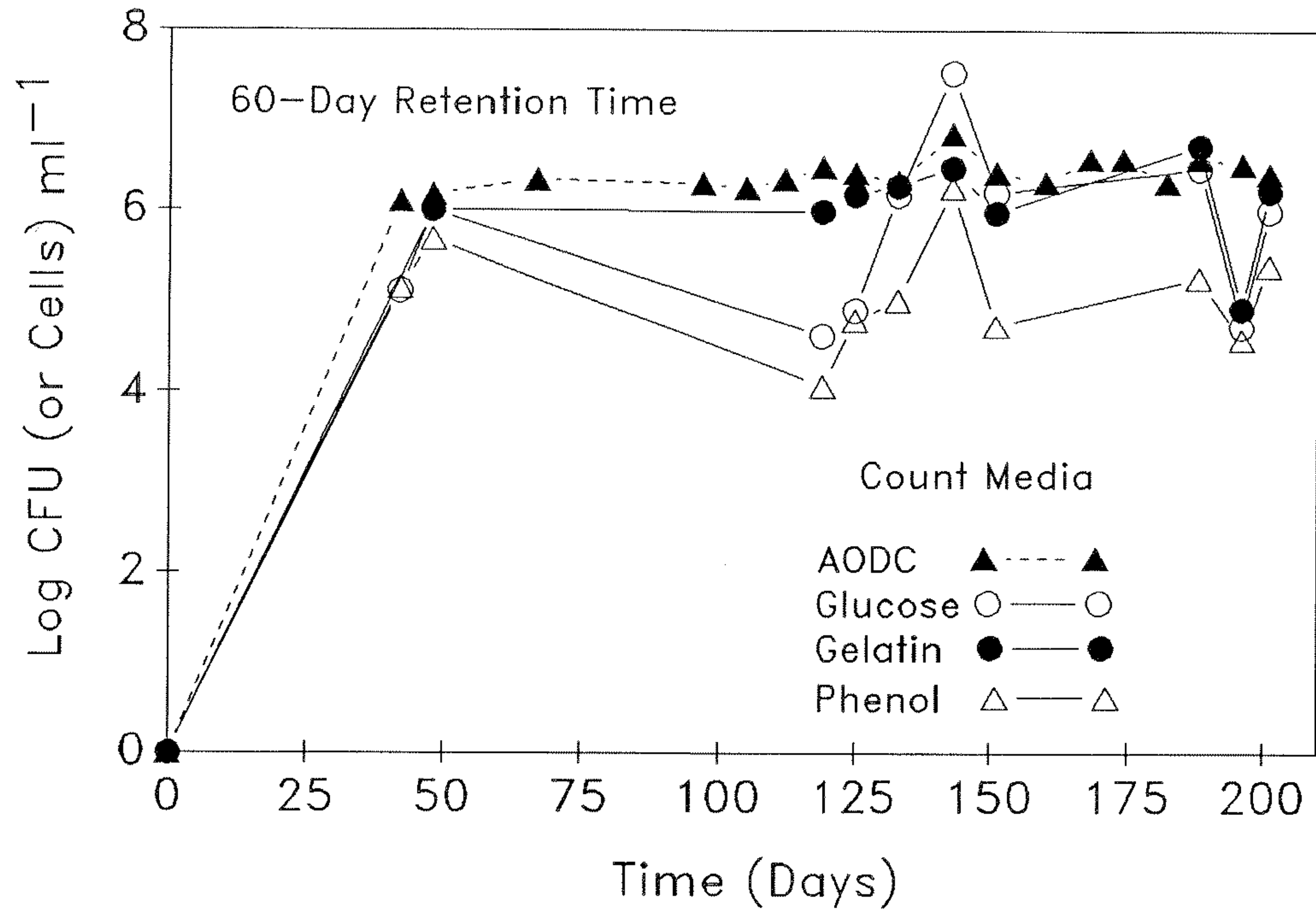
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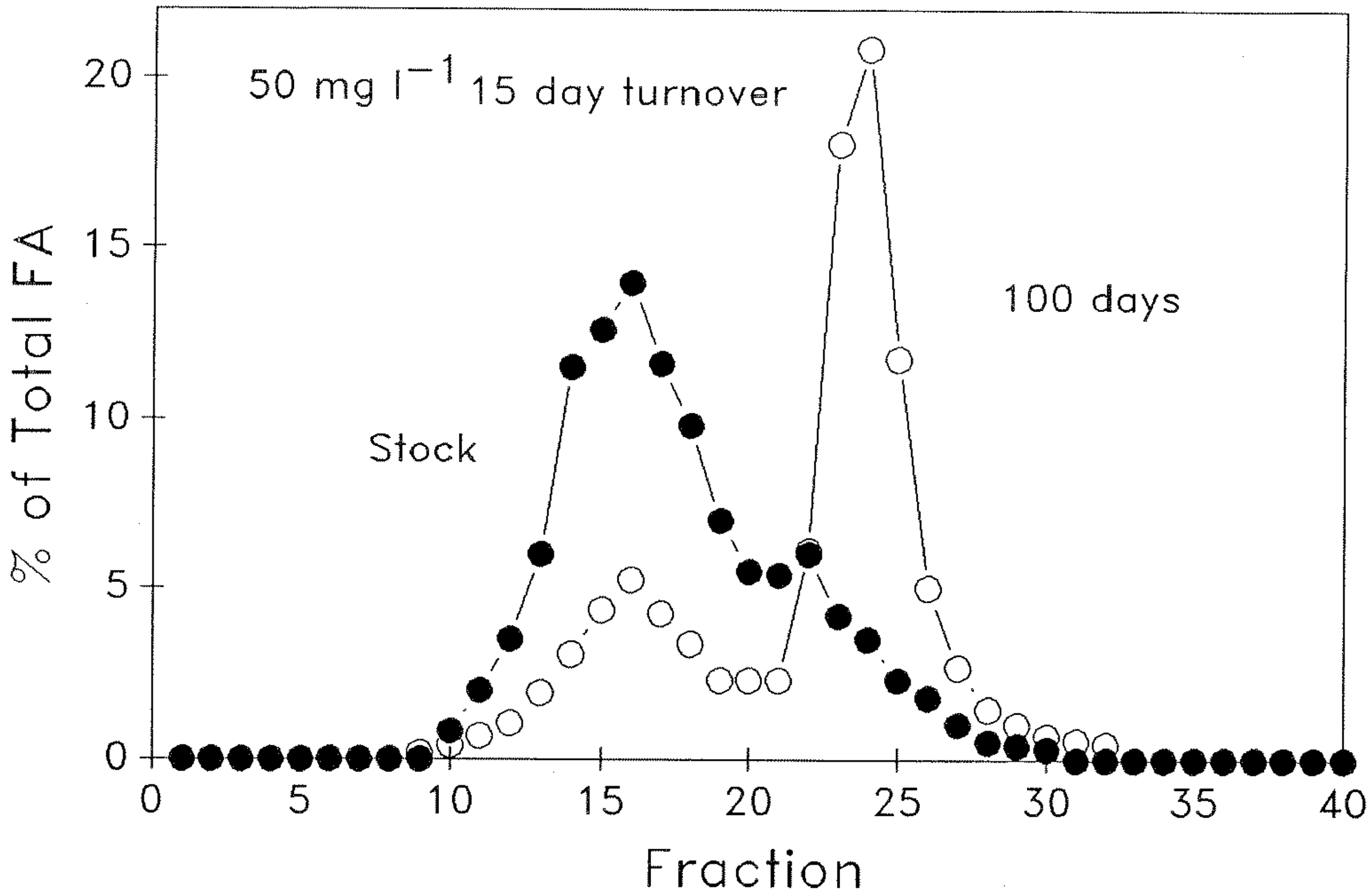
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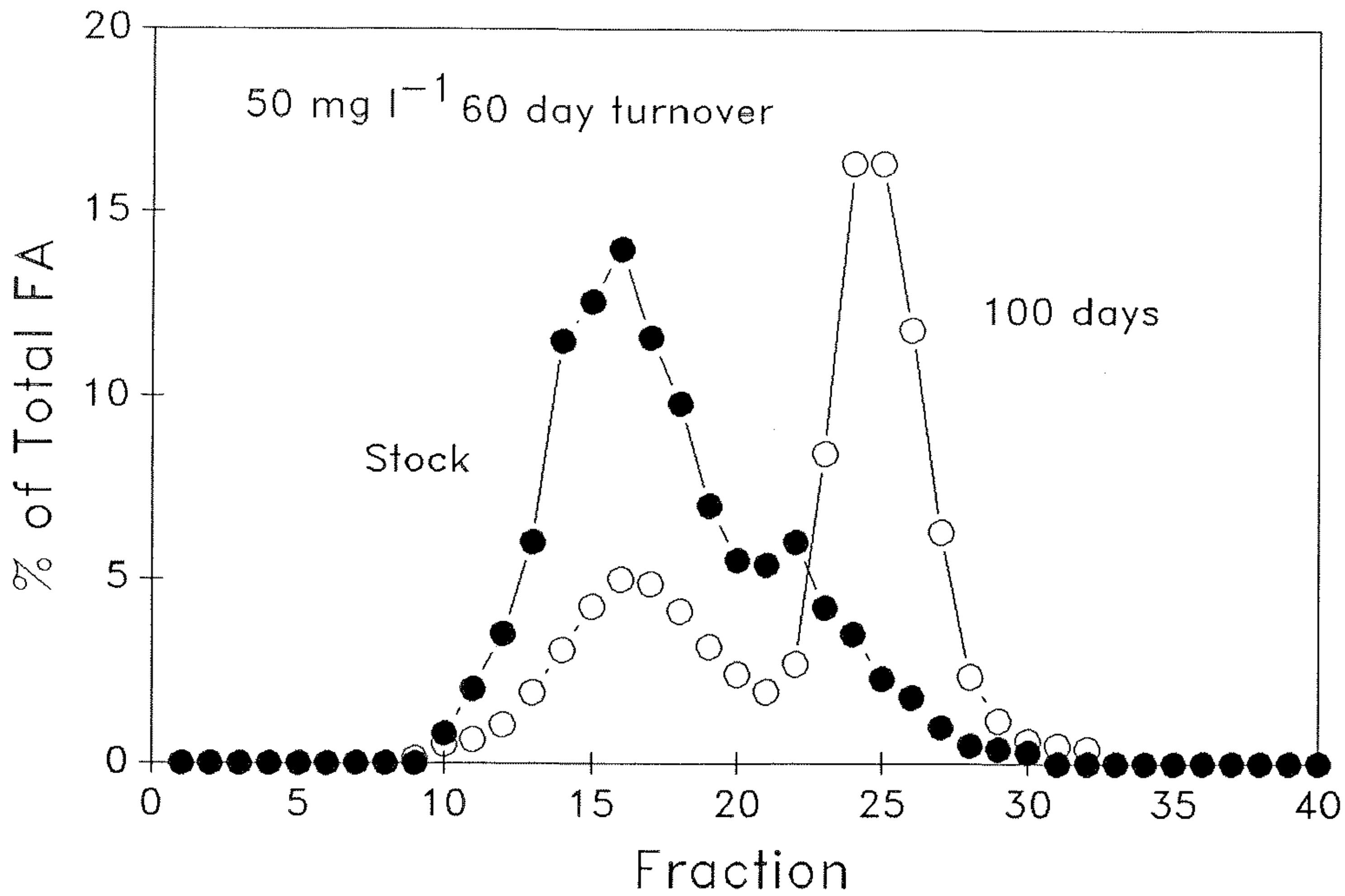
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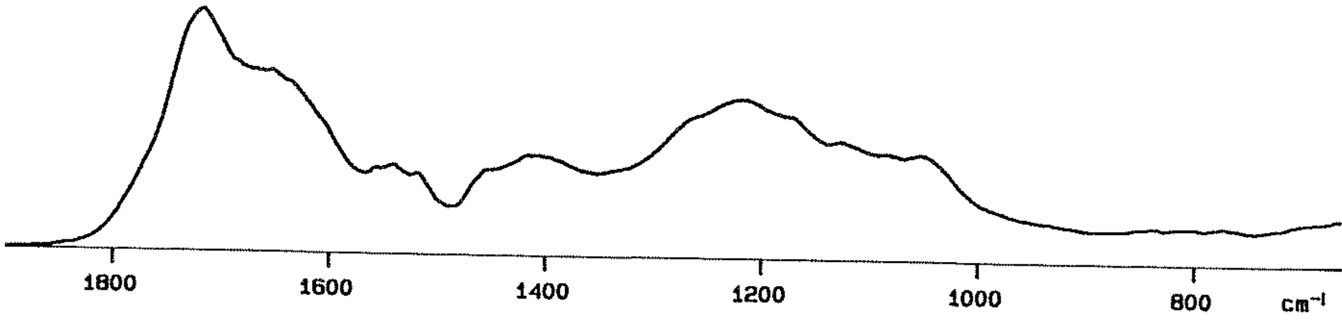
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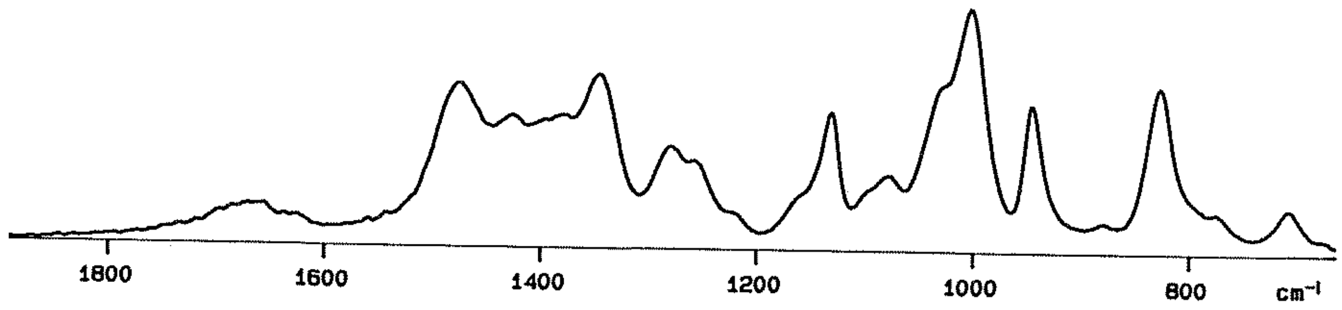
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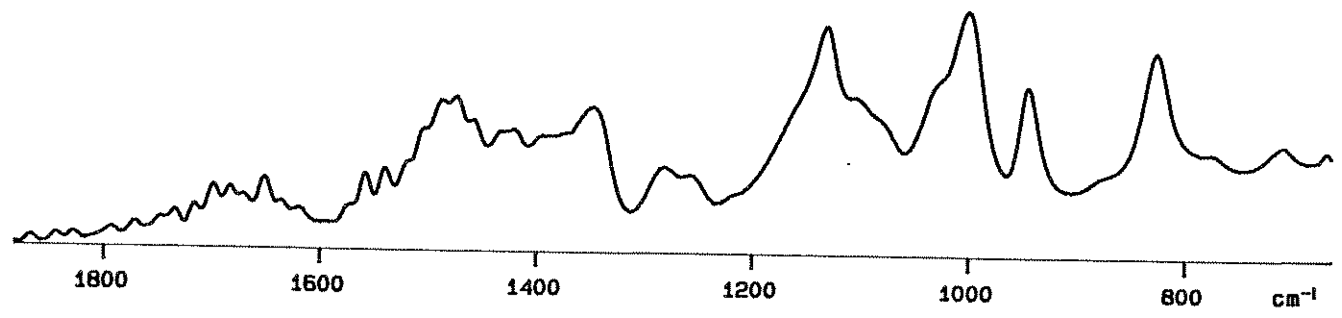
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