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BIODEGRADABILITY OF TNT: A THREE-YEAR PILOT PLANT STUDY

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The results of a three-year pilot plant study concerning the biodegradability of aqueous solutions of TNT with the 3,000-gallon NSWC-Oxidation Ditch Facility under a variety of conditions are reported. Best results were obtained with activated sludge microorganisms and supplemental nutrient. Under these conditions, TNT was 97 (±2%) bioreduced on a continual basis with the formation of a combined yield of 12 (±2%) of the corresponding two monoamine and two diamine isomers. Supportive C-14 TNT labeled studies show formation of less than

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0.4% $^{14}CO_2$ with the remainder of the C-14 activity about equally distributed between the solid, or bacterial floc, and aqueous phase. The various operation parameters of the Oxidation Ditch are discussed.

SUMMARY

This report describes the results obtained in a three-year pilot plant feasibility study concerning the microbiological degradation of TNT for the removal of TNT from large volumes of water. The results of this biological approach provide the necessary data needed for making choices between this and other physical and chemical approaches for TNT removal from waste waters. The work was performed under Task Number S0400-001, Biodegradability of TNT.

Julius W. Enig

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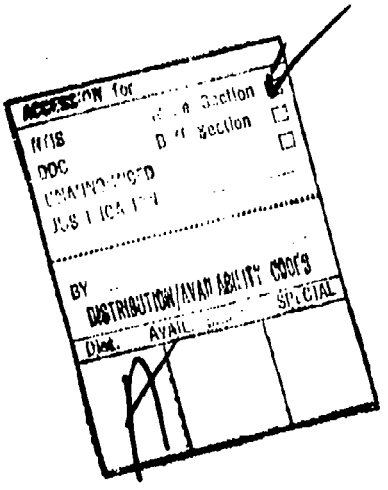

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I INTRODUCTION AND BACKGROUND

Emphasis on environmental protection has led to concern about contamination of water by explosives from military production, loading and demiling facilities. Particular attention has been paid recently to two bomb loading lines at NAD McAlester, Oklahoma. During loading and wash-down procedures large volumes of water become contaminated with explosives. Since these lines have been used mainly for loading tritonal (TNT/Al) for the past several years, the primary concern has been TNT contaminated water, and how this contamination might be eliminated economically and efficiently. The practice in the past has been to direct these waste waters into natural streams.

As early as 1965, chemists and NAD McAlester noted abundant plant growth in those streams which received the TNT contaminated water. It was suspected, then later reported[1] that TNT might be undergoing biological transformation in these streams and thus supplying nutrient for plant and aquatic life. In 1968, samples of these streams at various locations and distances from the point of introduction were analyzed for TNT content by NSWC chemists. The results of these analyses showed TNT concentrations in the 19 to 38 ppm range in the stream water at the point of introduction, but a rapid decrease in TNT concentration to less than a part per trillion (10^{-12} g/ml) down-stream and past a small lagoon. This decrease in concentration was much greater than could be accounted for by dilution alone and suggested TNT disappearance by some other means.

More convincing evidence for biological interaction with TNT in these streams was obtained by the isolation of two amine isomers closely related to TNT from the McAlester streams by NSWC chemists. These isomeric compounds were shown to be (a) 4-amino-2,6-dinitrotoluene (4A), and (b) 2-amino-4,6-dinitrotoluene (2A) by comparison to independently synthesized standards[2]. Furthermore,

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1. E. P. Pardee and J. D. Hodge, "Biological Degradation of TNT", paper presented at the Convention of the American Society of Agronomists, Washington, D. C., November 1967.

2. M. E. Sitzmann, "Chemical Reduction of 2,4,6-Trinitrotoluene - Initial Products", J. Chem. & Eng. Data, Vol 19, No. 2, pp. 179-181, April, 1974.

both 4A and 2A were produced in the laboratory under controlled conditions by biological action on TNT with the pure bacterial strains, E. coli, Ps. fluorescens, Ps. denitrificans, and Ps. putida, supplemented with glucose and inorganic salts. These microbiological studies were carried out by Hudock[3], and the solutions were chemically analyzed at NSWC.

In March and May of 1972, Heckly isolated a number of microorganisms from various stream locations at NAD McAlester, Oklahoma, and, in particular, from those streams where TNT contamination existed. Heckly's group at the Naval Biomedical Research Laboratory (NBRL), Oakland, California, found three species of pseudomonas microorganisms (designated I, II, and Y) isolated from the McAlester streams that were particularly adapted for TNT biological interaction. Subsequently, aqueous solutions of TNT were treated with these microorganisms at NBRL and chemically analyzed at NSWC in a cooperative effort[4,5]. It was shown that although TNT is itself toxic to the microorganisms, when supplemented with additional nutrient it undergoes biotransformation (Graph 1). Aqueous TNT samples were biotransformed batch-wise and in a continuous culture system. Best results were obtained in the pH range 6 to 8 with at least a 50/1 nutrient to TNT ratio.

In 1973, Dr. Orin H. Halvorson was called to NSWC as a consultant on the design of a test pilot-scale TNT biological treatment facility to be built at NSWC. The design of the 3,000 gallon NSWC TNT Oxidation Ditch Facility (constructed in the summer and fall of 1973; schematics 1, 2 and photographs 1, 2, and 3) was based on results from several large scale (20 liter) batch experiments with TNT and activated sludge microorganisms supplemented with additional nutrients. In these large batch experiments, 20 liters of 71 ppm aqueous TNT was 93% biotransformed in 61 hours. During this experiment, 5 liters of 100 ppm aqueous TNT were added in one liter portions between the 20th and 25th hour, while at these same times, one liter of reaction mixture was removed. This was done to simulate a continuous flow system. The results

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3. G. A. Hudock (Indiana University, Department of Zoology, Bloomington, Indiana, 47401), Final Report: "Biological Effects of TNT Wastes and a Description of Means for their Detoxification and Removal", U. S. Navy Research Contract N60921-71-C0272, June 10, 1972.

4. W. D. Won and R. J. Heckly (Naval Biomedical Research Laboratory, Oakland, California, 94625), Final Report, "Biodegradation of Trinitrotoluene", June 30, 1974. (Requests for this report must be referred to the Office of Naval Research, Arlington, Virginia, 22217, Attn: Code 443).

5. W. D. Won, R. J. Heckly, D. J. Glover, and J. C. Hoffsommer, "Metabolic Disposition of 2,4,6-Trinitrotoluene", Applied Microbiology, Vol. 27, No. 3, pp. 513-516, March 1974.

of these large-scale batch experiments showed that TNT supplemented with glucose nutrient could be efficiently biotransformed by a mixed culture of microorganisms from a sewage disposal plant. The rates of TNT biotransformation with the mixed bacterial strains (activated sludge) from the sewage plant were very nearly the same as the rates for TNT biotransformation with pure pseudomonas-type organisms (isolate Y) from the TNT contaminated streams at NAD McAlester, Oklahoma (Graph 2).

The objective of this task was to find an efficient biological process for destroying TNT in waste water. The process, ideally, should produce non-toxic products, and have no adverse effects on the ecology. Furthermore, this biological approach should be viewed as a feasibility study of an alternative method for removing TNT from water in place of the more conventional adsorption (i.e., resins, charcoal) methods [6,7]. A recent report by Tatyrek[8] discussed a number of other methods for the removal of TNT from waste water, including ultra-violet radiation, ozonolysis, combination ozonolysis/ultra-violet radiation, gamma radiation, incineration, catalytic wet oxidation, and composting/soil disposal.

The NSWC TNT Oxidation Ditch Facility was in continuous operation on a test pilot-plant basis beginning in July, 1974. Analytical support was performed continually during this time to monitor TNT concentrations in feed as well as effluent waters from the Ditch. In addition, small-scale laboratory experiments, including both batch and continuous culture experiments, were conducted in support of the NSWC Oxidation Ditch Facility. This report presents our results on the biotransformation of TNT for the removal of TNT from large volumes of waste water.

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6. Technical Report: "Wastewater Treatment in the Military Explosives and Propellants Production Industry", Vol. 3, Chapter 6, October 1975, prepared by The American Defense Preparedness Association for the U. S. Environmental Protection Agency Office of Research & Development, Washington, D. C. 20410.

7. R. K. Andren, J. M. Nystrom, and R. J. Erickson, "Treatment of TNT Munitions Wastewaters Using Polymeric Adsorption Resins", Pollution Abatement Div., U.S. Army Natick Laboratories, Natick, MA 01760.

8. A. F. Tatyrek, "Treatment of TNT Munitions Wastewaters - The Current State of the Art", Technical Report 4909, Picatinny Arsenal, Dover, New Jersey, October 1976.

II RESULTS AND DISCUSSION

The NSWC Oxidation Ditch Facility was operated continually for a total of 1,038 days in an attempt to optimize the parameters for TNT bioconversion and minimize amine by-product formation. Graphs 1 and 2 provide background information for TNT biotransformation and are referred to in the Introduction and Background Section. Schematics 1 and 2 outlining the design of the NSWC Oxidation Ditch Facility and the TNT Dissolution System are also included in the Introduction and Background Section. Photographs 1, 2, and 3, referred to in the Introduction and Background Section, show the NSWC Oxidation Ditch Facility and related auxiliary equipment. Tables 1, 2, and 3 give the results of experiments performed in the NSWC Oxidation Ditch Facility, and table 4 summarizes the small-scale laboratory studies for TNT bioconversion. In addition, tables 1 through 4 give detailed results of the individual experiments performed by referring to tables 5 through 14, graphs 3 through 12, and schematics 3 through 7.

In general, there were 4 continuous TNT runs totaling 564 days, Table 2. All four runs involved continuous TNT feed into the Ditch, while the cornsteep nutrient was continuously added together with TNT for runs 1 and 2, but added batch-wise for runs 3 and 4. The rate of TNT addition to the Ditch was approximately 8 liters/min (2 gal/min) for runs 1, 2, and 3, but only 0.25 liters/min for the extended TNT residence time experiment, run 4.

The results show an average TNT bioconversion of 97 $\pm$ 2% and an average 4-A formation of 9.5 $\pm$ 1.5% for all four runs, Table 2. The somewhat lower TNT bioconversion for run 3 appears to be more a function of the lower nutrient/TNT ratio than to any difference in the method of the nutrient addition, i.e., continuous vs. batch-wise addition.

In addition to the 4-A formation, three other amines have been identified; 2-A, 2,4-diA, and 2,6-diA. The average composition of the amine mixture for run 2, Table 2, was found to be: 4-A (80.5%), 2-A (8.3%), 2,4-diA (9.8%), and 2,6-diA (1.1%). From these data, the relative amounts (weight basis) were calculated to be: 4-A/2,4-diA/2-A/2,6-diA = 1.00/0.10/0.12/0.013. The average ratio of 4-A/2-A for runs 1, 3, and 4 was found to be 8.4 $\pm$ 1.0. These results suggest a similar biological process for TNT bioconversion and amine formation for all four runs. Since 4-A is 80.5% of the total amine mixture, the total yield of amines for runs 1 through 4, Table 2, can be estimated as 15%, 9.9%, 12%, and 9.9%, respectively, for an average of 12 $\pm$ 2% total amines.

Graphs 3 to 8 show the TNT feed and effluent concentrations with time for the four TNT runs, Table 2. Graph 9 shows the theoretical TNT dilution curves at 8 liters/min and 0.25 liters/min as a function of influent TNT concentration and time assuming that no bioconversion takes place and that the effluent rate is the same as the influent rate. Graph 3 shows the TNT concentration in the feed and effluent and also the 2-A and 4-A concentrations in the floc and effluent (89% of total amine mixture) for run 1. Although the bioconversion of TNT appears to be quite complex, several observations

may be made from these plots: (1) Changes in TNT feed are reflected by similar changes in amine formation, although this amine change may be offset by 3 to 5 days after the TNT change; (2) The concentration of amines in the floc is, in general, higher than in the effluent, Graph 3; (3) While 4-A is the most abundant and 2,6-diA is the least abundant of the total amines formed, the relative amounts of 2,4-diA and 2-A vary with time in a complicated manner, run 2, Graph 6; (4) Increasing the residence time of the TNT in the Ditch by a factor of 32 (0.25 liters/min compared to 8.0 liters/min) does not eliminate the 4-A in the effluent, run 4, Graph 8. In fact, the 4-A concentration is about the same as that observed in runs 1, 2, and 3. This is somewhat surprising when one considers the Batch studies for the disappearance of the amine intermediates, Table 4, experiment 2, Graphs 11 and 12.

The persistence of 4-A in the effluent for run 4, Table 2 and Graph 8, may be partially explained from the data for the slow disappearance of total amines from the floc, Tables 3 (experiments 1 and 2) and 5, and from the data for the corresponding slow disappearance of total amines from the effluent, Graph 10. During these experiments, no TNT was added to the Ditch, while tap water and cornsteep nutrient were added at 8 liters/min. From these observations, the following is offered as one possible explanation. TNT is adsorbed into the bacterial floc and is bioreduced to a mixture of amines, whereupon the bacteria die (Graph 1) encasing the amines. Next, the amines are slowly extracted into the water as this cell encasement ruptures or is degraded by other microorganisms. Support for this explanation is also found from the data shown in Table 3 (experiments 4, and 5), and Tables 7 and 8. More 2, 4-diA and 2,6-diA were found in the floc than in the effluent (Table 7), while Graph 11 shows that 4-A is converted to 2,4-diA only, but that 2-A is converted to a mixture of 2,4-diA and 2,6-diA. Furthermore, the data from Table 3 (experiment 5) and Table 8 show three to four times as much TNT and amines in the floc as on the floc with a substantially greater amount of amines in the floc. However, the data from Table 8 show no accumulative build-up of either TNT or amines in or on the floc. This non-accumulation of amines in the floc is also shown graphically for TNT run 1, Table 2, Graph 3.

The four amines accounted for only about 12 $\pm$ 2% by weight of the TNT feed on a continuous operation basis, Table 2, runs 1 to 4; however, C-14 labeled TNT batch bioconversion studies allowed a material balance to be made for the total distribution of C-14 activity after TNT bioconversion, Table 4 (experiment 1) and schematics 1 and 2. After 3 days, the C-14 activity was about equally distributed between bacterial floc and supernate or aqueous phase. Of the C-14 activity in the aqueous phase, about half (26.6% total C-14 activity) was accounted for by 4-A, 2-A, 2,4-diA, and 2,6-diA (25.9% by chemical analysis). In the floc, however, only about 8% of the total C-14 activity was accounted for by these amines both in and on the floc. The formation of 34% amines (26%

in supernate, 8% in floc) in this C-14 batch study was consistent with the 21% yield of amines (supernate) in the batch TNT bioconversion after three days, Graph 1.

After 30 days, the C-14 activity decreased in the supernate (aqueous) phase, but increased in the floc, schematic 2. The yield of amines in the aqueous phase has been drastically reduced to <0.28% (from 25.9%, schematic 1) while the amines in the floc were reduced to 2.8% (from 8%, schematic 1). These data are consistent with the disappearance of 4-A, 2-A, and 2,4-diA, and 2,6-diA in the batch experiments outlined in Table 4 (experiment 2) and shown in Graphs 11 and 12. Comparing C-14 activity distribution for schematics 1 (3 days) and 2 (30 days), both experiments show less than 0.4% $^{14}CO_2$ formation, a decrease in total amines from 34% to 3%, an increase in C-14 activity in the floc from 40% to 64%, and a slight increase in C-14 activity in the water soluble phase after benzene extraction from 25% to 33%. Also, less than 0.005 ppm of the tetranitro azoxytoluenes were found in the floc after 30 days. The absence of these compounds was shown by acetone extraction of the floc followed by thin-layer chromatographic and C-14 counting techniques. In both the C-14 labeled TNT experiments shown in schematics 1 and 2, the starting TNT concentration was approximately 20 ppm.

These data are consistent with the stepwise bioreduction of TNT to complex water soluble and insoluble products, as shown in (1),

(1) $Tol(NO_2)_3 \rightarrow Tol(NO_2)_x(NHOH)_y \rightarrow Tol(NO_2)_x(NH_2)_z$

polar products + polymer,

where x = 0 to 2; y = 1 to 3, and z = 1 to 3. A similar nitro reduction process has been observed for the soil degradation of the herbicide, trifluralin (α, α, α-trifluoro-2,6-dinitro-N-N-dipropyl-p-toluidine) to form aromatic diamines and other unidentified polar compounds[9]. Although none of the hydroxylaminonitrotoluenes of the type, Tol $(NO_2)_x(NHOH)_y$, have been observed in the NSWC Oxidation Ditch Experiments, their presence is indicated by the isolation of small amounts of the coupled tetranitro azoxytoluenes (< 1 ppm) from the floc of the batch experiments shown in graph 1. These azoxynitrotoluenes are postulated to arise through chemical disproportionation[10] of the $Tol(NO_2)_x(NHOH)_y$ as shown in (2),

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9. G. W. Probst and J. B. Tepe in "Degradation of Herbicides", edited by P. C. Kearney and D. D. Kaufman, published by Marcel Dekker, Inc., New York, 255-280 (1969).

10. F. Bell, J. Kenyon, P. H. Robinson, J. Chem. Soc., 1243 (1926) given in P.A.S. Smith, "The Chemistry of Open Chain Organic Nitrogen Compounds. Vol. II", W. A. Benjamin, Inc., New York, p. 4 (1966).

(2) $Tol(NO_2)_2(NHOH)_1 \rightarrow (NO_2)_2Tol\text{-}N{=}N\text{-}Tol(NO_2)_2 + Tol(NO_2)_2(NH_2)_1 \cdot O$

Since the reaction outlined (2) is complex and is postulated[10] to involve a series of bimolecular reactions, it is not surprising that the formation of the coupled tetranitroazoxy products was quite low or was absent and depended on the initial TNT concentration. For example, the initial TNT concentration in the C-14 experiments was 19.6 ppm, and less than 0.005 ppm of the coupled products were observed, while in the batch experiments shown in Graph 1, the initial TNT concentration was 90 ppm, and still only 0.97 ppm of the four possible mixed tetranitro azoxytoluenes[11] were found in the floc. It might also be pointed out that the presence of the tetranitro azoxytoluenes was not detected in the NSWC Oxidation Ditch TNT runs, Table 2.

Complete reduction of TNT would be expected to form 2,4,6-triaminotoluene according to (1). This amine has been found to be very reactive and forms both water soluble and insoluble products[12]. Therefore, it is not surprising that the C-14 activity in the floc was found to increase after 30 days. McCormick[13] has noted the formation of similar water insoluble products from TNT bioreduction. Cellular (floc) incorporation of C-14 activity derived from biotransformation of C-14 labeled TNT has also been observed by Traxler[14]. Although not proven, this material appears to be a high molecular weight, water insoluble, polymeric-like substance.

The small-scale continuous culture apparatus experiments, Table 4 (experiments 3 and 4), indicate that cornsteep and molasses are about equally effective (weight basis) for TNT bioconversion, Table 9. In both cases the highest TNT bioconversion occured where the nutrient /TNT ratio was 100 or greater (compare runs 1 and 5 with 4 and 7, Table 9). The average yield of 4-A + 2-A was 17% for all seven runs and was quite close to the 14% yield of 4-A + 2-A for run 1, Table 2.

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11. D. A. Kubose and D. J. Glover, NSWC/WOL/TR 76-96, "Structure Identification Via Mass Spectrometry. Tetranitro Azoxy and Azotoluenes", 10 Sep 1976.

12. E. G. Kayser, unpublished results, This Center.

13. Reported by N. McCormick in the Twenty-Fifth Conference on Microbiological Deterioration of Military Material, edited by B. J. Wiley, Report: Natick/TR-77/014, p 22, U.S. Army Natick Research and Development Command, Food Sciences Laboratory, DRXNM-YPB, Natick, MA 01760.

14. R. W. Traxler (University of Rhode Island), "Biodergradation of Trinitrotoluene (Alpha TNT) and Its Production Isomers", Report No. 76-48-FSL, p. 17, U.S. Army Natick Research and Development Command, Natick, MA 01760.

Batch studies showed, in general, that TNT bioconversion was greater and amine formation less under aerobic than under anaerobic conditions, Table 4 (experiment 7), and Table 13. However, continuous culture apparatus experiments show that partially anaerobic 24 hour cycle periods (8 hours under aerobic conditions followed by 16 hours under anaerobic conditions) did not adversely affect the percentage of TNT bioconversion or increase amines formation, Table 4 (experiment 4), and Table 10. The average yield of amines (4-A + 2-A) for cyclical anaerobic/aerobic 24 hour periods for a total time of 3 weeks (for each run) was 9%. This compares quite well with the 9.5% amine (4-A + 2-A) formation average for runs 1 to 4, Table 2, for all the TNT continuous runs in the NSWC Oxidation Ditch Facility.

Batch experiments showed no temperature effect on TNT bioconversion in the 3 to 53°C temperature range, Table 4 (experiment 5) and Table 11. These results agree with the NSWC Oxidation Ditch TNT bioconversion results shown in Table 2. The times for the runs in Table 2 were as follows: (1) November - February, (2) October - June, (3) August - September, and (4) March - May. The temperatures, for these runs varied considerably.

The effect of pH on TNT bioconversion and amine formation appears to be more pronounced in the batch studies than on a continuous TNT/nutrient feed basis (compare Table 3, experiment 3, and Table 6 with Table 4, experiment 6, and Table 12). Under Ditch conditions at a pH of 6.6 $\pm$ 0.1, an average amine yield of 32 $\pm$ 5% was found over a 16 day period with 99.9% TNT bioconversion whereas, under batch conditions, a 9.4% yield of amines was found with only 45% TNT bioconversion at pH 6.0 to 6.2. At pH 7.1 to 7.8 the agreement between ditch operations and batch experiments for TNT bioconversion and amine formation was quite good: Ditch, 98.5% TNT conversion, 7% amine formation; Batch: 99% TNT conversion, 8% amine formation. The relative amounts of the four amines were similar in the pH 7.1 to 7.8 range for batch and continuous addition, whereas, more 2,4-diA was formed at pH 6.6 (Table 6) under Ditch conditions than at pH 6.0 to 6.2 (Table 12) under batch conditions. The high yield of 77% for combined 4-A + 2-A observed in the batch studies in the pH range 8.7 - 9.1 (Table 12) and especially the greater preponderance of 2-A is quite unexpected.

The biological oxygen demand (BOD) was found to be about 0.4 of the COD value given in Table 1 for both steepwater feed nutrient and Ditch effluent (footnotes a and e). These results indicate that not all of the chemically oxidizable materials in the cornsteep water can be utilized by the activated sludge microorganisms. Since the TNT is present only to the extent of from 0.2 to 2% of the amount of cornsteep nutrient present, Table 2, the rather high COD values found for Ditch effluent, Table 1, appears to reflect the inability of the sludge microorganisms to fully degrade cornsteep nutrient.

Finally, the bioconversion of TNT does not seem to be affected by the presence of RDX, Table 4 (experiment 8) and Table 14. Under identical conditions, TNT alone was found to be 99.6% bioconverted, and also 99.6% bioconverted in the presence of RDX. By contrast, RDX was not observed to be biodegraded under these conditions either alone or in the presence of TNT.

III EXPERIMENTAL

NSWC Oxidation Ditch Facility. The NSWC Oxidation Ditch for TNT biotransformation studies (schematic 4) had a volume of approximately 3,000 gallons. It was built in the form of an oval, 59 feet long, 11 feet wide, with a dug out portion 3 feet wide and 3 feet deep. A 24 inch Cherne Aerotor (Cherne Industrial Inc., Edina, Minnesota) with 8 perforated fiberglass blades, driven by a 3 horsepower electric motor via a variable speed hydraulic transmission, aerated and moved the Ditch liquid mixture. The speed of the rotor was adjusted so that the flow around the Ditch was approximately 1 foot/sec. Approximately every half hour for a period of 5 minutes a portion of the water/sludge mixture was pumped into a 350 gallon fiberglass setting tank (Photograph 1). Here the sludge (floc) was allowed to settle and the clear effluent was drained off at the top via a weir/pipe/gravity flow system and discharged into a near-by stream. The settled floc was then pumped back into the Ditch for approximately 5 minutes out of every half hour. Both the water/sludge mixture and the settled floc were pumped at a rate of about 7.5 gallons/min.

A TNT Dissolution System (schematic 2) was located between the inlet water line and the 190 gallon polyethylene tank. This system consisted of a 30 inch long and 6 inch diameter pipe packed with 15 to 20 pounds of flake TNT through which water was pumped. The TNT saturated water from the Dissolution System was then fed into the 190 gallon tank and mixed with water. Water containing TNT from this 190 gallon tank was pumped directly into the Ditch at a rate of approximately 2 gal/min. At the same time, a 50/50 mixture of water and cornsteep nutrient (Argo Steepwater, E-801, CPC International Inc., International Plaza, Englewood Cliffs, New Jersey 07632) was metered (Cole-Parmer, Masterflex, Model 7565) via a quick disconnect into the TNT-water line before being added to the Ditch as shown (schematic 4).

The rates of the addition of the 50/50 cornsteep-water mixture varied from 10 to 45 ml/min (Operation Days, 1 to 753, Table 1). There was often clogging of the input nutrient line due to water insolubles in the cornsteep-water mixture. Stirring of this mixture with a macerator was usually necessary in order to help prevent clogging of the input line. Later on, the cornsteep-water mixture was added batch-wise three times daily about four hours apart (Operation Days 753 to 1,038, Table 1).

Chemical Analysis. The TNT feed line was sampled just prior to entering the Ditch (schematic 4), while the effluent line from the settling tank was sampled just prior to entering the drain. Aqueous samples were extracted (1:1 by volume) with benzene, and analyzed with a gas chromatograph equipped with a ^{63}Ni electron capture detector[15,16]. A Hewlett-Packard Model 5750 research gas chromatograph was used with a 4 ft x 1/4 in. glass column packed with 2.95% Dexsil GC 300 on Chromosorb W AW DMCS, 80 - 100 mesh; column temperature, 175°C; injection port, 180°C; carrier gas, argon-methane (95:5), flow rate 192 ml/min; ^{63}Ni detector temperature, 290°C; pulse, 150 microsec.; attenuation, X 80. The concentrations of compounds were adjusted to give the following approximate concentrations (in grams/microliter): TNT, 2.6×10^{-10}; 4-A and 2-A, 2×10^{-9}; 2,4-diA and 2,6-diA, 4×10^{-8}. Retention times were approximately as follows: TNT, 2 min.; 4-A, 5 min.; 2-A, 6 min.; 2,4-diA, 5 min.; 2,6-diA, 6 min. (The diamines were separated chemically prior to gas chromatographic analysis[16].) Relative height responses were (gram basis): TNT/4-A/2-A/2,6-diA/2,4-diA = 1.0/0.12/0.082/0.0048/0.0036. Samples were analyzed by reference to standard solutions containing known concentrations of the authentic compounds[2,17].

NSWC Oxidation Ditch Operation Parameters. Measurements of pH were made with a Leeds and Northrup pH Meter (Cat. No. 7401) standardized against at least two buffers of known pH.

Dissolved oxygen (DO) content of the Ditch was determined with a Fieldlab Oxygen Analyzer (Beckman Model #100800).

The chemical oxygen demand (COD) of the cornsteep nutrient feed into the Ditch and effluent from the Settling Tank were determined by refluxing an appropriate volume of the sample for 2 hours with a known volume of 0.250 N standard $K_2Cr_2O_7$ in 50% sulfuric acid, and back-titrating excess $K_2Cr_2O_7$ with standard ferrous ammonium sulfate according to the procedure outlined in Standard Methods for the Examination of Water and Wastewater[18]. The COD values, Table 1,

15. J. C. Hoffsommer, "Quantitative Analysis of Nitro Compounds in the Micro- to Picogram Range by a Combination of Thin-Layer and Vapor Phase Chromatography with the Nickel-63 Electron Capture Detector", J. Chromatog., 51, 243-251 (1970).

16. D. J. Glover, J. C. Hoffsommer, D. A. Kubose, "Analysis of Mixtures of 2-Amino-4,6-Dinitrotoluene; 4-Amino-2,6-Dinitrotoluene; 2,4-Diamino-6-Nitrotoluene; and 2,6-Diamino-4-Nitrotoluene", Anal. Chem. Acta, 88, 381-384 (1977).

17. M. E. Sitzman, "Synthesis of 2,6-Diamino-4-Nitrotoluene", J. Chem. & Eng. Data, Vol. 21 No. 2, p 242 (1976).

18. Standard Methods for the Examination of Water and Wastewater, published by the American Public Health Association, Inc., 1790 Broadway, New York, N. Y. 10019, 12th Edition, Ed., H. P. Orland, pp. 510-514 (1965).

are given in ppm oxygen and provide a measure of the oxygen equivalent of that portion of the organic matter in a sample which is susceptible to oxidation by a strong chemical oxidant.

The floc levels of the Ditch, Table 1, were determined by filtering one liter of the Ditch mixture (or an appropriate smaller volume for the continuous culture apparatus) through a tared Whatman #1 filter paper and drying in an oven set at 90°C for at least two hours. To speed the filtration, it was often convenient to allow the Ditch mixture to settle and first filter the clear supernate followed by the concentrated aqueous floc. Floc settling was usually 80 to 90% complete in a half hour.

In some instances, the biological oxygen demand (BOD) values of the ditch effluent were determined (Biospherics Incorporated, Rockville, Maryland 20852) by the procedure outlined in Standard Methods for the Examination of Water and Wastewater[19]. Adenosine triphosphate (ATP) values in mg ATP/liter were in some cases determined (Biospherics Incorporated, Rockville, Maryland 20852) on Ditch effluent samples based on the rapid method of Chappelle and Levin[20]. ATP values give a direct relationship to the number of live bacteria in a system. For example, an ATP value of 0.34 mg ATP/liter show in the case of Pseudomonas fluorescens that there are 1.1×10^{12} cells/liter of sample[20]. However, the amount of ATP for different bacteria was found to vary from 0.28×10^{-13} mg ATP/cell (Acrobacter acrogenes) to 8.9×10^{-13} mg ATP/cell (Mycobacterium smegmatis)[20]. Activated sludge would be expected to contain a mixed colony of bacteria.

Suspended solids (SS) of the Ditch effluent were measured by Biospherics Incorporated, Rockville, and were found to be on the average about 40 mg/liter, although in some instances values as high as 190 mg/liter were found.

Continuous Culture Apparatus Experiments. Small-scale continuous culture apparatus experiments (Table 4, experiments 4 and 5) were performed with the Bio-Matic Bench Scale Activated Sludge Plant (Princeton Aqua Science Model CF-235, schematic 3). The apparatus, which was modeled to approximately 1/1000 the size of the NSWC Oxidation Ditch Facility, is composed of a 9 liter transparent acrylic aeration chamber with a built-in ceramic air sparger and a settling basin of transparent acrylic with a maximum capacity of 2 liters. The sludge, or settled floc, was returned from the settling basin to the aeration chamber through a pinch valve which was automatically

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19. Ibid., pp 415-421.

20. E. W. Chappelle and G. V. Levin, "Use of the Firefly Bioluminescent Reaction for Rapid Detection and Counting of Bacteria", Biochem. Medicine, 2, 41-52 (1968).

energized by a pre-set timer. The TNT, and nutrient solution were metered (Cole-Parmer Masterflex, Model 7565) into the aeration chamber containing about 4 liters of the nutrient, floc, and water mixture.

C-14 Labeled TNT Studies. Seven-carbon labeled C-14 TNT was obtained from Nuclear Equipment Chemical Corporation, Farmingdale, New York, 11735, and had an activity of 3.5 millicuries/gram. Gas chromatographic analysis of the TNT showed it to be at least 99% alpha TNT. A coincidence - type liquid scintillation counter (Nuclear Chicago, Model 6853) was used with Aquasol (New England Nuclear, Boston Mass.) counting cocktail for counting C-14 activity according to standard counting techniques. The counting efficiency for C-14 under these conditions was 90%. A 19.6 ppm TNT was found to give 1.35×10^5 counts/minute. The set-up for the C-14 labeled TNT study is shown in schematic 6 while the C-14 work-up procedure is given in schematic 7.

Batch TNT Bioconversion Studies. Batch TNT bioconversion studies were usually done in 100 ml aerated erlenmyer flasks. Bacterial floc was obtained from a separate nutrient-activated sludge micro-organism mixture growing in the absence of TNT. Before the experiment, 100 ml of this mixture was centrifuged, the supernate discarded, and the floc transfered to a clean erlenmyer flask with a known volume of water. An aqueous solution of known concentration of TNT was then added to bring the total volume to 100 ml. At the same time, a weighed amount of cornsteep nutrient was added to the flask to maintain the desired nutrient/TNT ratio. The mixture was aerated with a glass frit and 5 ml portions were drawn off at various times for chemical analysis. This general scheme was employed in the C-14 labeled TNT studies.

IV SUMMARY AND CONCLUSIONS

The results of this three year pilot plant study on the biodegradability of TNT are summarized with the following conclusions:

(1) TNT (2,4,6-trinitrotoluene), at a concentration between 10 and 50 ppm in water, was found to be 97 ± 2% biotransformed when fed continuously into an aerated Oxidation Ditch Facility containing bacterial floc grown from activated sludge microorganisms supplemented with cornsteep water nutrient. The combined yield of the aromatic nitro amines found in the effluent was 12 ± 2% of the TNT feed concentration. The relative amounts of these amines, on a weight basis, were found to be: 4-A/2-A/2,4-diA/2,6-diA = 1.00/0.12/0.10/0.013.

(2) TNT biotransformation appears to be a step-wise bioreduction of the aromatic nitro groups to form amines which eventually react to form complex polar products.

(3) TNT biotransformation studies with completely labeled C-14 TNT show less than 0.4% $^{14}CO_2$ formation, with the remainder of the C-14 activity about equally distributed between the bacterial floc (solid) and effluent (liquid) phase. These results give no evidence of (do not lend support for) aromatic cleavage during TNT bioconversion.

(4) RDX was found not to be biodegraded either alone or in the presence of TNT. However, TNT bioconversion was not inhibited by the presence of RDX.

(5) The best conditions for TNT biotransformation in the 3,000 gallon NSWC Oxidation Ditch Facility were: (a) TNT feed and effluent rates, 2 gallon/min.; (b) cornsteep/TNT ratio, 100; (c) pH, 7 to 8; (d) floc, 4 to 5 g/liter; and, (e) biological loading factor (cubic ft. Ditch/lb BOD), 10 to 20. Under these conditions the quality of the effluent was as follows: (a) COD, 100 to 300; (b) BOD, 40 to 120; (c) ATP, 0.1 to 1.5; and, (d) suspended solids, 40 mg/liter.

(6) To achieve an effluent concentration of 1 ppm or less of combined amines and TNT using this biological approach, the feed concentration of TNT must not exceed 7 ppm.

(7) The results of this study are significant in that this is the first time that the biodegradability of TNT has ever been investigated on a pilot-plant scale over an extended period of time. Also, it is noteworthy that although TNT is toxic to microorganisms, TNT may be biologically transformed on a continual basis by a relatively simple process in the presence of supplemental nutrient.

(8) The biological approach for the removal of TNT from large volumes of water has a number of drawbacks in that a close control of a number of parameters, such as pH, nutrient/TNT ratio, and a low initial concentration of TNT, is required. This is necessary to keep the toxic, amine by-products in the effluent at an acceptable low level. Therefore, in our opinion, favorable operating conditions would be necessary to offset these operational drawbacks. For example, a free nutrient supply and a low initial TNT concentration makes the biological approach for TNT removal from water much more attractive. A detailed assessment of the utility of this biological approach as compared to more conventional methods, such as carbon and resin adsorption techniques, must await a comparative cost analysis. Such a cost comparison is currently in progress at SRI International, Menlo Park, California.

V ACKNOWLEDGMENT

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VI REFERENCES

1. E. P. Pardee and J. D. Hodge, "Biological Degradation of TNT", paper presented at the Convention of the American Society of Agronomists, Washington, D. C., November 1967.

2. M. E. Sitzmann, "Chemical Reduction of 2,4,6-Trinitrotoluene - Initial Products", J. Chem. & Eng. Data, Vol 19, No. 2, pp. 179-181, April, 1974.

3. G. A. Hudock (Indiana University, Department of Zoology, Bloomington, Indiana, 47401), Final Report: "Biological Effects of TNT Wastes and a Description of Means for their Detoxification and Removal", U. S. Navy Research Contract N60921-71-C0272, June 10, 1972.

4. W. D. Won and R. J. Heckly (Naval Biomedical Research Laboratory, Oakland, California, 94625), Final Report, "Biodegradation of Trinitrotoluene", June 30, 1974. (Requests for this report must be referred to the Office of Naval Research, Arlington, Virginia, 22217, Attn: Code 443).

5. W. D. Won, R. J. Heckly, D. J. Glover, and J. C. Hoffsommer, "Metabolic Disposition of 2,4,6-Trinitrotoluene", Applied Microbiology, Vol. 27, No. 3, pp. 513-516, March 1974.

6. Technical Report: "Wastewater Treatment in the Military Explosives and Propellants Production Industry", Vol. 3, Chapter 6, October 1975, prepared by The American Defense Preparedness Association for the U. S. Environmental Protection Agency Office of Research & Development, Washington, D. C. 20410.

7. R. K. Andren, J. M. Nystrom, and R. J. Erickson, "Treatment of TNT Munitions Wastewaters Using Polymeric Adsorption Resins", Pollution Abatement Div., U.S. Army Natick Laboratories, Natick, MA 01760.

8. A. F. Tatyrek, "Treatment of TNT Munitions Wastewaters - The Current State of the Art", Technical Report 4909, Picatinny Arsenal, Dover, New Jersey, October 1976.

9. G. W. Probst and J. B. Tepe in "Degradation of Herbicides", edited by P. C. Kearney and D. D. Kaufman, published by Marcel Dekker, Inc., New York, 255-280 (1969).

10. F. Bell, J. Kenyon, P. H. Robinson, J. Chem. Soc., 1243 (1926) given in P.A.S. Smith, "The Chemistry of Open Chain Organic Nitrogen Compounds. Vol. II", W. A. Benjamin, Inc., New York, p. 4 (1966).

11. D. A. Kubose and D. J. Glover, NSWC/WOL/TR 76-96, "Structure Identification Via Mass Spectrometry. Tetranitro Azoxy and Azotoluenes", 10 Sep 1976.

12. E. G. Kayser, unpublished results, This Center.

13. Reported by N. McCormick in the Twenty-Fifth Conference on Microbiological Deterioration of Military Material, edited by B. J. Wiley, Report: Natick/TR-77/014, p 22, U.S. Army Natick Research and Development Command, Food Sciences Laboratory, DRXNM-YPB, Natick, MA 01760.

14. R. W. Traxler (University of Rhode Island), "Biodergradation of Trinitrotoluene (Alpha TNT) and Its Production Isomers", Report No. 76-48-FSL, p. 17, U.S. Army Natick Research and Development Command, Natick, MA 01760.

15. J. C. Hoffsommer, "Quantitative Analysis of Nitro Compounds in the Micro- to Picogram Range by a Combination of Thin-Layer and Vapor Phase Chromatography with the Nickel-63 Electron Capture Dectector", J. Chromatog., 51, 243-251 (1970).

16. D. J. Glover, J. C. Hoffsommer, D. A. Kubose, "Analysis of Mixtures of 2-Amino-4,6-Dinitrotoluene; 4-Amino-2,6-Dinitrotoluene; 2,4-Diamino-6-Nitrotoluene; and 2,6-Diamino-4-Nitrotoluene", Anal. Chem. Acta, 88, 381-384 (1977).

17. M. E. Sitzman, "Synthesis of 2,6-Diamino-4-Nitrotoluene", J. Chem. & Eng. Data, Vol. 21 No. 2, p 242 (1976).

18. Standard Methods for the Examination of Water and Wastewater, published by the American Public Health Association, Inc., 1790 Broadway, New York, N. Y. 10019, 12th Edition, Ed., H. P. Orland, pp. 510-514 (1965).

19. Ibid., pp 415-421.

20. E. W. Chappelle and G. V. Levin, "Use of the Firefly Bioluminescent Reaction for Rapid Detection and Counting of Bacteria", Biochem. Medicine, 2, 41-52 (1968).

VII GLOSSARY OF TERMS

1. 4-A : 4-Amino-2,6-Dinitrotoluene.
2. 2-A : 2-Amino-4,6-Dinitrotoluene.
3. 2,4-diA : 2,4-Diamino-6-Nitrotoluene.
4. 2,6-diA : 2,6-Diamino-4-Nitrotoluene.
5. Tetranitro Azoxytoluenes : A mixture of the following compounds, (a) 2,2',6,6'-tetranitro-4,4'-azoxytoluene, (b) 4,4',6,6'-tetranitro-2,2'-azoxytoluene, (c) 2,4'-dimethyl-3,3', 5,5'-tetranitro-ONN-azoxybenzene, and (d) 2',4-dimethyl-3,3',5,5'-tetranitro-ONN-azoxybenzene.
6. COD : Chemical Oxygen Demand.
7. BOD : Biological Oxygen Demand.
8. DO : Dissolved Oxygen.
9. BLF : Biological Loading Factor.
10. SS : Suspended Solids.
11. ATP : Adenosine Triphosphate.
12. Floc : Solid part of bacterial growth, measured in grams/liter.
13. Activated Sludge : A mixed colony of microorganisms obtained from a sewage treatment plant.
14. Batch Bioconversion : A bioconversion study of relatively short duration under controlled conditions whereby all ingredients are combined simultaneously in a small volume.

TABLE 1

Chronological NSWC Oxidation Ditch Facilities Operation Parameters of TNT Bioconversion

Operation, Days	Experiment Event	COD (feed) ppm[a]	BLF[b]	pH[c]	DO[d]	Floc, g/l	COD[e] (Effluent)
0-39	Floc Build-up	400-500	43	6.4-6.7	5-7	3.0[f]	50-70
39-142	Floc Build-up	1500-2000	14	6.0-6.6	4-8	4.8[f]	100-150
142-150	BLF Change	1800-2300	9.4	5.7-6.4	4-8	5.1-5.8	100-130
150-226[g]	TNT Run	1900-2500	9.4	7.6-8.0	6-9	5.6-7.8	150-250
226-359[h]	Amine Wash-out, Floc	1900-2800	9.4	6.0-8.0	5-9	5.0-8.0	100-250
359-488	BLF Change	1900-2800	8.6	5.2-7.4	3-5	4.0-6.8	150-300
488-708[i]	TNT Run	2000-2800	8.6	6.1-7.9	5-7	4.0-7.0	150-300
708-753[j]	Amine Washout, Effluent	1500-2000	17.3	5.4-6.7	6-9	2.7-4.7	200-300
753-975[k]	TNT Run	Batch Nutrient Addition	19.4	6.0-7.4	6-9	3.5-5.5	150-300
975-992	Preliminary to last TNT Run	Batch Nutrient Addition	19.4	7.4-8.0	8-9	4.5-5.3	150-300
992-1038[l]	TNT Run	Batch Nutrient Addition	19.4	6.2-7.9	8-9	4.0-5.1	175-250

(a) Continuous cornsteep nutrient at ~8 liters/min. COD is measured value. BOD was about 0.4 of COD value given.

(b) BLF = biological loading factor = cubic feet of ditch/lb. BOD. Nominal values of BLF were calculated from pounds of cornsteep added in one day and ditch liquid volume, 385 cubic feet.

TABLE 1 (Cont'd)

(c) pH maintained within given values by the addition of lime, sodium hydroxide or ammonia gas, as needed.

(d) DO = dissolved oxygen content in ppm O_2.

(e) Measured total COD of uncentrifuged effluent. COD of the centrifuged effluent supernate was 25 to 50% less than uncentrifuged effluent. BOD was on the average about 0.4 of the COD value given. ATP values in mg adenosine triphosphate/liter were found to vary from 0.1 to 1.5. Suspended solids (SS) were found to be around 40 mg/liter on the average.

(f) Final value.

(g) 76 Day continuous TNT/nutrient feed (Graph 3).

(h) See Table 5.

(i) 220 Day continuous TNT run (Graphs 4, 5, and 6).

(j) See Graph 10.

(k) 222 Day continuous TNT run (Graph 7).

(l) 46 Day TNT extended residence time run (Graphs 8 and 9).

TABLE 2

Results of NSWC Oxidation Ditch Facilities Operations Concerning Amine Formation and TNT Bioconversion

TNT Run Experiment[a]	Influent, TNT[b] feed, ppm	Average Cornsteep/ TNT	Effluent, TNT, ppm	Average Amines, ppm	Percentage Amines[c] Formed	TNT Loss
(1) 76 Day Run with Continuous TNT/Cornsteep Feed (Graph 3) (150-226)	12	200	0.012	1.7	14[d] (12)	99.9
(2) 220 Day Run with Continuous TNT/Cornsteep Feed (Graphs 4, 5, 6) (488-708)	21	128	0.7	2.1	10[e] (8)	96.7
(3) 222 Day Run with Continuous TNT Feed and Batch Addition of Cornsteep, 3X Daily (Graph 7) (753-975)	16	44	1.2	1.8	11[f] (10)	92.6
(4) 46 Day Extended TNT Residence Time in Ditch with Batch Addition of Cornsteep, 3X Daily (Graphs 8, 9) (992-1038)	53[g]	463	0.27	1.7	(8)[h]	99.3

(a) Numbers in parentheses indicate operations days, Table 1.

(b) ~8 liters/min. into ~1.0 X 10^4 liter ditch volume.

(c) Based on average TNT feed. Numbers in parentheses indicate yield of 4A only.

(d) 4A + 2A in the ratio, 1.00/0.14. Floc contains 2.9 $\pm$ 0.8 ppm 4A + 2A.

(e) 4A + 2A + 2,4-diA + 2,6-diA in the ratio, 1.00/0.10/0.12/0.013.

TABLE 2 (Cont'd)

(f) 4A + 2A in the ratio 1.00/0.12. A 28 day portion showed: 4A/2A/2,4-diA/2,6-diA = 1.00/0.12/0.036/0.005.

(g) Average TNT feed, 53 ± 11 ppm at a rate of 0.25 liters/min. into $\sim 1.0 \times 10^4$ liter volume.

(h) 4A formation based on expected TNT dilution concentration at any time, t, (days) given by $(53)(1-e^{-0.036t})$. 4A and 2A were found in the ratio, 1.00/0.11 for a 5 day measured portion of the run.

TABLE 3

Results of NSWC Oxidation Ditch Facilities Operations for TNT Bioconversion - Supplementary Experiments Concerning Amine Formation and Amine Washout

Experiment[a]	Results
(1) 133 Day Amine Wash-Out/ Disappearance from Floc (Table 5) (226-359)	Total amine wash-out/disappearance from floc is fairly linear at ~2.6%/day for 27 days.
(2) 46 Day Amine Wash-Out/ Disappearance from Effluent/ Supernate (Graph 10) (708-753)	Total amine wash-out/disappearance from effluent is non-linear and is 11 times slower than would be expected from dilution alone. After 6 days, the rate of amine wash-out is ~2.2%/day, and indicates amine extraction from floc by water.
(3) Amine Formation as a Function of pH (Table 6) (457-473; 644-659; 537-539)	Amine formation increases from 7% at pH 7.5 to 32% at pH 6.6.
(4) Relative Composition of Amines in Effluent and Floc (Table 7) (150-226; 488-708; 753-975; 992-1038 for effluent, and 303, 368, 688, 771, and 872 for floc)	Relative amounts of 4A and 2A in effluent and floc are about the same. Relative amounts of 2,4-diA and 2,6-diA (to 4A) in the floc are substantially greater than in effluent and suggests bioconversion of 4A and 2A to these products in the floc.
(5) TNT/Amines In and On Floc During Ditch Operations (Table 8) (159-1029)	TNT and amine adsorption in and on floc were on the average 0.003% and 0.5%, respectively, of the amount of TNT added to the ditch. There was no accumulative build-up of either TNT or amines in the floc.

(a) Numbers in parentheses indicate operation days, table 1.

TABLE 4

Results of Supportive Laboratory Experiments for TNT Bioconversion

Experiments	Results
(1) C-14 Labeled TNT (all seven carbons) Bioconversion Studies - Batch Studies (Schematics 3, 4, 5, 6)	Less than 0.4% formation of $^{14}CO_2$. 34% C-14 in amines (floc + effluent) 40% C-14 in solids (floc) 25% C-14 in water solubles (effluent)
(2) Bioconversion of Amine Intermediates - Batch Studies (Graphs 11, 12)	4A converted to 2,4-diA 2A converted to 2,4-diA and 2,6-diA Relative rates: 2A>4A>2,4-diA≅ 2,6-diA
(3) Small Scale Continuous Culture Apparatus (Princeton Aqua Science) Experiments for TNT Bioconversion - Cornsteep vs. Blackstrap Molasses Nutrients-Effect of Nutrient/TNT Ratio (Table 9, Schematic 7)	No significant differences between cornsteep or blackstrap molasses nutrients for TNT bioconversion and amine by-product formation. 97-98% TNT bioconversion when nutrient/ TNT = 100.
(4) Small Scale Continuous Culture Apparatus (Princeton Aqua Science) Experiments for TNT Bioconversion - Cyclic Continuous (Aerobic)/Static (Anaerobic) Runs (Table 10)	TNT bioconversion was > 99% and 4A + 2A formation was 8 ± 3% for four runs. 4A was 96% of amine mixture.
(5) Temperature Effects for TNT Bioconversion - Batch Studies (Table 11)	TNT bioconversion was 96.5% to 99.8% for all runs in the temperature range, 3°C to 53°C.
(6) pH Effects on TNT Bioconversion Batch Studies (Table 12)	Only 45% TNT biocinversion at pH 6.0 to 6.2, but 97 to 99% TNT bioconversion between pH 7.4 and pH 9.1. The % amine formation increases from 9% (pH 6) to 78% (pH 9.1)

TABLE 4 (Cont'd)

(7) TNT Bioconversion Under Aerobic/Anaerobic Conditions - Batch Studies (Table 13)	Under the same conditions, TNT is bioconverted to a greater extent in an aerobic system (99.5%) than in an anaerobic system (69%),
(8) Bioconversion of TNT in the Presence of RDX - Batch Studies (Table 14)	TNT is 99.6% bioconverted in the presence of RDX but RDX remains unchanged (0% RDX bioconversion).

TABLE 5

Amine Washout/Disappearance From Bacterial Floc, NSWC Oxidation-Ditch Facility

Consecutive Days[a]	Total Amines[b], ppm	4A[c], ppm	2A[d], ppm	2,4-diA[e], ppm
0	1.77	1.03	0.30	0.44
14	1.10	0.68	0.13	0.29
21	0.91	0.64	0.07	0.20
27	0.41	0.32	0.026	0.062
77	0.069	0.05	~.0	0.019

(a) Operation days, 226-253, Table 1.
(b) -0.046 ppm (2.6%)/day.
(c) -0.026 ppm (2.5%)/day.
(d) -0.01 ppm (3.3%)/day.
(e) -0.011 ppm (2.5%)/day.

TABLE 6

Amine Formation as a Function of pH, NSWC Oxidation Ditch Facility

pH[a]	% TNT Conversion	% Amine Formation	% of Total Amine 4A + 2A	% of Total Amine 2,4-diA + 2,6-diA	Relative Amine Order
(1) 6.6 ± 0.1 (16)	99.9 ± 0.2	32 ± 5[b]	70.5	29.5	4A/2,4-diA/2,6-diA/2A 1.0/0.04/0.023/0.018
(2) 7.1 ± 0.1 (16)	98.1 ± 1.5	5.6 ± 1.8[c]	92.7	7.3	4A/2A/2,4-diA/2,6-diA 1.0/0.16/0.078/0.012
(3) 7.5 ± 0.1 (3)	98.9 ± 0.3	7 ± 2[d]	91.9	8.1	4A/2A/2,4-diA/2,6-diA 1.0/0.16/0.085/0.016

(a) Numbers in parentheses indicate number of consecutive days of run.
(b) Operation days, 459-475, Table 1.
(c) Operation days, 644-660, Table 1.
(d) Operation days, 537-539, Table 1.

TABLE 7

Relative Compositions of Amines in Floc and Effluent, NSWC Oxidation Ditch Facility

System	Relative Amine Compositions 4A	2A	2,4-diA	2,6-diA
Effluent[a]	1.0	0.12	0.07	0.009
Floc[b]	1.0	0.14	0.48	0.10

(a) Average relative amine composition in effluent from four TNT bioconversion runs Table 2.

(b) Average relative amine composition in floc taken at 303, 368, 688, 771, and 872 operation days, Table 1. These values represent the total amines in the floc and adsorbed on the floc. The average percentage of amines in and on the floc are 75% and 25%, respectively.

TABLE 8

Total Amount of TNT and Amines In and On Floc During NSWC Oxidation Ditch Operations for TNT Bioconversion

Operation Days	Accumulative TNT Added, g	Total Amount In/On Floc[a] TNT[b], g	Amines[c], g
159	1,226	0.097	15.2
170	2,725	0.19	32.1
191	5,586	0.14	42.3
198	6,540	0.26	58.8
206	7,630	0.06	54.4
212	8,448	0.04	118.7
226	10,355	0.014	17.5
688	66,487	-	79.8
771	76,497	3.4	58.4
872	86,051	-	8.9
997	96,815	0.14	16.1
1029	97,409	0.62	40.8

(a) Floc was continually recycled to ditch. A portion of the ditch mixture was cycled to the settling tank every 30 to 40 minutes, the floc settled by gravity and the supernate discharged as effluent. The settled floc was pumped back into the ditch to complete the cycle.

(b) Of this total amount of TNT, an average of 18% was adsorbed on the floc while 82% was found inside the floc. The % TNT in and on the floc was 0.003 ± 0.002% of the accumulative amount of TNT added for each day of operation.

(c) Of this total amount of amines, an average of 25% was adsorbed on the floc while 75% was found inside the floc. The % amines adsorbed in and on the floc was 0.5 ± 0.4% of the accumulative amount of TNT added for each day of operation.

TABLE 9

Small-Scale Continuous Culture Apparatus Experiments (Princeton Aqua Science, Bench-Scale Activated Sludge Plant) - Cornsteep vs. Blackstrap Molasses Nutrient Feeds

Run[a]	TNT Feed, ppm	Nutrient	Nutrient/TNT	% TNT Conversion	% Amines[b] Formation
1	16.4	cornsteep	14.9	88	11
2	15.8	cornsteep	26.4	85	18
3	12.0	cornsteep	67.8	96	29
4	12.8	cornsteep	101.0	98	22
5	13.2	molasses	16.00	87	20
6	14.9	molasses	29.0	82	19
7	14.0	molasses	110.0	97	9

(a) Values for each run correspond to the average value obtained over a three to four week period during which both TNT and nutrient were fed continuously. Floc levels were 5 to 6 g/l during these runs.

(b) Claculated from TNT feed for 4A + 2A amines. The yield of 4A was 84 $\pm$ 6% of the 4A + 2A mixture for all runs.

TABLE 10

Small-Scale Continuous Culture Apparatus Experiments (Princeton Aqua Science, Bench-Scale Activated Sludge Plant) - Static/Continuous TNT Bioconversion

Run[a]	TNT Feed, ppm	Cornsteep/TNT	% TNT Conversion	% Amine[b] Formation
1	1.3	315	99.5	10
2	3.7	152	99.9	5
3	4.8	114	99.9	14
4	10.9	92	99.7	7

(a) For each run, TNT and cornsteep were fed continuously into the continuous culture apparatus for 8 hours, then for a period of 16 hours both feeds were discontinued and the system allowed to become anaerobic. This cyclic procedure was repeated continuously for a total of three weeks for each run. Values reported are an average for this three week period.

(b) The percentage amines (4A + 2A) are based on the feed TNT. The 4A was 96% of the 4A + 2A mixture.

TABLE 11

Temperature Effects for TNT Bioconversion - Batch Experiments

Temperature Range	% TNT Bioconversion[a]
1 - 3 C	96.5
21 - 23 C	99.7
49 - 53 C	99.8

(a) Batch experiments were for a total of 24 hours. The cornsteep/TNT ratio was approximately 100 for each temperature range.

TABLE 12

pH Effects for TNT Bioconversion - Batch Experiments

Run[a]	pH	% TNT Bioconversion	% Amine Formation 4A + 2A	% Amine Formation 2,4-diA + 2,6-diA
1[b]	6.0 - 6.2	45	9.0	0.41
2[c]	7.4 - 7.8	99	8.1	0.24
3[d]	8.7 - 9.1	97	77	1.2

(a) Experiments were for a total of 24 hours. Cornsteep/TNT was approximately 100.
(b) Total amines, 9.4%; 4A/2A/2,4-diA/2,6-diA = 1.0/0.6/0.07/0.007.
(c) Total amines, 8.3%; 4A/2A/2,4-diA/2,6-diA = 1.0/0.5/0.04/0.005.
(d) Total amines, 78.2%; 2A/4A/2,4-diA/2,6-diA = 1.0/0.9/0.02/0.01.

TABLE 13

TNT Bioconversion Under Aerobic and Anaerobic Conditions - Batch Studies

System[a]	pH	%TNT Bioconversion	% Amine Products 4A	2A	2,4-diA	2,6-diA
Aerobic[b]	6.8 - 7.5	99.5	7.0	4.1	0.71	0.09
Anaerobic[c]	6.8 - 7.3	69.0	22.0	7.9	3.8	0.45

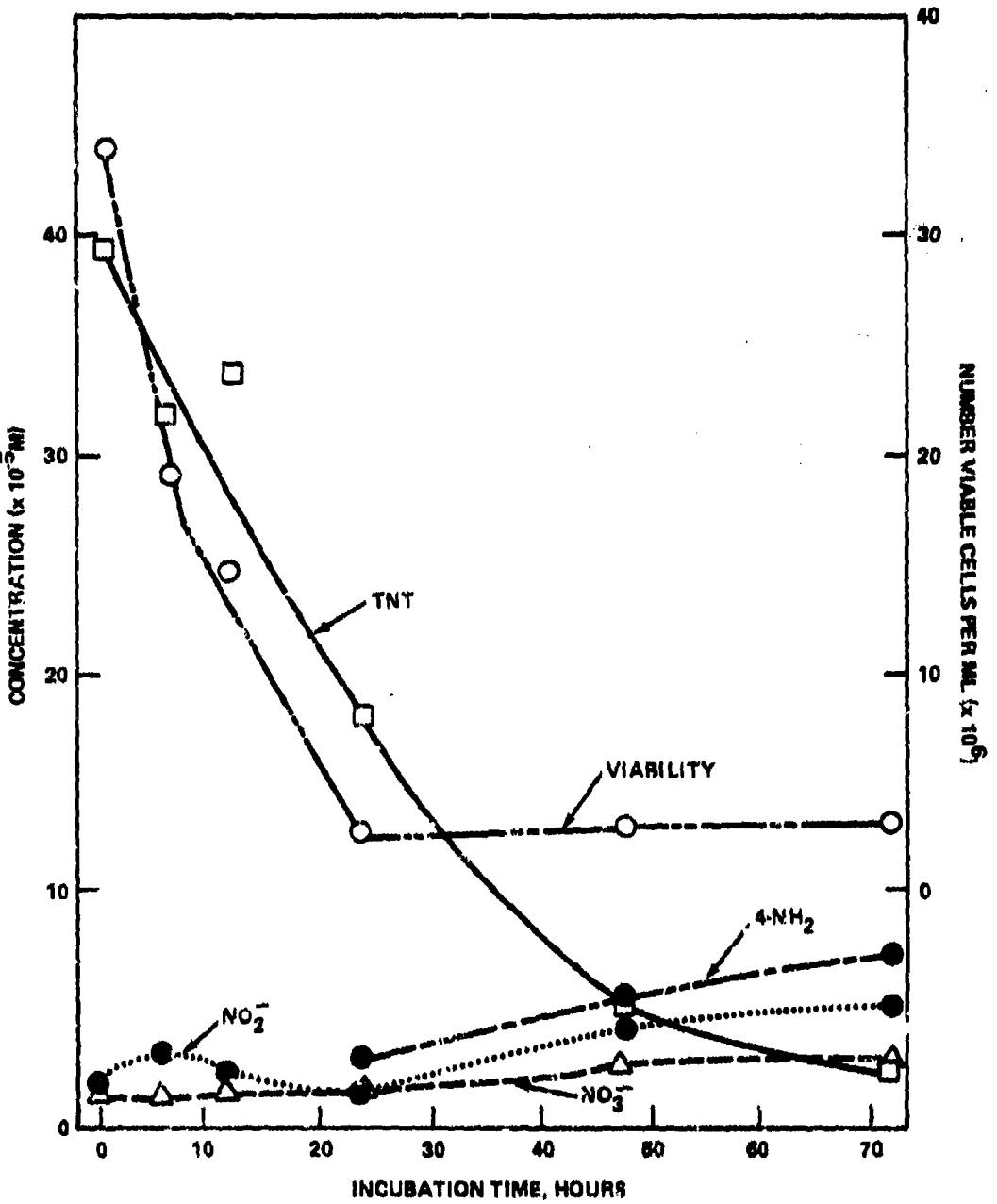
(a) Experiments were for a total of 24 hours. CS/TNT $\cong$ 100.
(b) Total amines, 11.9%; 4A/2A/2,4-diA/2,6-diA = 1.0/0.58/0.10/0.012.
(c) Total amines, 34.2%; 4A/2A/2,4-diA/2,6-diA = 1.0/0.35/0.17/0.020.

TABLE 14

Bioconversion of TNT in the Presence of RDX - Batch Studies

Compound/Mixture	% Bioconversion[a]
TNT[b]	99.6
RDX[c]	0
TNT/RDX Mixture[d]	99.5 (TNT ; 0 (RDX)

(a) % Bioconversion in 24 hours. CS/TNT $\cong$ 100. Activated sludge organisms.
(b) Initial TNT concentration, 20.7 ppm.
(c) Initial RDX concentration, 7.3 ppm.
(d) Initial concentrations of TNT and RDX mixture, 15.1 ppm and 7.3 ppm, respectively.



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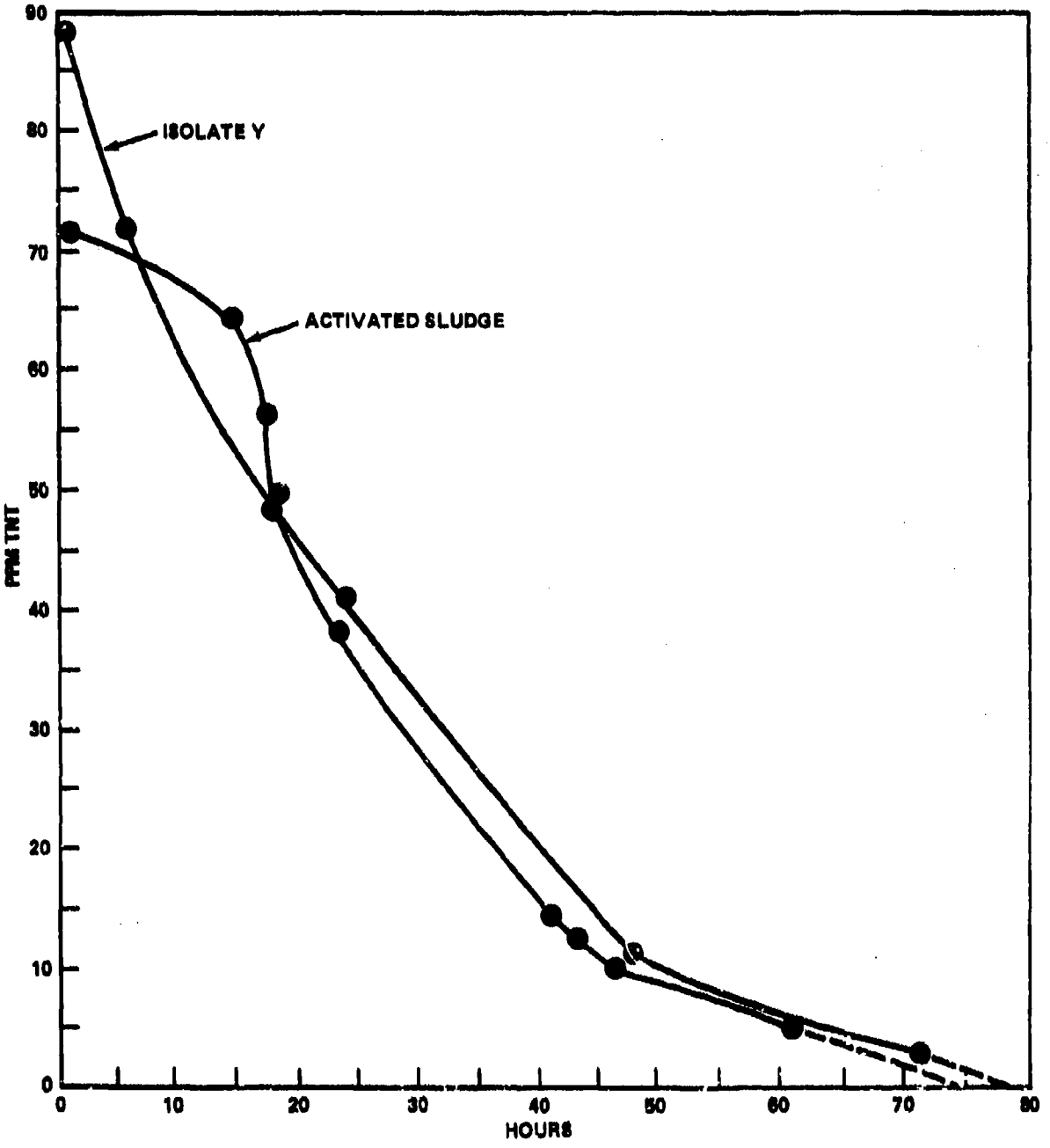
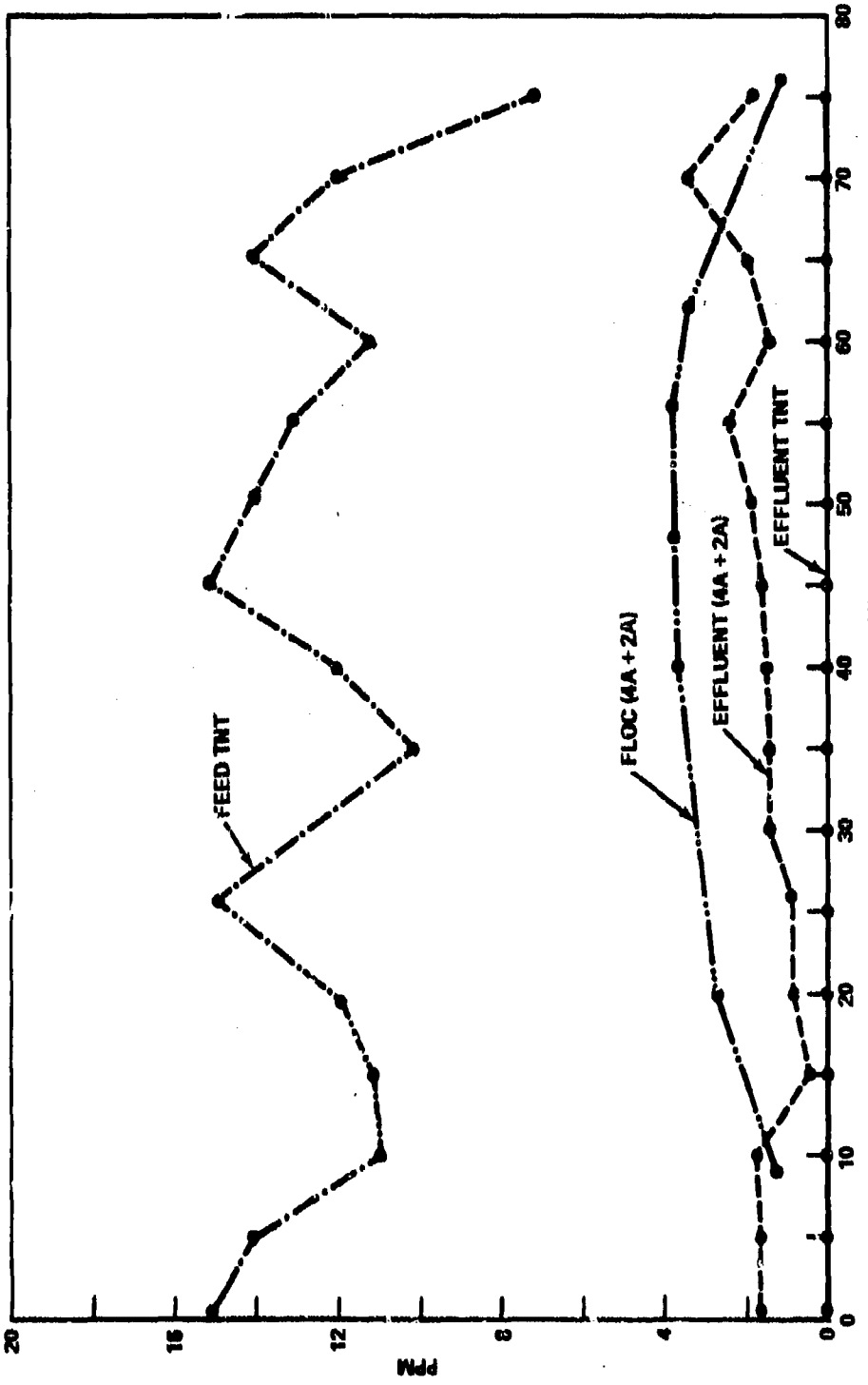
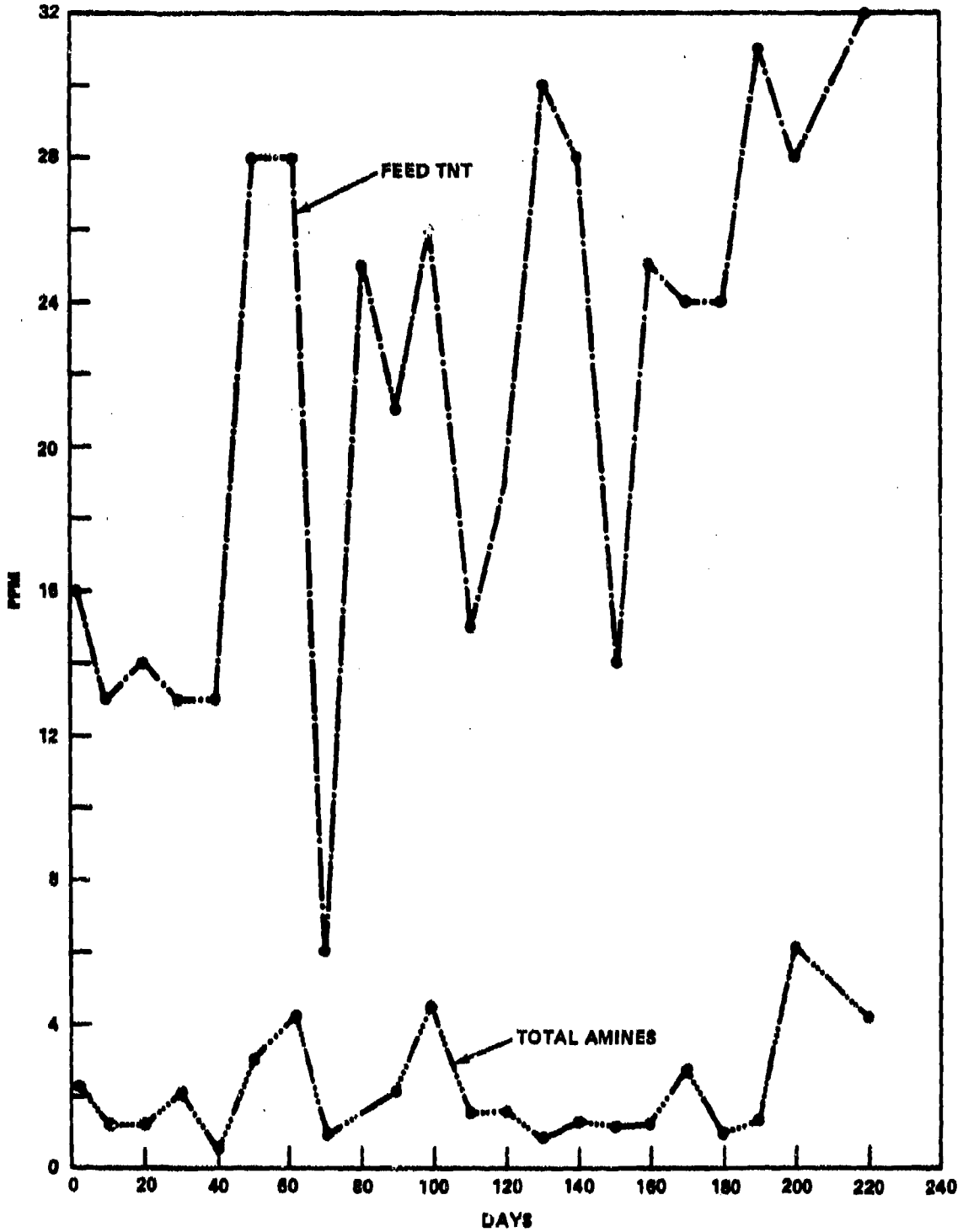


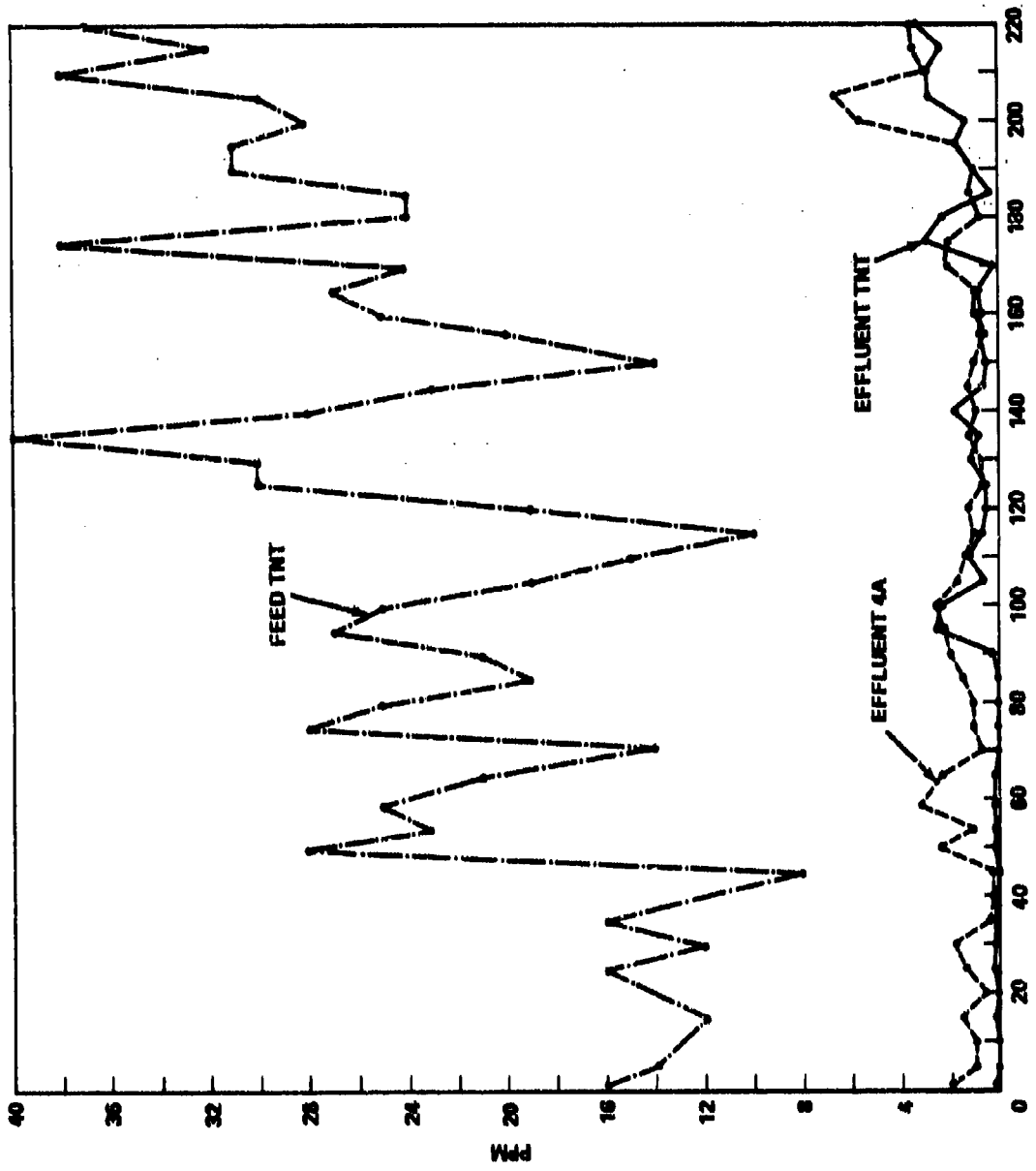
GRAPH 2 BIOTRANSFORMATION OF TNT WITH ACTIVATED SLUDGE AND WITH ISOLATE Y FROM NAD McALESTER



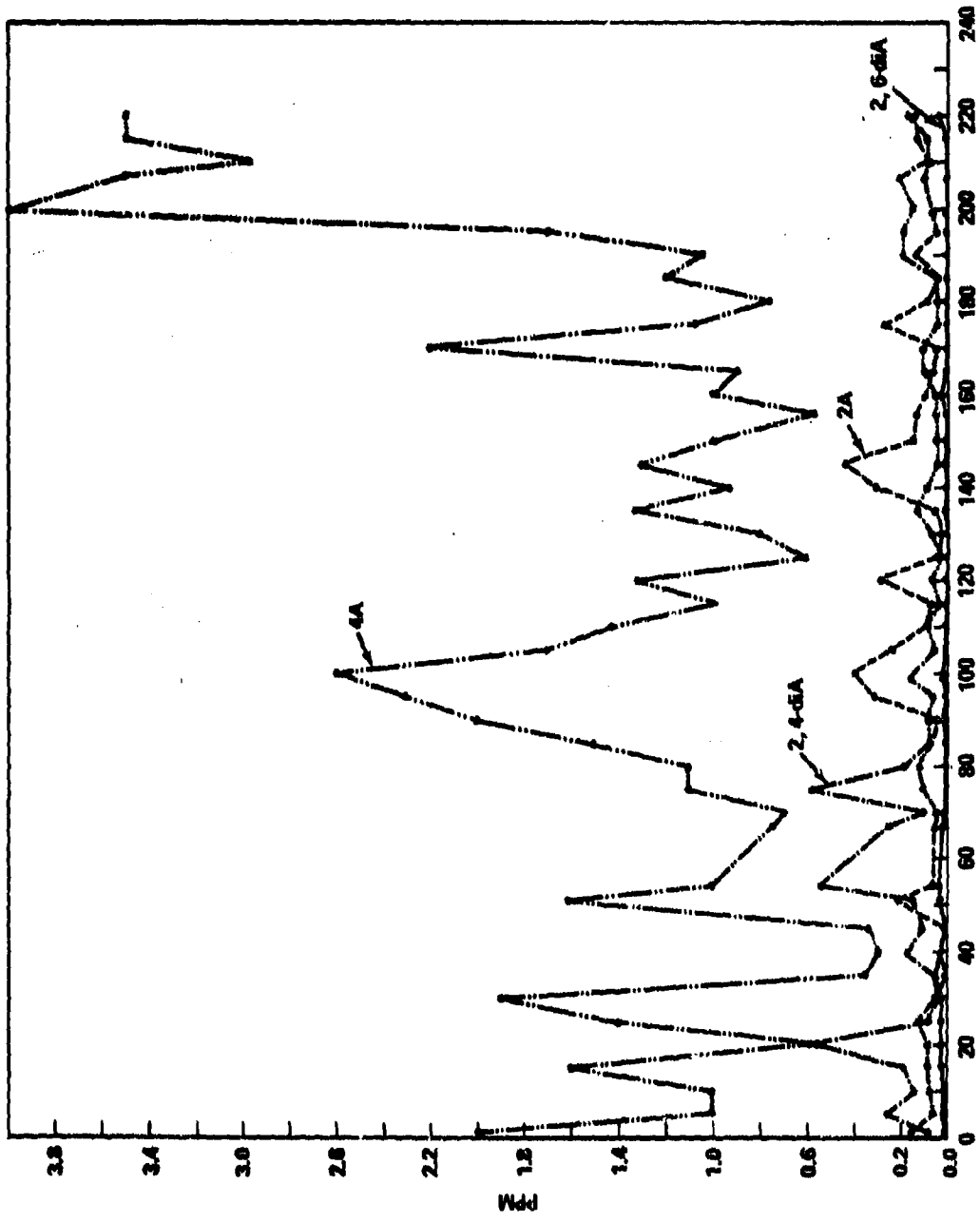
GRAPH 3 BIOTRANSFORMATION OF TNT - 76 DAY CONTINUOUS RUN - TNT FEED AND AMINE CONCENTRATIONS IN EFFLUENT AND FLOC AS A FUNCTION OF TIME



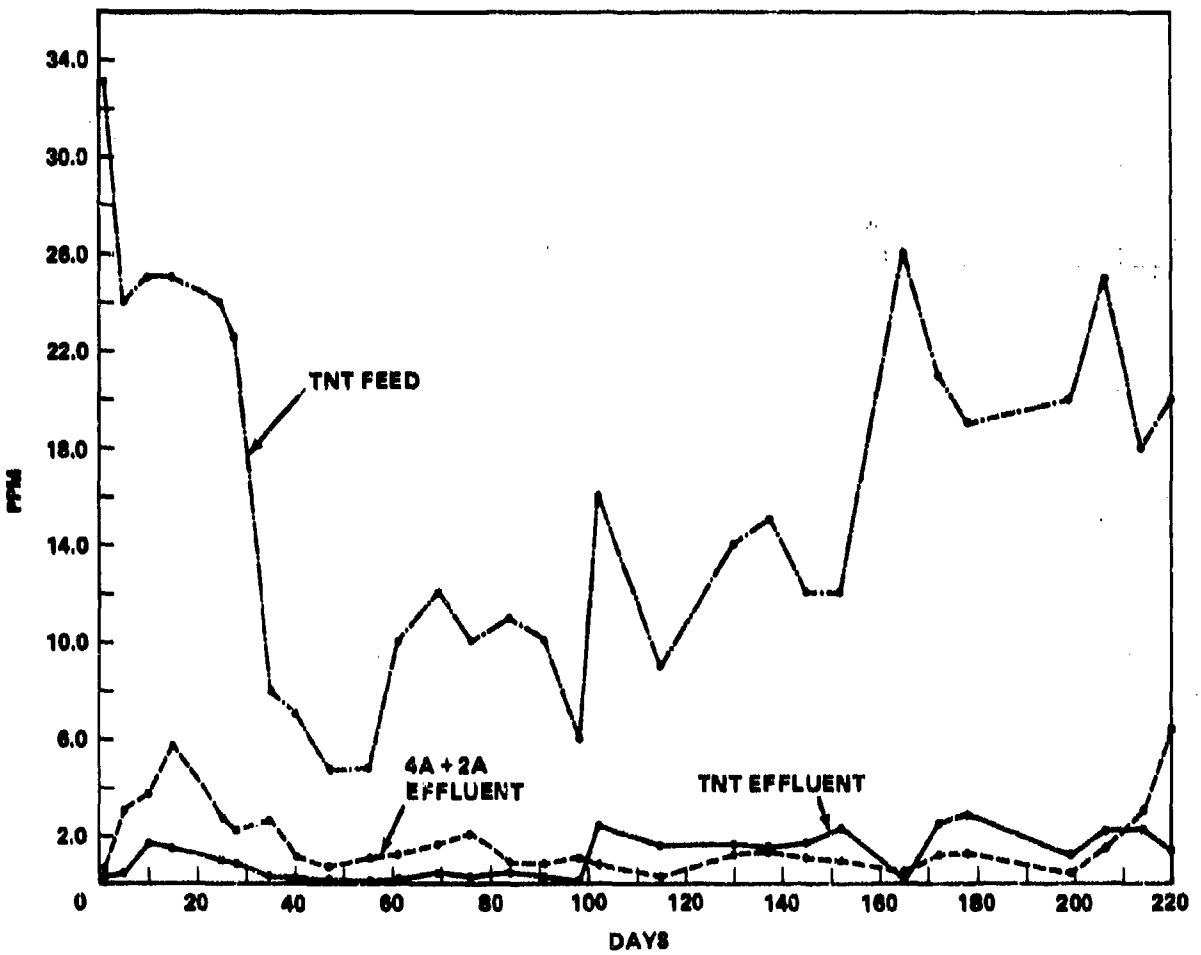
GRAPH 4 BIOTRANSFORMATION OF TNT - 220 DAY CONTINUOUS RUN - TNT FEED AND EFFLUENT TOTAL AMINE CONCENTRATIONS AS A FUNCTION OF TIME



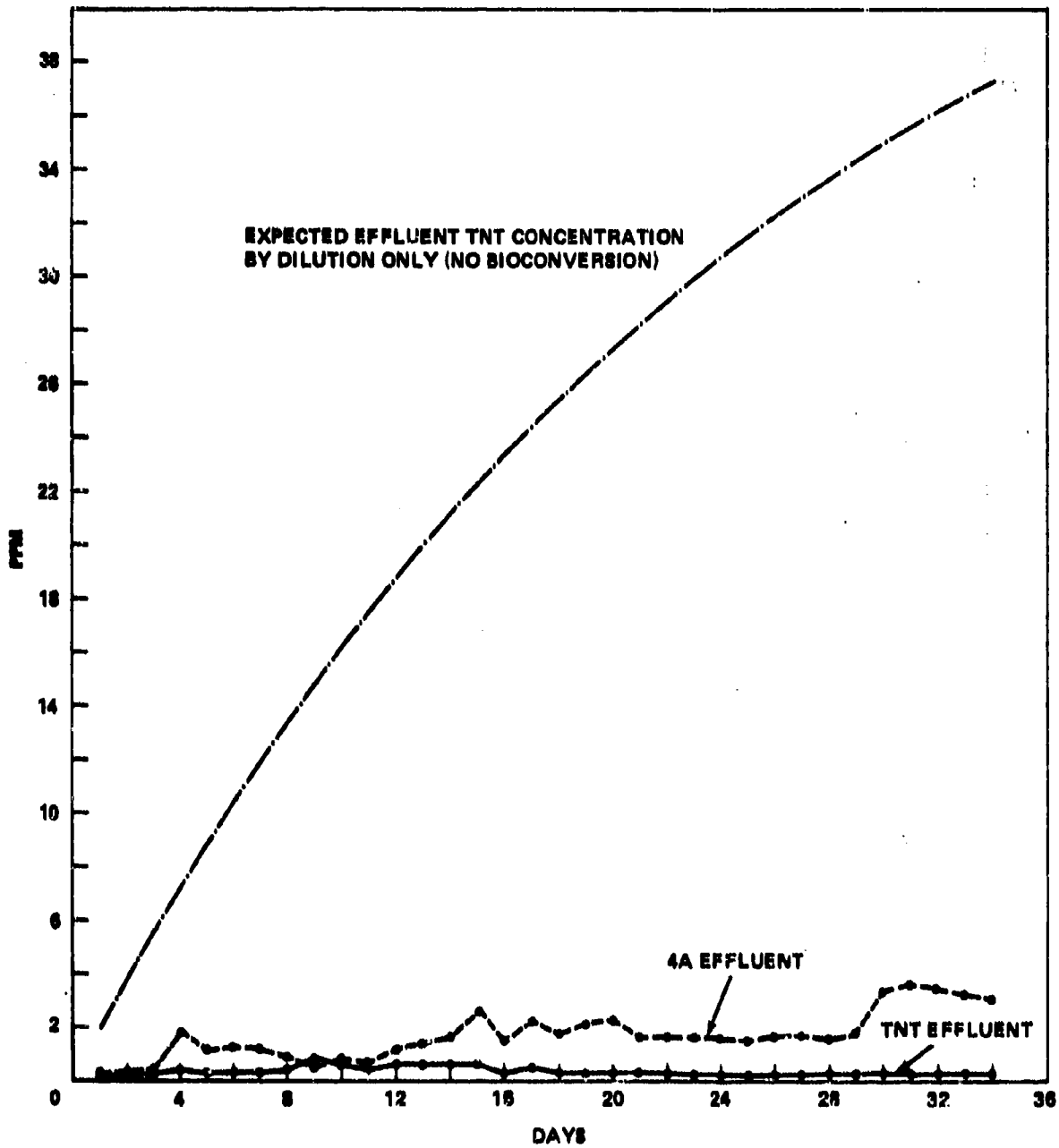
GRAPH 5 BIOTRANSFORMATION OF TNT - 220 DAY CONTINUOUS RUN - TNT FEED AND EFFLUENT 4A AND TNT CONCENTRATIONS AS A FUNCTION OF TIME



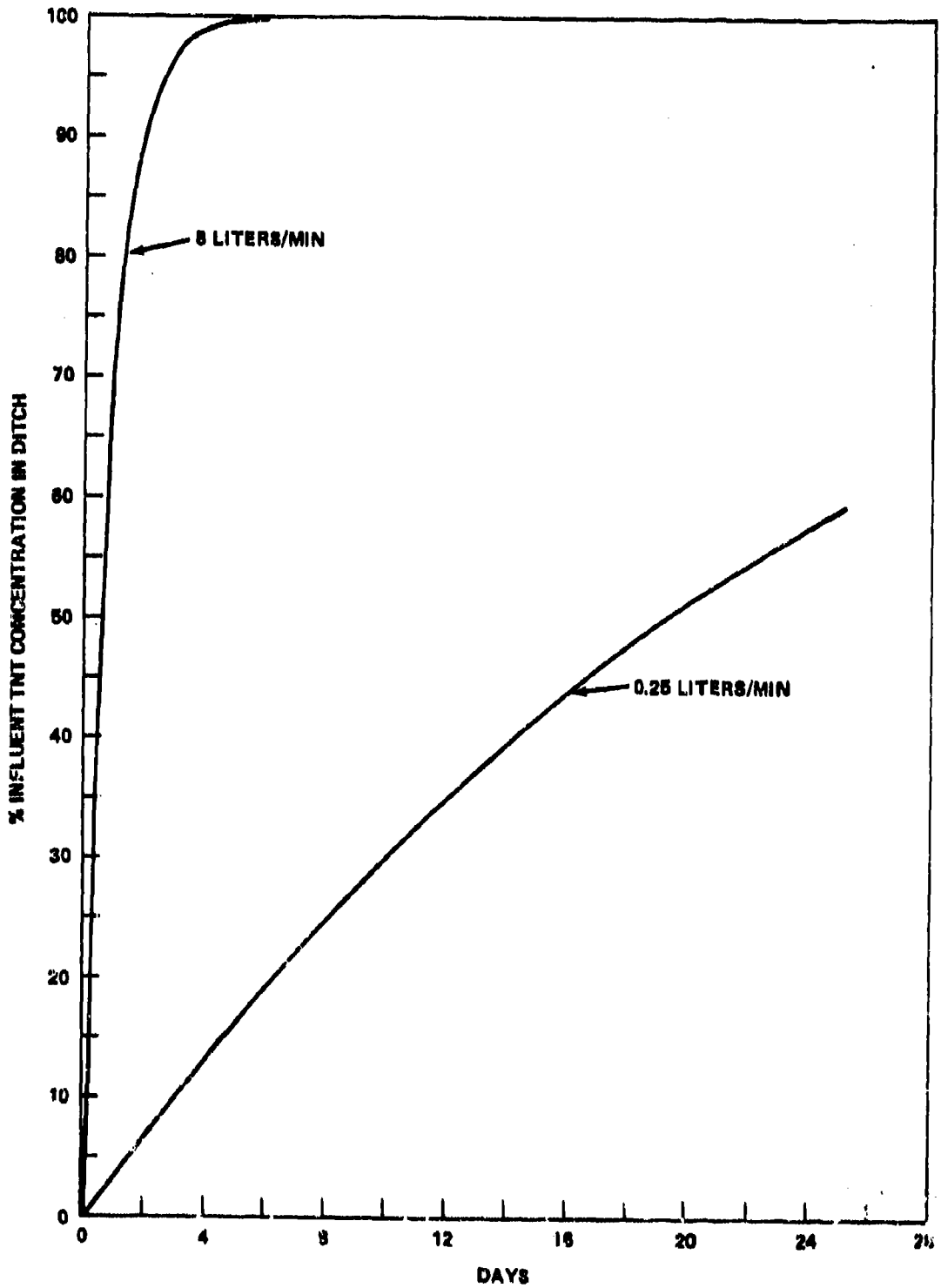
GRAPH 6 BIOTRANSFORMATION OF TNT - 220 DAY CONTINUOUS RUN - INDIVIDUAL AMINE (4A, 2A, 2, 6-diA, AND 2, 4-diA) CONCENTRATIONS AS A FUNCTION OF TIME



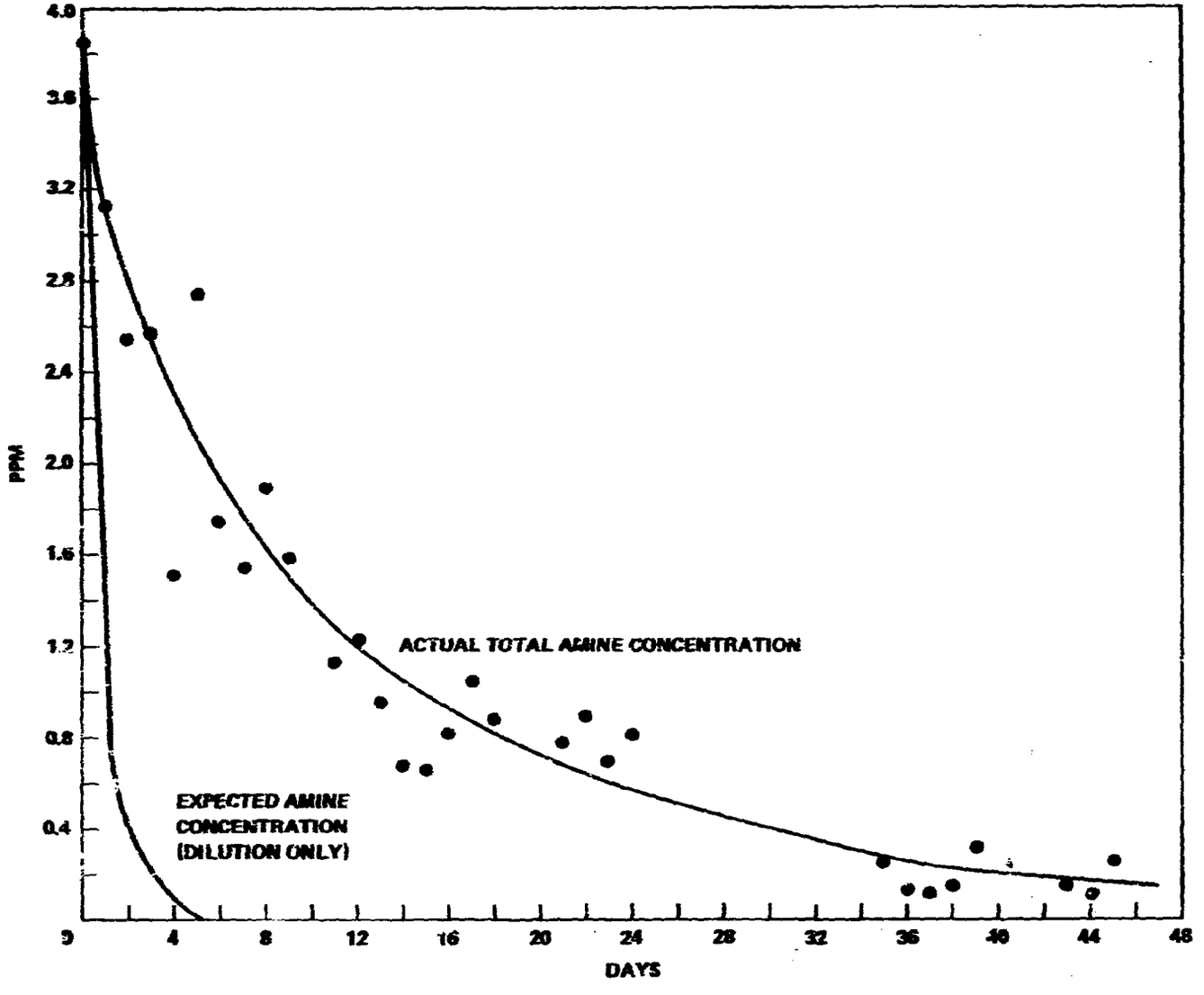
GRAPH 7 BIOTRANSFORMATION OF TNT - 222 DAY BATCH NUTRIENT (C8) FEED AND CONTINUOUS TNT FEED WITH AMINE AND TNT EFFLUENT CONCENTRATIONS AS A FUNCTION OF TIME



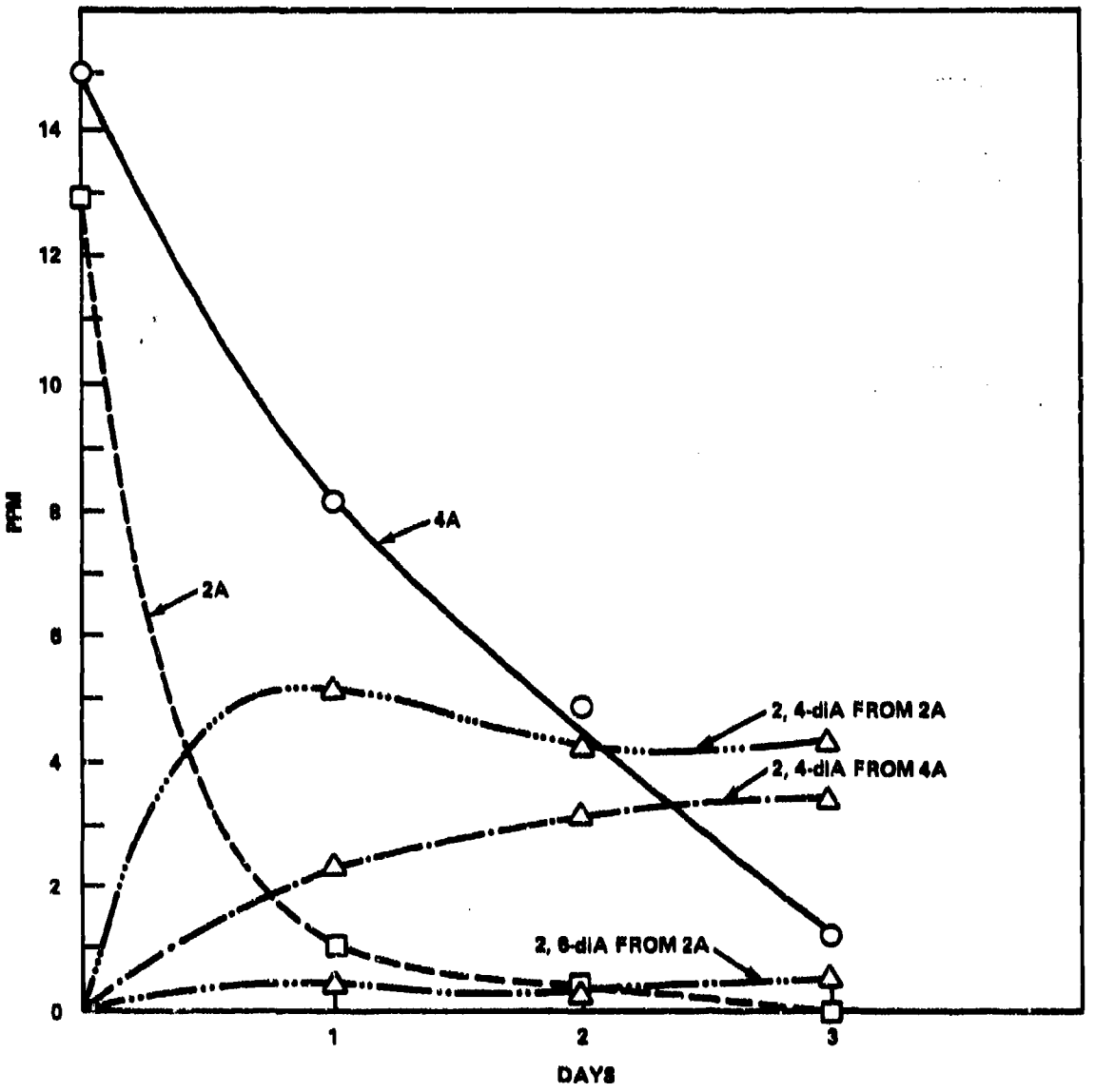
GRAPH 8 BIOTRANSFORMATION OF TNT - 46 DAY EXTENDED TNT RESIDENCE TIME EXPERIMENT - TNT FEED AND EFFLUENT 4A AND TNT CONCENTRATIONS AS A FUNCTION OF TIME



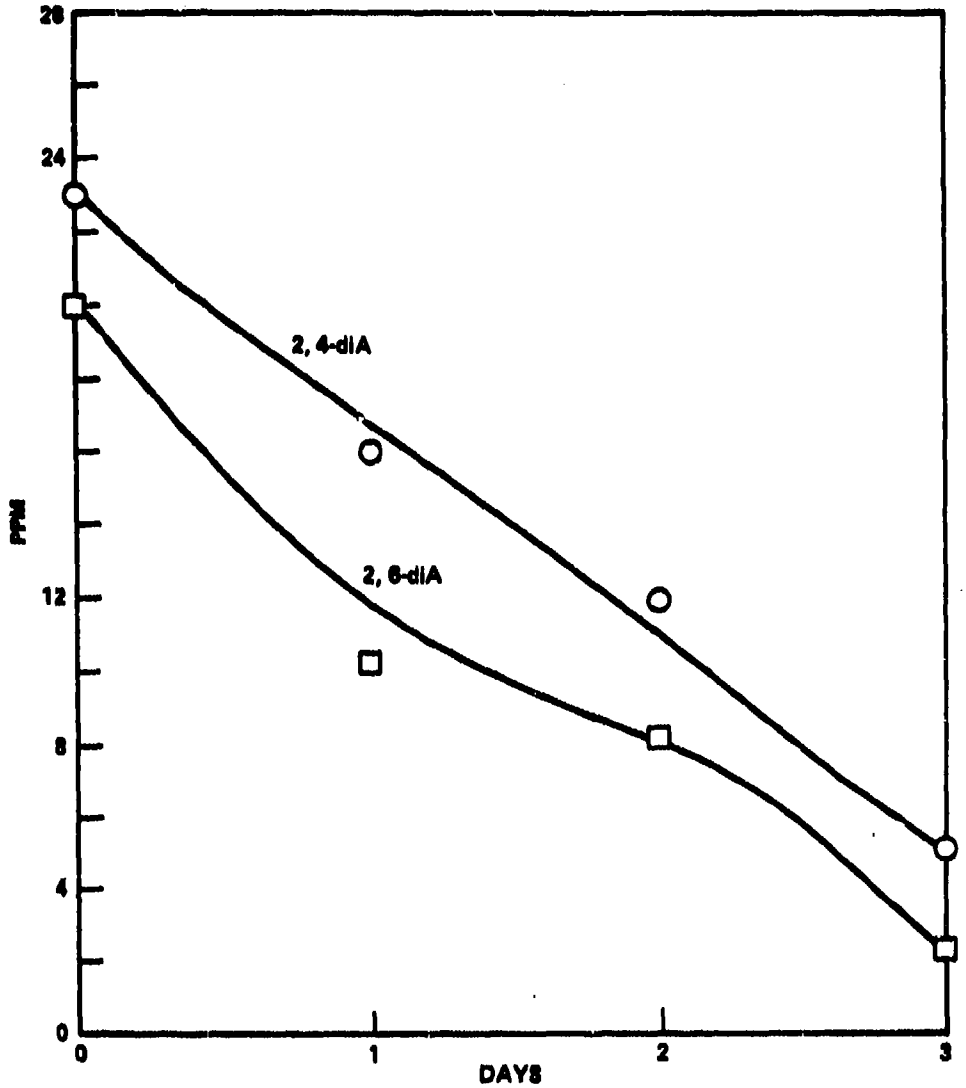
GRAPH 9 EXPECTED EFFLUENT TNT CONCENTRATION BY DILUTION ONLY (NO BIOCONVERSION) AS A FUNCTION OF INFLUENT TNT CONCENTRATION AND TIME FOR TWO DIFFERENT ADDITION RATES



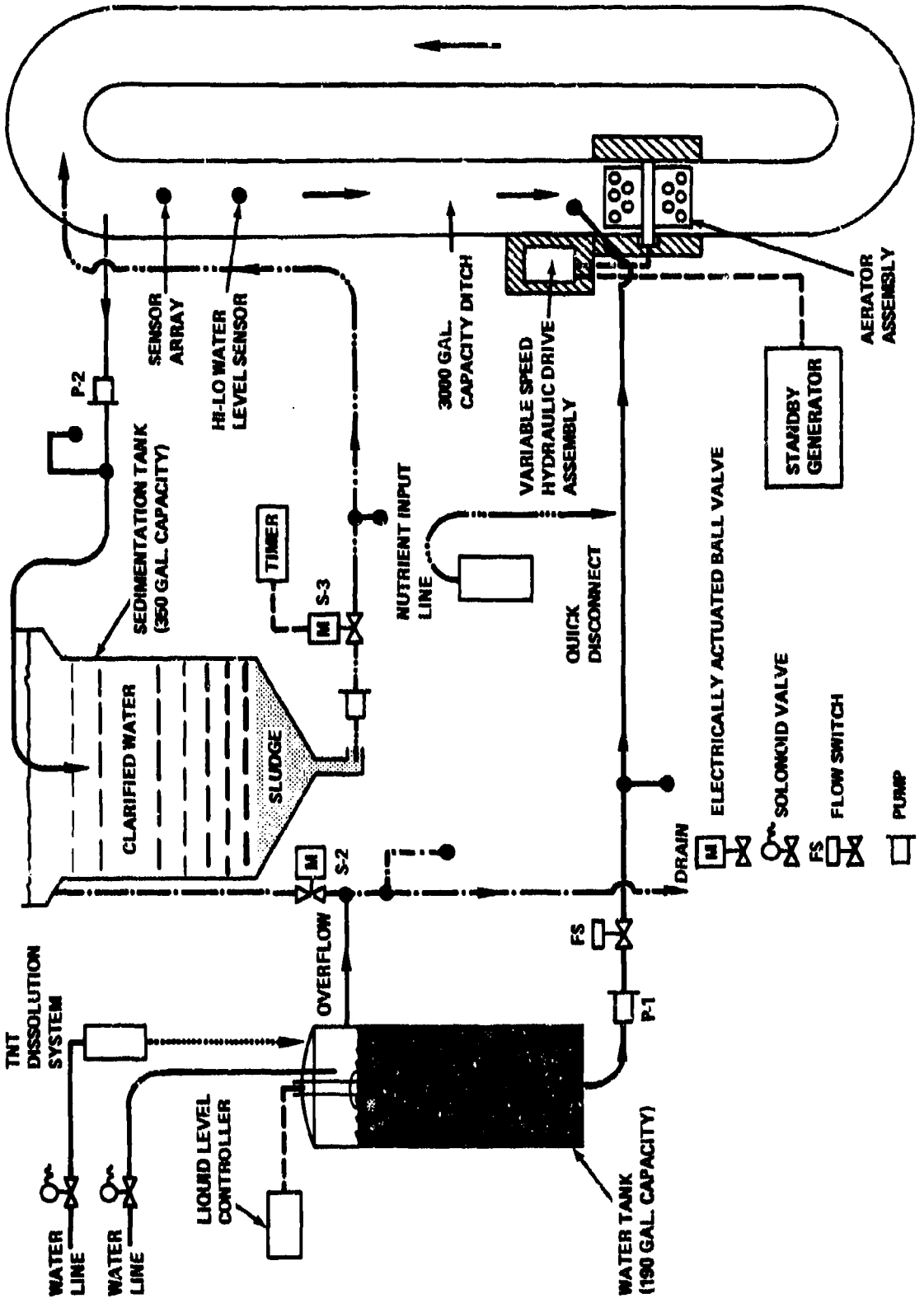
GRAPH 10 TOTAL AMINE WASHOUT/DISAPPEARANCE FROM EFFLUENT - NSWC/WOL OXIDATION DITCH FACILITY



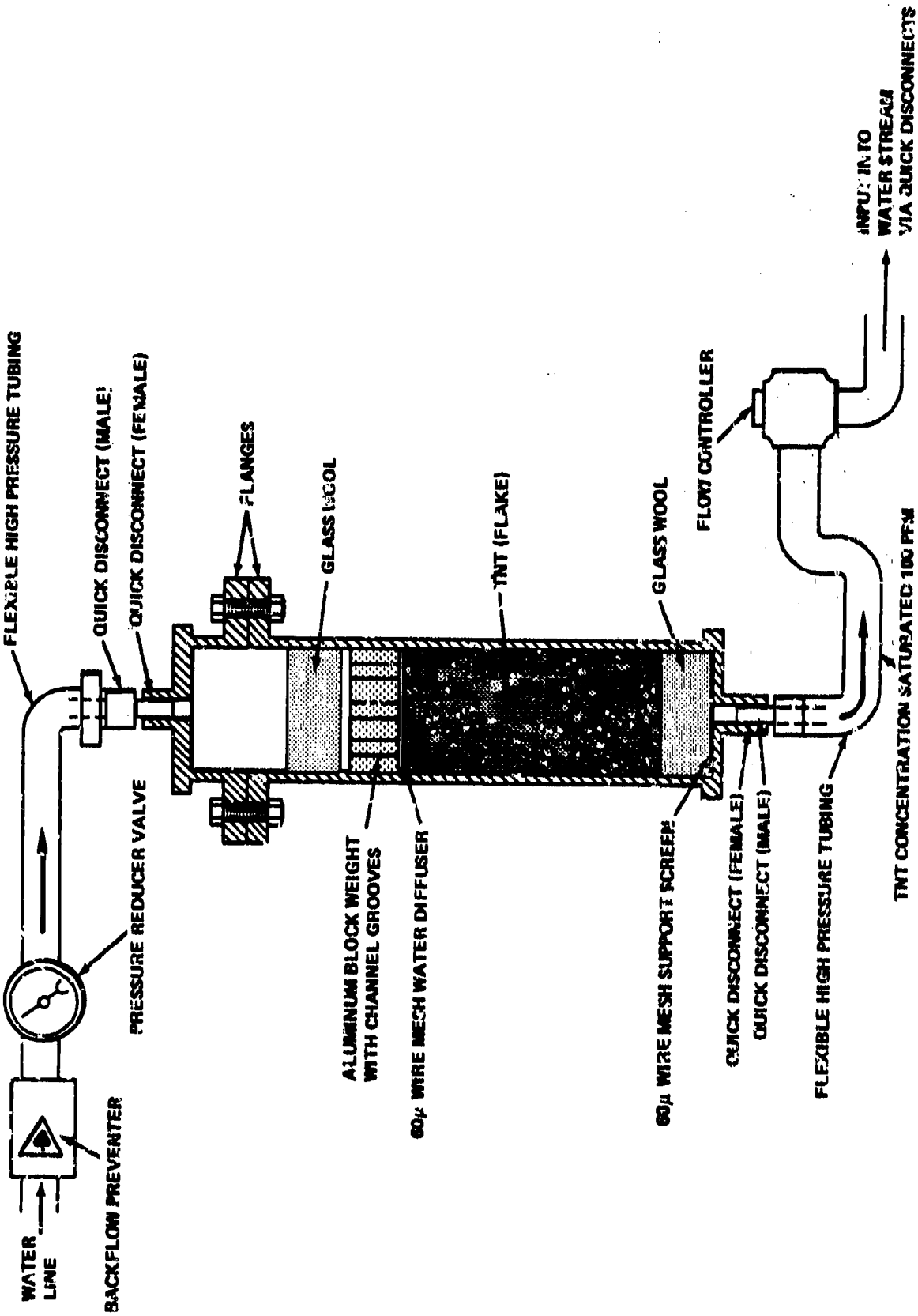
GRAPH 11 PRODUCTION OF 2, 4-diA AND 2, 6-diA FROM BIOTRANSFORMATION OF 2A AND 4A - BATCH STUDIES



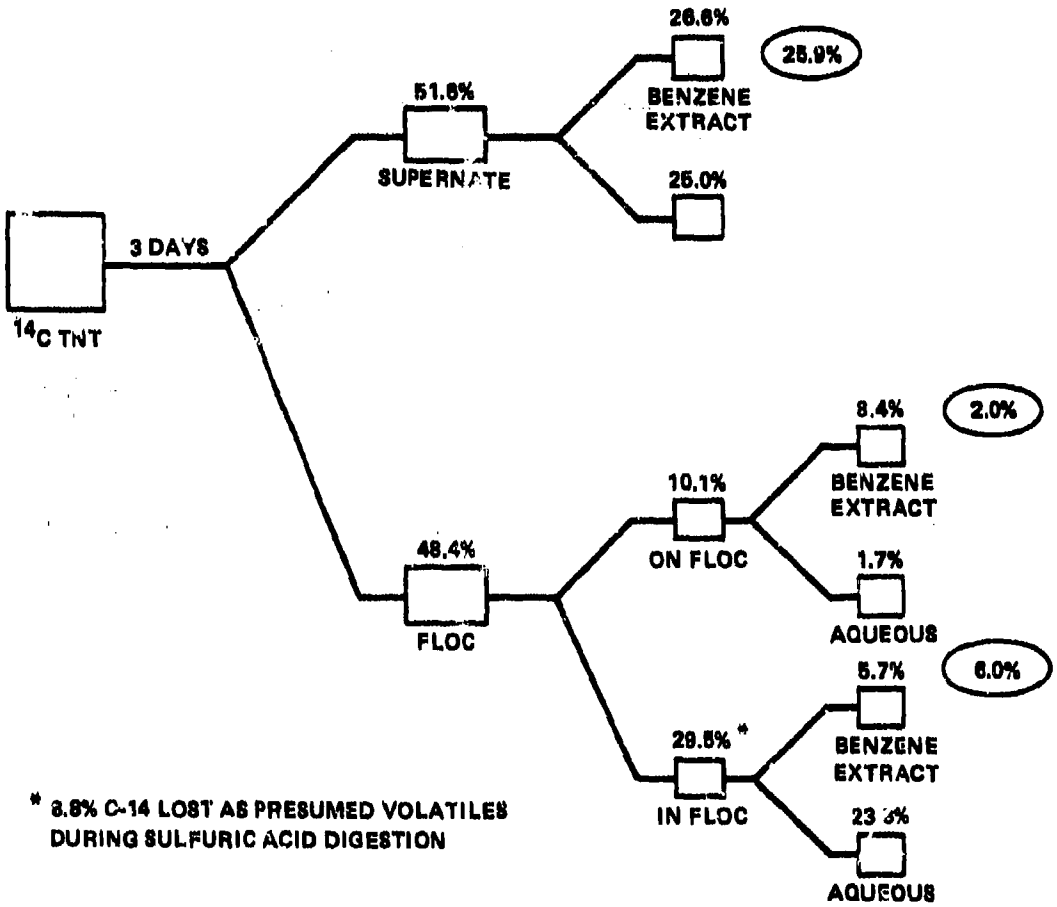
GRAPH 12 BIOTRANSFORMATION OF 2, 4-dIA AND 2, 6-dIA – BATCH STUDIES



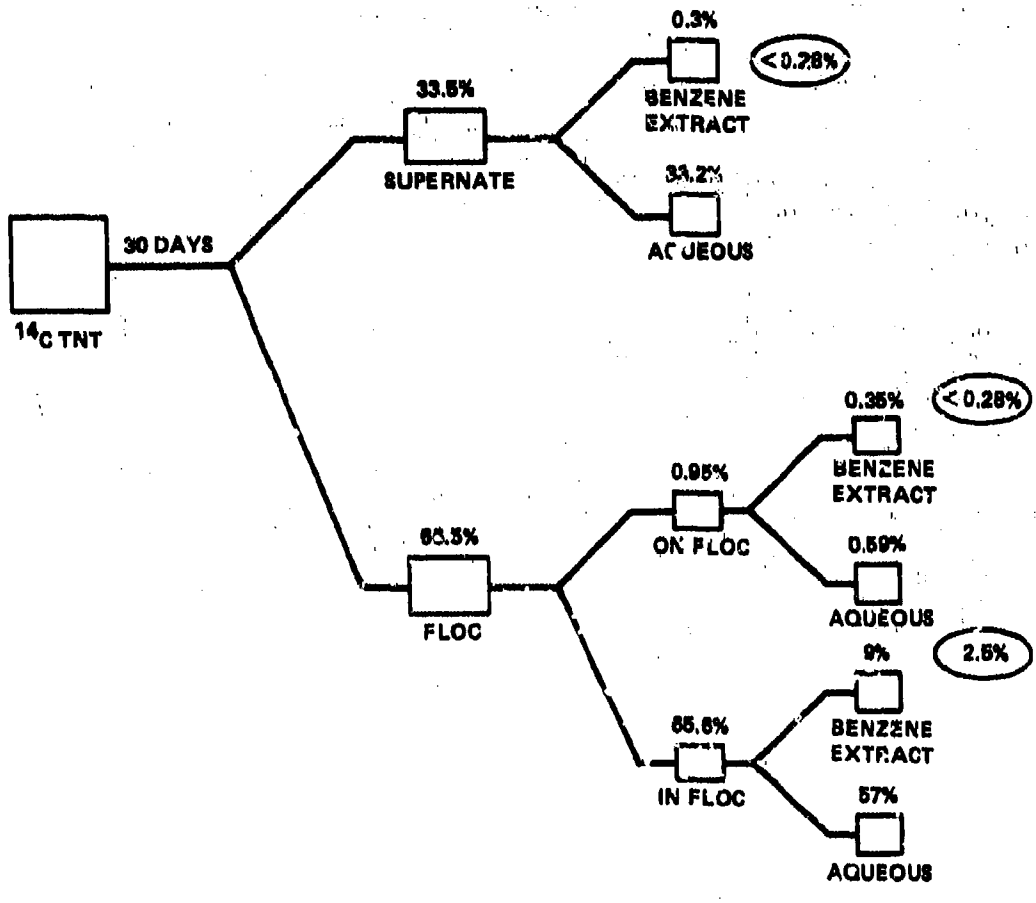
SCHEMATIC 1 NSWC/WOL OXIDATION DITCH FACILITY



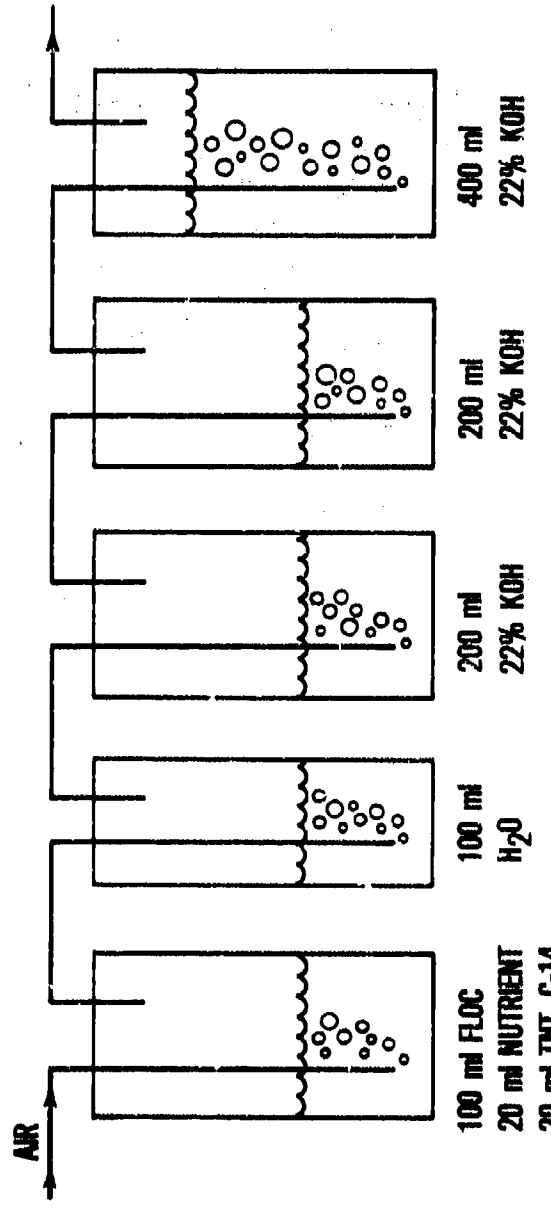
SCHEMATIC 2 TNT DISSOLUTION SYSTEM



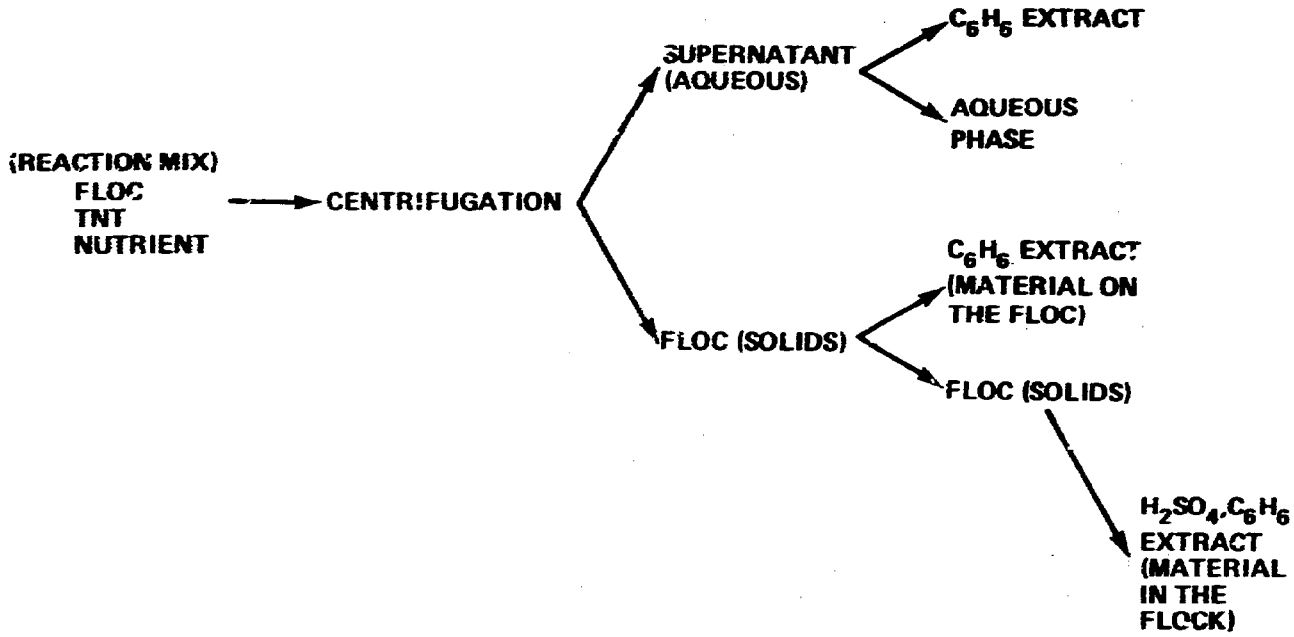
SCHEMATIC 3 DISTRIBUTION OF ^{14}C ACTIVITY AND ITS COMPARISON TO THE DISTRIBUTION OF MEASURED BIOTRANSFORMATION PRODUCTS (CIRCLED VALUES) i.e., RESIDUAL TNT, 2A AND 4A; AND 2, 4-diA AND 2, 6-diA AFTER 3 DAYS



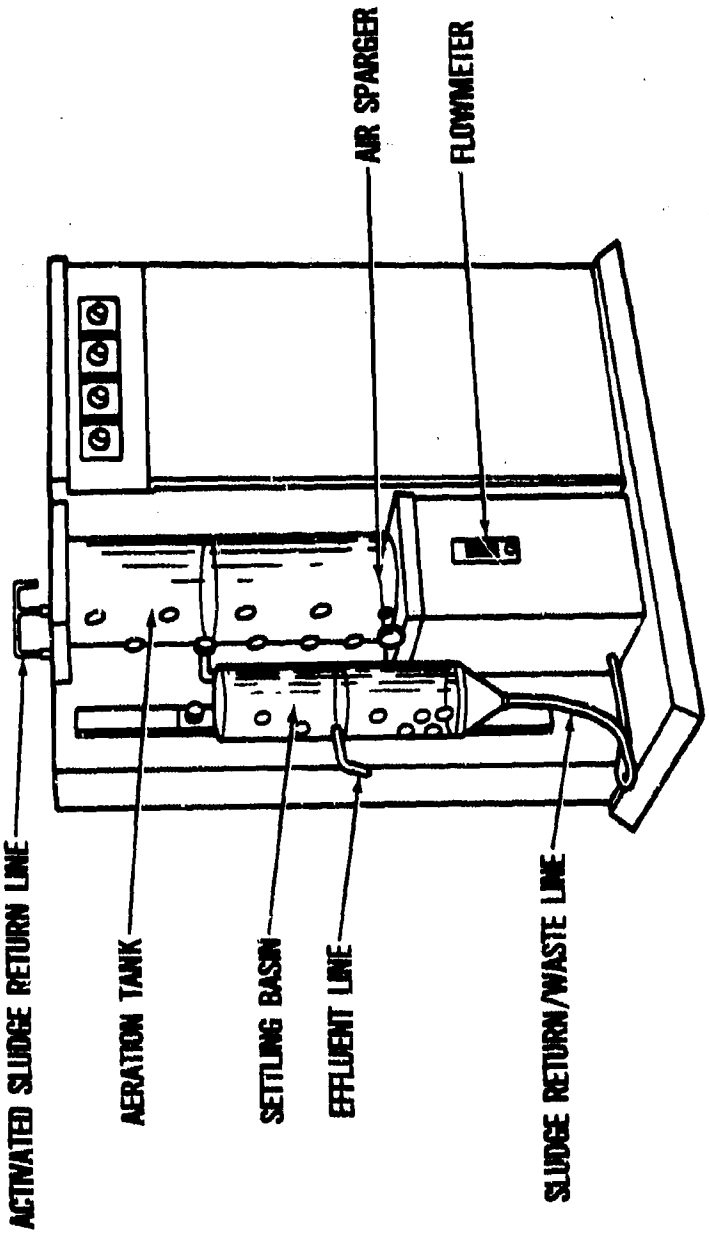
SCHEMATIC 4 DISTRIBUTION OF ^{14}C ACTIVITY AND ITS COMPARISON TO THE DISTRIBUTION OF MEASURED BIOTRANSFORMATION PRODUCTS (CIRCLED VALUES) i.e., RESIDUAL TNT, 2A AND 4A; AND 2, 4-diA AND 2, 6-diA AFTER 30 DAYS



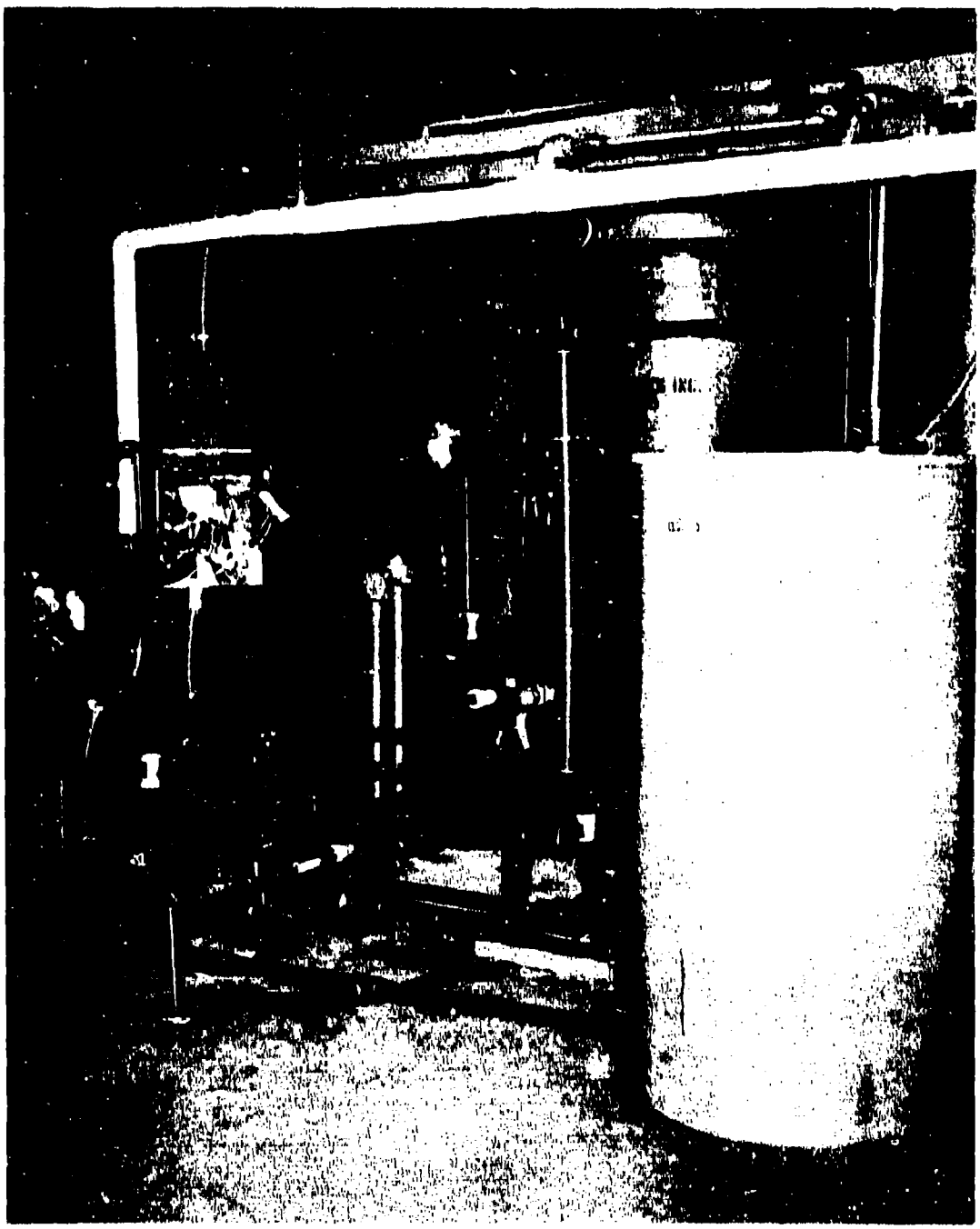
SCHEMATIC 5 CARBON-14 LABELED TNT STUDY SCHEMATIC



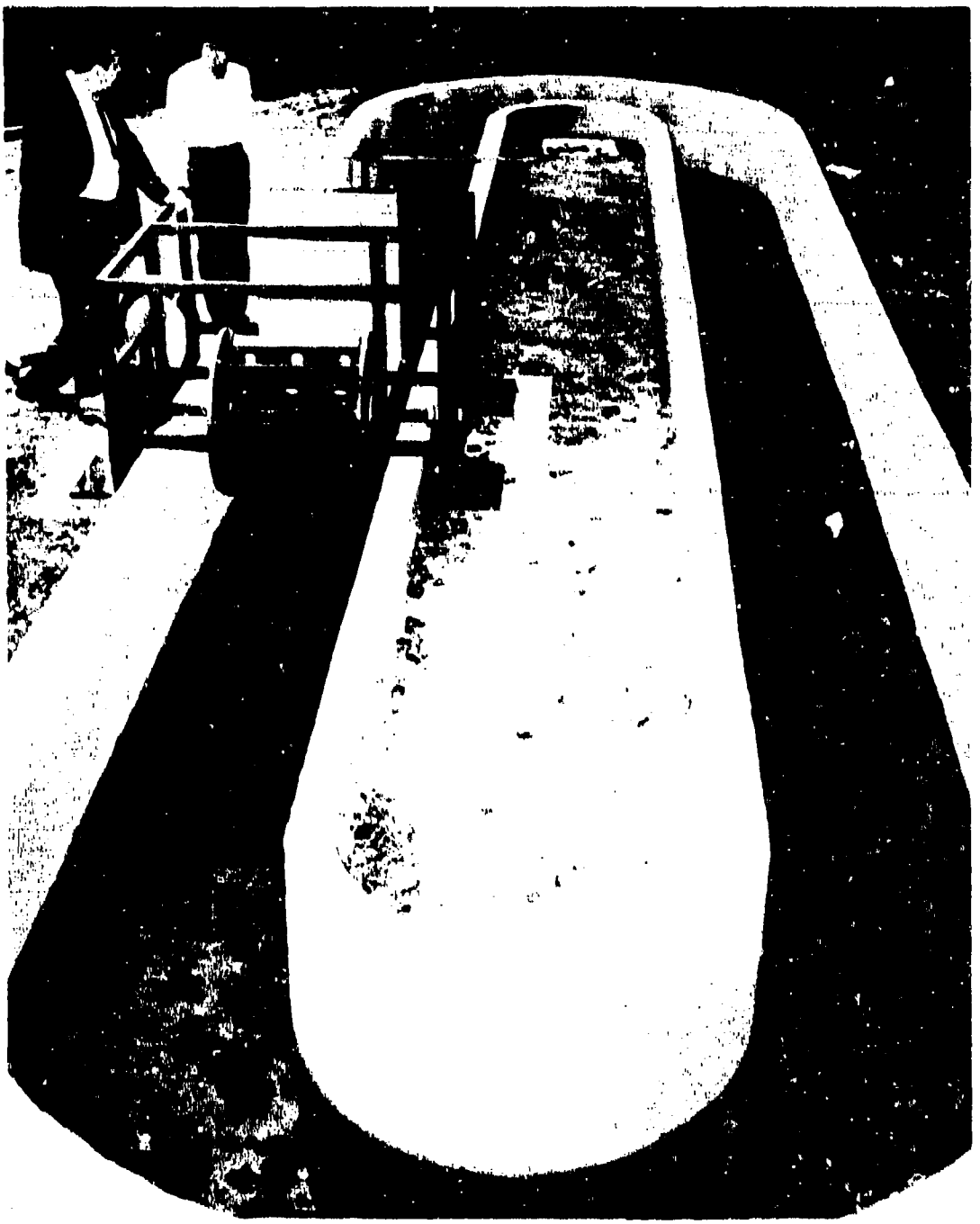
SCHEMATIC 6 C-14 WORK-UP PROCEDURE



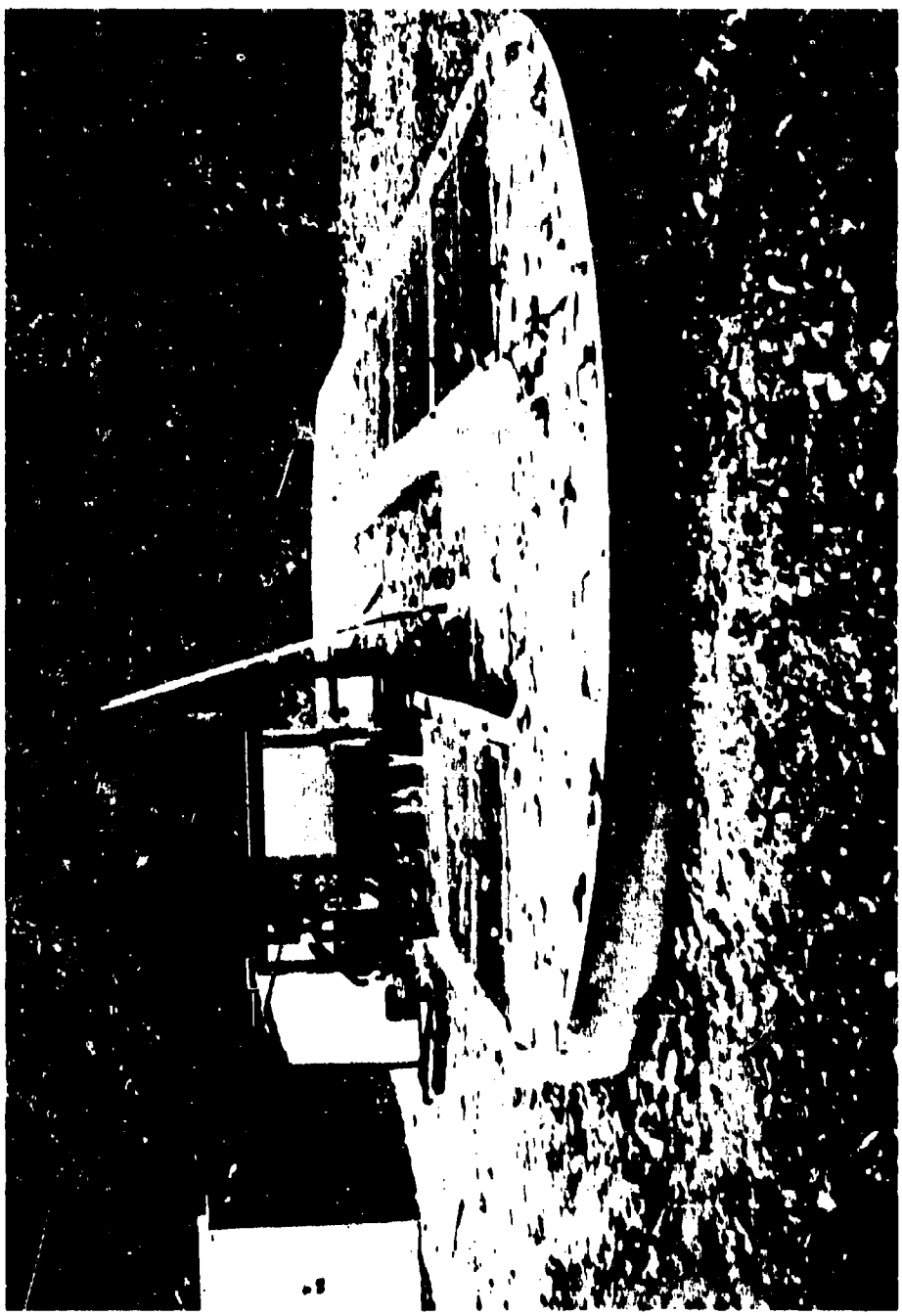
SCHEMATIC 7 PRINCETON AQUA SCIENCE BIO-MATIC BENCH-SCALE ACTIVATED SLUDGE PLANT



PICTURE 1 NSWC/WOL OXIDATION DITCH SLUDGE SETTLING TANK AND AUXILIARY EQUIPMENT



PICTURE 2 NSWC/WOL OXIDATION DITCH SHOWING RACETRACK, ROTOR, TNT/NUTRIENT FEED LINE (FOREGROUND), LINES TO SETTLING TANK AND LEVEL CONTROLLERS



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