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NITROGEN ISOTOPE FRACTIONATION IN
CHEMICAL AND MICROBIOLOGICAL SYSTEMS

by



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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled NITROGEN ISOTOPE FRACTIONATION IN CHEMICAL AND MICROBIOLOGICAL SYSTEMS, submitted by Robert Persey Wellman in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Nitrogen isotope fractionation was investigated in a number of chemical and microbiological systems with the aims of elucidating mechanisms and examining sample conversion techniques.

The basic approach differed from that of other laboratories in that increments of the product were examined isotopically rather than the integrated or accumulated product. This approach proved to be superior in interpreting isotope effects.

Problems were encountered in the accepted conversion of ammonia to nitrogen by the hypobromite technique. These were traced to the presence of oxygen in the samples and were remedied by heating the nitrogen product with copper filings.

In the chemical reduction of nitrate to ammonia, the isotope effects were consistent with N-O bond rupture being rate-controlling. However, it was found that poisoning of the catalyst may prevent this reaction from going to completion.

The isotope effects in the chemical reduction of nitrite to ammonia were smaller than those in nitrate reduction. Further, the fractionation factor between product NH_3 and the nitrogen species in solution were larger during earlier stages of the reduction. This is interpreted in terms of the build up of an intermediate pool. If the step which depletes this pool is not isotopically selective while at the same time competes for rate control, then the fractionation factor decreases with time since at

any instant this pool represents an isotopic average of the nitrite reduced over all the preceding reaction time.

The alkaline hydrolysis of urea was very interesting in that inflections were found in the curve of isotopic composition of the instantaneous product ammonia versus percentage reaction. This is consistent with the two step mechanism of urea \rightarrow carbamate + ammonia and carbamate \rightarrow carbonate + ammonia. The product ammonia is produced in both steps and can give rise to the inflections found.

The microbiological reduction of nitrate and nitrite to nitrogen in all cases revealed faster reduction of the N^{14} species by a factor of about 2 percent. In nitrate reductions, the effects were not as large as in the inorganic chemical reductions, while in the case of nitrite reduction, the biological and chemical isotope fractionations were comparable. The isotope fractionation depended upon the organism, medium, and other factors in a complex manner. The isotope effects show that N-O bond rupture is not rate-controlling and this is consistent with the findings of conventional kinetics that intermediates build up during the conversions. The results show that transfer of nitrogen from the terrestrial inorganic pool (nitrates, nitrites) through the biosphere results in the return of N_2 to the atmosphere which is depleted significantly in N^{15} in comparison to the inorganic pool.

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CHAPTER 1

INTRODUCTION

1.1 Discovery of Isotopes

Investigations carried on with radioactive substances during the early part of this century, revealed that many substances, considered "pure" in a chemical sense, actually consisted of mixtures whose components differed in their radioactive properties. BOLTWOOD (1906) reported the existence of a substance called "IONIUM" which was chemically identical to thorium. SODDY (1910) extended this type of investigation to other elements and named those species of atoms which were chemically similar, but radioactively different, "isotopes". THOMSON (1914) demonstrated the presence of isotopes for elements of low atomic weights. He established the fact that isotopes need not be radioactive, but that they are different nuclear forms of an element. Carbon, nitrogen, and oxygen isotopes were found to exist through studies of molecular spectra; the nuclear mass having a measurable effect on the observed vibrational and rotational spectra. (KING and BIRGE, 1929; GIAUQUE and JOHNSTON, 1929; NAUDE, 1930).

ASTON (1919-21) and DEMPSTER (1918-22) improved THOMSON'S positive ray apparatus and constructed, in effect, the first mass spectrograph. With this apparatus, they were able to make isotopic abundance determinations of a large number of elements.

The discovery of UREY et al (1931, 1935) of deuterium and its subsequent

concentration through distillation of liquid hydrogen and also by electrolysis of water provided a highlight in the quest of stable isotopes.

1.2 Mass Spectrometry

J.J. THOMSON pioneered in the mass analysis of ion beams. In his device, a beam of ions composed of different masses with a range of velocities passed transversely through uniform parallel electric and magnetic fields. The ion beam was split by the fields to form a family of parabolas which were detected on a photographic plate or a fluorescent screen. Each parabola corresponded to a particular charge-to-mass ratio while its length depended on the energy spread in the molecular beam. DEMPSTER and ASTON modified Thomson's apparatus by incorporating focussing arrangements. Dempster's apparatus served in many respects as a prototype for modern mass spectrometers. Ions diverging from the source were deflected 180 degrees by a uniform magnetic field and were directionally focussed. A mass separation is realized if the initial energy spread is small. The beam was focussed on an exit slit and detected electrically.

In the period around 1930, a group at the University of Minnesota, under Professor J.T. TATE designed an ion source with greatly improved characteristics. A tightly magnetically collimated beam of electrons was used to produce positive ions. Collisions between neutral atoms and the electron beam resulted in the formation of a nearly monoenergetic beam of ions.

NIER (1940) incorporated the "Minnesota" source into a spectrometer

of the Dempster type. He also was the first to use a magnetic deflection of less than 180° with electronic detection.

In recent years, the rapid development of electronics has resulted in substantial improvements in mass spectrometer design. By the use of double focussing and other special techniques, nuclidic masses can be determined to eight significant figures. Through the use of simultaneous collection, differences in isotopic abundance ratios of less than $\pm 0.01\%$ can be reproducibly measured. The use of electron multipliers in the beam detection system and ion counting techniques permit isotopic analysis of samples of less than one microgram.

1.3 Physical Isotope Effects

Purely physical processes such as diffusion result in a degree of isotopic selectivity. In many cases, the isotopic diffusion coefficients are inversely proportional to the square root of the isotopic masses in accordance with Graham's law. This rule also holds for interstitial diffusion in solids as demonstrated in a number of cases including Li^6 and Li^7 diffusion in rutile (JOHNSON and KROUSE, 1966). The isotope effect for other diffusion mechanisms can be very complex (SCHOEN, 1958).

Phase partitioning leads to isotopic fractionation. The heavier isotope is usually favoured in the more condensed phase. Such effects, however, are generally small compared with chemical and biological isotope effects. For example in the Quemont Mine, the higher melting point sulfides, pyrite and pyrrhotite are enriched in S^{34} with respect to the lower melting

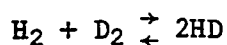
point chalcopyrites and sphalerites by only 1 %. (RYZNAR et al, 1967). However, an interesting exception exists in the hydrologic cycle. The polar regions of the earth experience precipitation depleted in H_2O^{18} by as much as 5 percent as compared with equatorial waters. (EPSTEIN and SHARP, 1959). This arises simply because H_2O^{16} has a higher vapour pressure than H_2O^{18} .

1.4 Chemical Equilibrium Isotope Effects

Failure of initial attempts to separate isotopes by chemical means, and the knowledge that chemical properties are determined by electronic configurations led to the belief that isotopes of an element were chemically identical. This concept was abandoned when UREY et al (1931) discovered and isolated deuterium.

The large mass difference between deuterium and protium made it very evident that isotopes react at different rates in many chemical systems.

RITTENBERG and UREY (1934) using statistical mechanics calculated values for the equilibrium constant for the isotope exchange reaction:



The equilibrium constant was different from four, the value depending on the temperature. FARKAS and FARKAS (1934) made similar calculations for the water-hydrogen exchange reaction. There was good agreement between theory and experiment. The calculations of UREY and GRIEFF (1935) for

isotopic exchange of many of the lighter elements (boron, carbon, nitrogen, oxygen, and chlorine) predicted differences of as high as 10 percent for the equilibrium constants. Data pertinent to exchange reactions has been summarized by UREY (1947).

1.5 Kinetic Isotope Effects

Following the discovery of deuterium, various investigators demonstrated the mass-dependence of reaction rates. WASHBURN and UREY (1932) found that electrolysis of water favours the evolution of protium. This process is still used as a means of concentrating deuterium. BONHOEFFER et al (1934) discovered that the reaction of deuterium with bromine proceeds at one third the rate of that of protium. FARKAS and FARKAS (1934) reported similar results for photo-chlorination reactions. Reviews of kinetic isotope effects have been given by UREY and TEAL (1935) and EIDINOFF (1953).

Studies of kinetic isotope effects have been extended to other elements as an aid in elucidating reaction mechanisms. Carbon isotope effects in the decarboxylation of oxalic acid and the decomposition of malonic acid were studied isotopically by LINDSAY et al (1948). Kinetic effects have been reported for reactions involving oxygen, nitrogen and sulfur; (see review article, McMULLEN and THODE, 1963); selenium (KROUSE and THODE, 1962; REES and THODE, 1966); germanium (BROWN and KROUSE, 1964); and tellurium (SMITHERS and KROUSE, 1968).

1.6 Microbiological Isotope Effects

Of special interest are kinetic isotope effects which occur during metabolic reactions in living systems. Microorganisms have been found capable of demonstrating isotopic selectivity. For example, ROSENFELD and SILVERMANN (1959) found that methane, produced during bacterial fermentation of methanol, was enriched in C^{12} by over 7 percent in comparison to the methanol present.

Since SZABO et al (1950) suggested that biological activity may explain the variations in terrestrial S^{34}/S^{32} abundances, a great deal of effort has been devoted to isotopic selectivity by S-bacteria. Direct verification for sulfur isotope fractionation in nature has been found for a number of locations. Some of these are the Cyrenaican Lakes, Africa (MACNAMARA and THODE, 1951); Texas-Louisiana Salt Domes (THODE et al, 1951); and Western North American thermal springs (SASAKI and KROUSE, 1968). Although the major sulfur isotope fractionation has been identified with a kinetic isotope effect during $SO_4^{=}$ reduction, KAPLAN and RITTENBERG (1964) have shown that isotopic selectivity can occur to a lesser extent in other processes in the sulfur cycle.

In contrast, the nitrogen cycle has not received such a systematic study. It is well known that large variations in the terrestrial N^{15}/N^{14} ratio occur. However, prior to this thesis, large alterations to the nitrogen isotope abundance had not been demonstrated with controlled biological experiments.

CHAPTER 2

THEORY OF ISOTOPE EFFECTS

2.1 Introduction

A well developed theory exists for equilibrium isotope exchange reactions. Shortly after the existence of isotopes became established, UREY and RITTENBERG (1933-34) reported marked differences in the equilibrium constants for exchange reactions. UREY and GRIEFF applied statistical mechanics to the problem of isotopic exchange, and were able to derive theoretical expressions for the equilibrium exchange constants. Later, simplifications by BIGELEISEN and MAYER (1947) and by UREY (1947) made it possible to calculate the constants from a knowledge of only the vibrational frequencies associated with the isotopic molecules. The situation is often complicated in that these frequencies are usually known only for the more prevalent isotopic species. However, it is possible to calculate vibrational frequencies for isotopic species of minor abundance from force constants which can be evaluated from a knowledge of the molecular configuration and infrared spectra-data.

In a number of cases, including nitrogen, the availability of highly enriched isotopic compounds has made possible the direct observation of the vibrational spectra. In such cases, the number of calculations are reduced and the isotopic partition function ratios are more accurate.

2.2 Equilibrium Exchange Reactions

A typical isotope exchange reaction can be written as:



where the subscripts 1 and 2 refer to the light and heavy isotopes respectively. A and B are molecules which have the exchanged atom as a common constituent. The equilibrium constant for this exchange reaction is written as:

$$K = \frac{\left[\frac{Q_{A_2}}{Q_{A_1}} \right]^a}{\left[\frac{Q_{B_2}}{Q_{B_1}} \right]^b} \quad (2)$$

where the Q's are the total partition functions of the molecules. UREY (1947) and BIGELEISEN and MAYER (1947) have shown that this constant can be expressed in terms of isotopic partition function ratios Q_2'/Q_2' which depend only on the vibrational frequencies of the isotopic molecules.

Bigeleisen and Mayer's expression is

$$\frac{Q_2'}{Q_1'} = \frac{\sigma_1}{\sigma_2} \left[1 + \sum_i \left(\frac{1}{2} - \frac{1}{u_{2i}} + \frac{1}{e^{u_{2i}-1}} \right) \Delta u_i \right] \quad (3)$$

Here $u_i = hc\omega_i/kT$ and ω_i is the i 'th vibrational frequency in cm^{-1} . The summation is over i fundamental frequencies, a d -fold degeneracy being counted d times. The ratio of symmetry numbers σ_1/σ_2 is unity if the molecule under consideration contains only one atom of the element being exchanged or if the molecule contains more than one such atom, but these atoms occupy indistinguishable positions in the molecule and are all

exchanged. $\Delta u_i = u_{1i} - u_{2i}$ is always positive. The function $G(u_i) = \frac{1}{2} - \frac{1}{u_{2i}} + \frac{1}{e^{u_{2i}} - 1}$ has been tabulated by Bigeleisen and Mayer for values

$$0 \leq u \leq 25.$$

2.3 Kinetic Isotope Effects

The general theory of reaction rates has been developed from a statistical viewpoint by EYRING (1935) and by EVANS and POLANYI (1935). BIGELEISEN (1947) has used their "theory of absolute rates" to develop an expression for the ratio of reaction rates for the competing isotopic reactions.



In such processes, the lighter isotopic species reacts at a faster rate.

$$\frac{k_1}{k_2} = \frac{K_1}{K_2} \cdot \frac{\sigma_1}{\sigma_2} \cdot \frac{\sigma_2^\ddagger}{\sigma_1^\ddagger} \cdot \frac{v_{1L}^\ddagger}{v_{2L}^\ddagger} \left\{ 1 + \sum_i^{3n-6} G(u_i) \Delta u_i - \sum_i^{3n-7} G(u_i^\ddagger) \Delta u_i^\ddagger \right\} \quad (5)$$

Here $G(u_i)$ is the free-energy function previously defined. \ddagger refers to the activated complex of absolute reaction rate theory, σ_i are symmetry numbers, 1 and 2 refer to light and heavy isotopes respectively and v_L^\ddagger is the imaginary frequency of vibration along the reaction coordinate. The ratio $v_{1L}^\ddagger/v_{2L}^\ddagger$ is generally given the value $(\mu_2^*/\mu_1^*)^{1/2}$ where μ^* denotes the reduced mass along the reaction coordinate (BIGELEISEN and WOLFSBERG, 1958;

SLATER, 1953). The ratio of transmission coefficients K_1/K_2 has been shown by HIRSCHFELDER and WIGNER (1939) to be nearly one at and above room temperatures. Equation (5) is difficult to apply because of our limited knowledge of the activated complex and hence our inability to calculate the term

$$\sum_i^{3n-7} G(u_i^\ddagger) \Delta u_i^\ddagger$$

In general, however, the activated complex will be intermediate in form between the reactants and products, enabling two limits to be calculated for the ratio of rate constants. One limit arises when it is assumed that the activated complex is like the reactant, i.e.

$$\sum_i^{3n-6} G(u_i) \Delta u_i = \sum_i^{3n-7} G(u_i^\ddagger) \Delta u_i^\ddagger \quad (6)$$

In this case, the ratio of rate constants is the ratio of the reduced masses and is temperature independent.

If the activated complex is like the product, then

$$\sum_i^{3n-7} G(u_i^\ddagger) \Delta u_i^\ddagger = \sum_i^{3n-6} G(u_i) \Delta u_i \quad \text{product} \quad (7)$$

In the case of a diatomic reactant molecule, this term is zero since there is no vibrational frequency.

Experimental values for the ratio of rate constants usually fall within these limits.

2.4 Theoretical N^{14} - N^{15} Calculations

Theoretical N^{14} - N^{15} calculations have been carried out as follows:

- UREY (1947) - frequency shifts, isotopic partition ratios, and equilibrium constants for a number of nitrogen compounds.
- SPINDEL (1954) - frequency shifts, partition function ratios for oxides of nitrogen.
- BEGUN and FLECHER (1960) - partition function ratios for oxides of nitrogen based on observed spectra of N^{15} -substituted compounds.
- MONSE (1960) - equilibrium constants for several exchange reactions involving N_2O_4 .
- SAUNDERS (1963) - kinetic isotope effects in the rupture of the hypothetical activated complex $(CH_4)_4-N^+$.
- MCCREA (1951) - intramolecular kinetic isotope effects in nitrous oxide.
- BROWN and DRURY (1965) - kinetic isotope effects in the decomposition of diazonium salts.
- BROWN and DRURY (1967) - kinetic isotope effects for the reduction of nitrate, nitrite, and hydroxylamine.

2.5 Dependence of Isotopic Fractionation on Percentage Reaction

During a chemical reaction, the isotopic composition of both the product and the reactant will change. A competition exists between the

isotopic species.



where the subscripts 1 and 2 refer to lighter and heavier isotopic species respectively. If the reaction is first order in A,

$$\frac{dX_1}{dt} = k_1[B]^n (A_{01} - X_1) \quad (3)$$

$$\frac{dX_2}{dt} = k_2[B]^n (A_{02} - X_2) \quad (4)$$

The initial conditions are $X_1 = 0$, $X_2 = 0$, $A_1 = A_{01}$, $A_2 = A_{02}$.

Dividing [3] by [4] and integrating yields

$$\frac{k_1}{k_2} = \frac{\ln \left(\frac{A_{01}}{A_{01} - X_1} \right)}{\ln \left(\frac{A_{02}}{A_{02} - X_2} \right)} \quad (5)$$

The fraction of molecules that have reacted is given by

$$f = \frac{X_1 + X_2}{A_{01} + A_{02}}$$

The fractionation observed on a mass spectrometer after a fraction f of the reaction has taken place is given by:

$$r = \frac{X_2/X_1}{A_{02}/A_{01}}$$

if the initial reactant is taken as a reference. For the cases of carbon, oxygen, nitrogen and sulfur, the heavier isotopes are rare so

$$1 + A_{02}/A_{01} \approx 1$$

$$1 + \frac{X_2}{X_1} \approx 1$$

Equation (5) can be expressed on terms of the ratios A_{02}/A_{01} and X_2/X_1 , and r and f

$$\frac{k_1}{k_2} = \frac{\ln \left[1 - f \cdot \frac{1 + A_{02}/A_{01}}{1 + X_2/X_1} \right]}{\ln \left[1 - rf \cdot \frac{1 + A_{02}/A_{01}}{1 + X_2/X_1} \right]} = \frac{\ln(1-f)}{\ln(1-rf)} \quad (6)$$

NAKAI and JENSEN (1964) have developed for a first order reaction an expression for the fractionation factor "R" which is the ratio of $\frac{X_2}{X_1} / \frac{A_2}{A_1}$ where both X and A refer to the same percentage reaction. They find

$$R = \frac{F (k_2/k_1)^{-1} - F}{1 - F} \quad (7)$$

where F is the ratio of the amount of residual reactant at time t to the initial reactant. The use of such equations is limited. They apply only to single step first order processes. For more complicated systems, different equations arise.

CHAPTER 3

NITROGEN CHEMISTRY

3.1 Introduction

Elemental nitrogen, as found in the earth's atmosphere, is diatomic. RASETTI (1929) gives an internuclear distance of 1.095 Å and indicates a triple-bond Lewis structure. The molecule possesses great stability and is unreactive under any but drastic conditions, as indicated by a high dissociation energy of 9.764 e.v. or (225.2 Kcal./mole). (GAYDON, 1944, 1945; GLOCKLER, 1948).

Nitrogen exists in combined forms in oxidation states ranging from -3 to +5, with two extremes, ammonia and nitric acid as principal compounds.

In plants and animals, nitrogen is found in the form of proteins, nucleic acids and other compounds which average in composition 51% C, 25% O, 16% N, 7% H, 0.4% P and 0.4% S.

Nitrogen in nature undergoes a series of chemical reactions often called the "nitrogen cycle". This process is represented schematically, in greatly simplified form in figure 3.1. The atmosphere functions as a reservoir from which and to which nitrogen is depleted or added. The process by which nitrogen is incorporated into other molecular structures is called "nitrogen fixation".

There are currently two known processes of natural nitrogen fixation. Lightning discharges promote the formation of NO which is spontaneously

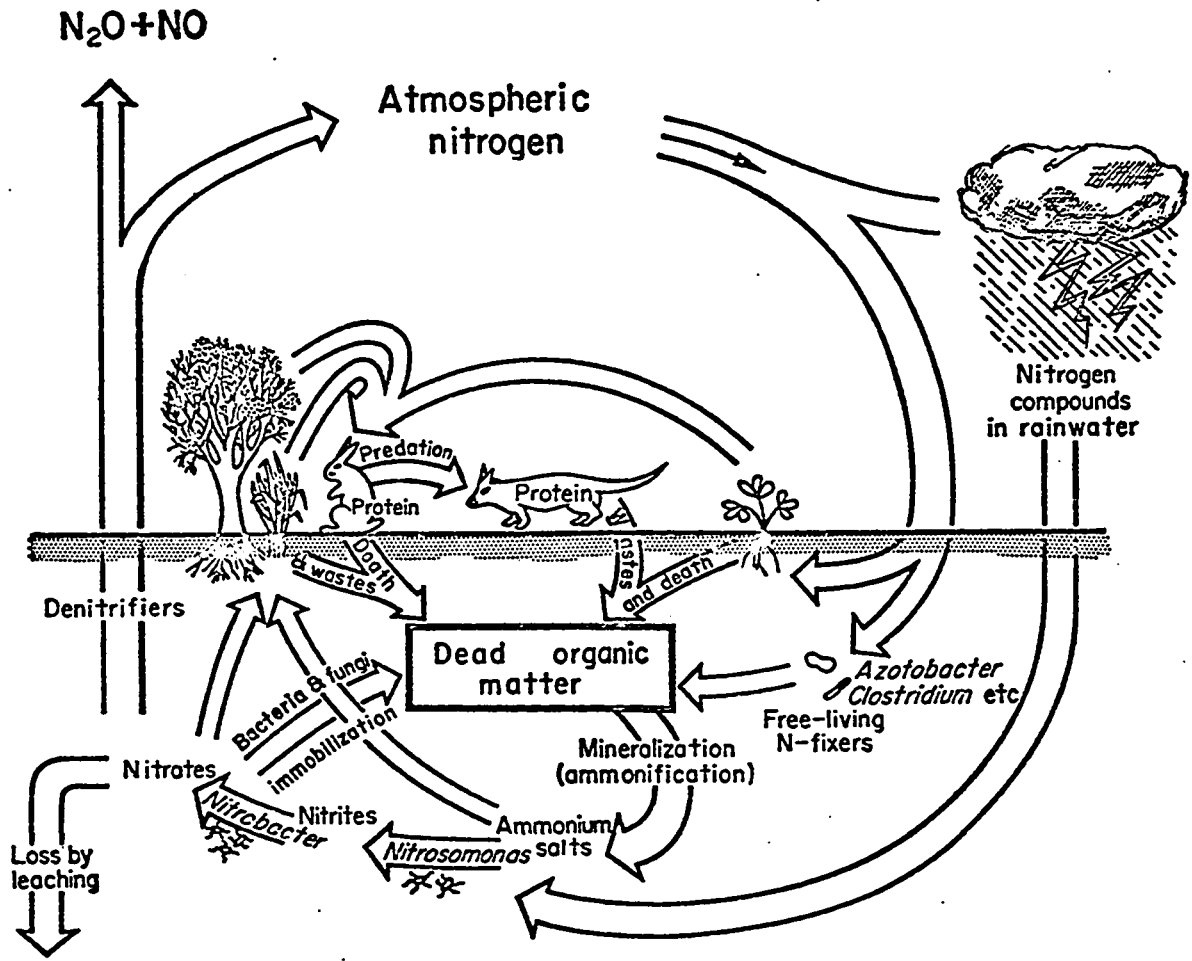


Figure 3.1 The nitrogen cycle

oxidized to NO_2 . This gas dissolves in rain water and disproportionates to form nitrates and nitrites which are returned to the soil.

The actual mechanism probably involves formation of free diradicals. At low gas pressures, an electric discharge is known to produce a chemically active and comparatively long lived afterglow with characteristic molecular spectra. Soil microorganisms are able to reduce the nitrate or nitrite to ammonia or to compounds in which the nitrogen has intermediate oxidation states, or to elemental nitrogen.

Biological fixation of nitrogen is a second method. This type of fixation is brought about by two classes of organisms; those that live in symbiotic association with higher plants, and those that are free-living or nonsymbiotic. Members of the bacterial genus Rhizobium are the most important symbiotic nitrogen fixers in agriculture. These bacteria fix nitrogen only when they are living in the roots of leguminous plants - clover, alfalfa, peas, etc. Nonsymbiotic nitrogen fixation is accomplished by a number of soil organisms particularly those assigned to bacterial genera, Azotobacter and Clostridium.

Nitrogen fixation has been accomplished on an industrial scale through use of the Haber process. At a temperature of 500°C and under a pressure of 1000 atmospheres, nitrogen and hydrogen will combine to form ammonia with about a 50% yield. This is currently the most economical method of commercial nitrogen fixation.

Organisms that give off gaseous forms of nitrogen during growth are abundant in soils, fresh waters and the ocean. These organisms, commonly called denitrifiers, use oxidized forms of nitrogen, particularly nitrate

and nitrite in the absence of oxygen as terminal electron acceptors during respiration. Denitrification is, of course, agriculturally undesirable because it results in a net loss of "fixed" nitrogen. Some common denitrifying bacteria found in nature and their metabolic products are listed in Table 3.1.

The chemistry of nitrogen is varied, complex, and interesting. Compounds range from the nearly inert to the highly explosive and of course occupy a central position in the chemistry of living systems. In spite of the great importance of nitrogen, the mechanisms of most of its chemical reactions are unknown. In fact, slight changes in reaction conditions often drastically influence the mechanism and outcome of a particular process.

The following summary is taken from two principle sources; MOELLER, Inorganic Chemistry, John Wiley and Sons, New York, 1952; MORRISON and BOYD, Organic Chemistry, Allyn and Bacon Inc., 1966. For this reason, few literature references will be given. The treatment has been abridged only to include reactions which either have been studied isotopically, or could be so studied and also those pertinent to sample preparation. Equations are intended to indicate known chemical processes. In some cases, where a mixture of products may be formed, the equations are not balanced.

3.2 Production of N₂

Nitrogen gas is a product of a number of reactions. From the analytical

TABLE 3.1

COMMON DENITRIFYING BACTERIA

Organism	Product of Reaction	Optimum Temperature Range °C
<u>Pseudomonas stutzeri</u>	1	30-37
<u>Bacillus sp. 625</u>	1	30-37
<u>Bacillus steareothermophilus</u>	1	65
<u>Bacillus sp. 405</u>	1	55
<u>Micrococcus denitrificans</u>	1	30-37
<u>Psuedomonas aeruginosa</u>	1	37
<u>Agrobacterium radiobacter</u>	2	28
<u>Agrobacterium tumefaciens</u>	2	28
<u>Cytophaga sp.</u>	2	28
<u>Alkaligenes faecalis</u>	3	28
<u>Rhizobium meliloti</u>	3	28
<u>Cytophaga sp.</u>	4	28
<u>Spirillum itersonii</u>	4	37
<u>Aerobacter aerogenes</u>	5	37
<u>Bacillus subtilus</u>	5	37
<u>Pseudomonas maltifilia</u>	6	37
Strain of <u>Escherichia coli</u>	6	37

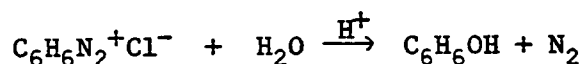
-
- | | |
|---|---|
| 1. Nitrate reduced to N ₂ | 4. Nitrite only reduced to N ₂ O |
| 2. Nitrate reduced to N ₂ O | 5. Nitrate reduced to Nitrite |
| 3. Nitrite only reduced to N ₂ | 6. Nitrite and nitrate reduced to ammonia |

viewpoint, these reactions are important, since ultimately, isotopic analysis are usually performed with N_2 .

(1) Oxidation of Ammonia Derivatives

The cold oxidation of ammonia, urea, or hydrazine, by chlorine, bromine, hypochlorite, or hypobromite yields nitrogen. The hot oxidation of ammonia by CuO , NO_2^- or $Cr_2O_7^-$ also yields nitrogen.

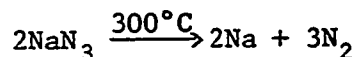
(2) Decomposition of Diazonium Compounds



Compounds such as benzene diazonium chloride decompose with first order kinetics to yield nitrogen gas. The nature of these compounds will be treated later.

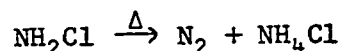
(3) Thermal Decomposition of Azides

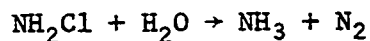
Alkali and alkaline earth metal azides decompose to the metals and nitrogen when heated above $300^\circ C$



Other metals in this group give only partial decomposition. Lead (II), Mercury (II), and Thallium (I) azides detonate when heated or struck and are used as primers. The decomposition products are similar but the mechanism seems different.

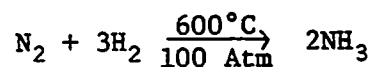
(4) Thermal Decomposition or Hydrolysis of Chloramine



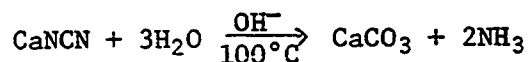


3.3 Production of Ammonia

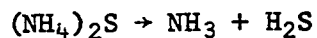
Ammonia is produced by direct synthesis (Haber Process)



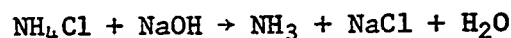
or by the alkaline hydrolysis of calcium cyanamide.



Ammonium salts are all thermally unstable. Carbonates and sulfides yield ammonia as one decomposition product.



The usual laboratory preparation involves heating an ammonium salt with a metal oxide or hydroxide.



Salts containing an oxidizing anion (NO_2^- , NO_3^- , $\text{Cr}_2\text{O}_7^{2-}$, etc.) yield various oxidation products of ammonia.

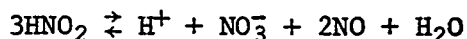
All known ammonium salts are soluble except the hydrogen tartarate, hexanitrocobaltate (III), hexachloroplatinate (IV) and the perchlorate.

Ammonia can be determined gravimetrically by precipitation of the chloroplatinate, followed by ignition. It can also be analysed colorimetrically by the use of Nessler's reagent (mercuric iodide) with which it forms a slightly soluble yellow precipitate ($\text{NH}_2\text{I}\cdot\text{H}_2\text{O}$)

3.4 Oxygen Acids of Nitrogen

Hyponitrous acid ($\text{H}_2\text{N}_2\text{O}_2$) has been prepared as a white crystalline compound. It decomposes on heating, sometimes explosively. Its aqueous solutions decompose slowly to yield nitrous oxide. Hyponitrites have both reducing and oxidizing properties.

Nitrous acid (HNO_2) has not been isolated. Solutions are prepared by treating nitrites with protonating agents. These undergo reversible decomposition



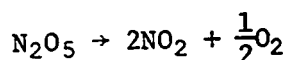
Strong oxidant such as Cl_2 , or MnO_2 convert nitrous acid into nitric acid. Atmospheric oxygen will convert nitrites to nitrates in alkaline media.

The behavior of nitrous acid as an oxidizing agent is complicated. For example, reaction with hydrogen sulfide gives nitric oxide and sulfur in acidic media, ammonia and sulfur in sodium hydrogen carbonate buffers, and ammonia, sulfur and thiosulfate in unbuffered sodium nitrite solutions. Other important oxidations are iodide to iodine, ammonium ion to nitrogen, sulfamate to nitrogen and sulfate and urea to nitrogen and carbon dioxide. Potassium iodide and starch form a quick spot test for nitrite if other oxidants are absent. The sulfanilamide test will be discussed later.

3.5 Oxides of Nitrogen

+6 State; Nitrogen trioxide (NO_3) is a blue gas which decomposes at room temperature, or in water to form nitric acid and oxygen. It is soluble in ether. The structure is unknown. It is formed by reactions between ozone and nitrogen pentoxide or nitrogen tetroxide. Fluorine reacts with nitric acid to yield the dimer N_2O_6 which decomposes rapidly with a loss of oxygen.

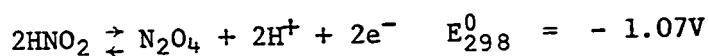
+5 State; Dinitrogen Pentoxide (N_2O_5) is the acid anhydride of nitric acid (HNO_3) and can be prepared by dehydrating nitric acid with phosphoric oxide (P_4O_{10}). It is a white solid which decomposes slowly into NO_2 and oxygen. In sulfuric, nitric and phosphoric acid, it exists as NO_3^- and NO_2^+ . The solid presumably exists as nitronium nitrate ($\text{NO}_2^+\text{NO}_3^-$). Its decomposition is known to follow first order kinetics

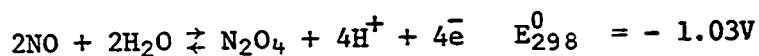


+4 State; Nitrogen dioxide, NO_2 is formed when concentrated nitric acid is reduced with metals or by the thermal decomposition of PbNO_3 , CuNO_3 or HgNO_3 . A low temperature favours formation of N_2O_4 as indicated. (20% dissociation at 27°C ; 90% dissociation at 100°C). In nitric acid, it exists as NO^+ and NO_3^- ions and in sulfuric acid as NO^+ and NO_2^+ ions. At 600°C , it decomposes:

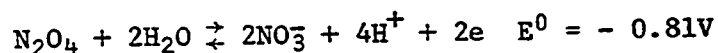


The oxides are active oxidizing agents comparable in strength to bromine.



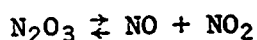


they are mild reducing agents,

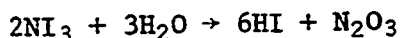


and are oxidized only by strong oxidants such as permanganates. They are toxic and corrode metals.

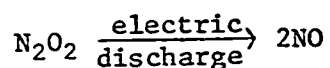
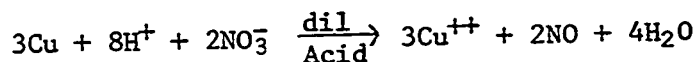
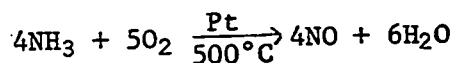
+3 State; Nitrogen sesquioxide N_2O_3 , which is the anhydride of nitrous acid, probably exists only at low temperatures in the gaseous and liquid phase. The dissociation

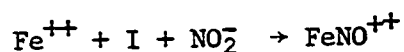
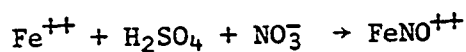


is reversible. Condensation of equimolar amounts of NO and NO_2 at -20°C gives the sesquioxide as a blue liquid. LEIFER (1940) has carried out N^{14} - N^{15} exchange studies which showed that the two N's in N_2O_3 were equivalent. N_2O_3 is formed in the hydrolysis of nitrogen triiodide

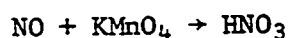
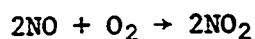
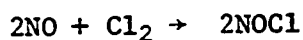


+2 State; Nitric Oxide, NO is a reactive colourless gas which does not dimerize appreciably in the gaseous phase although 97% N_2O_2 exists in the liquid phase at -163°C , as shown by paramagnetic studies. NO is produced in several ways:





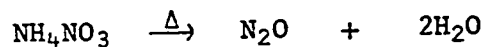
It is easily oxidized to NO_2 in air



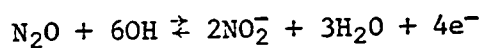
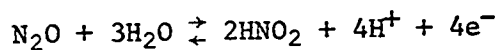
The air oxidation is known to obey third order kinetics and is favoured by low temperature. It forms nitroso complexes with transition metals such as iron.

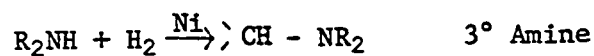
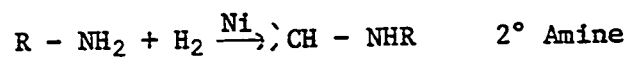
+1 State; Nitrous oxide, N_2O , is a linear unsymmetrical molecule, which is inert at low temperatures but decomposes at temperatures $\geq 565^\circ\text{C}$ into N_2 and O_2 . The kinetics are complex. It supports combustion and has appreciable solubility in fats and oils. It has found use as a propellant for shaving cream etc. It is formally the anhydride of hyponitrous acid, however, it does not react with water although it shows a high solubility. (HINSHELWOOD and BURK, 1924).

Nitrous oxide is prepared (1) by the thermal decomposition of ammonium nitrate.



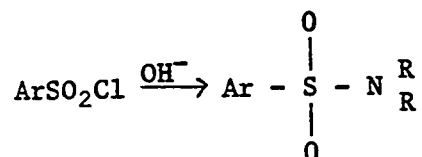
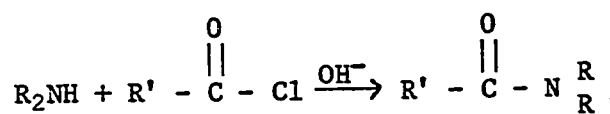
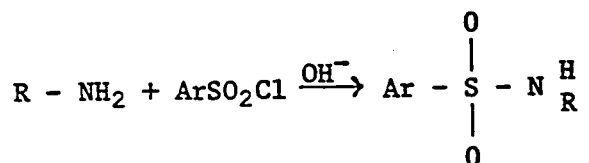
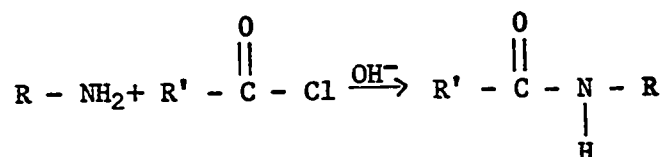
(2) by the controlled reduction of nitrites or nitrates





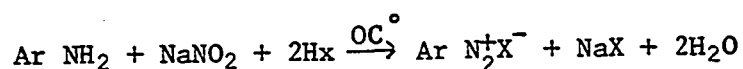
Important reactions are:

(1) conversion to amides (Hinsberg Reaction - primary and secondary amines only)



(2) Diazotisation Reaction

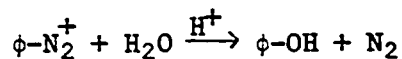
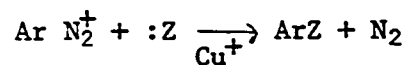
Sodium nitrite reacts with primary aromatic amines in the presence of mineral acid such as HCl at zero degrees centigrade.



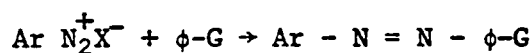
The diazonium salts are crystalline solids and many are explosive. They undergo two types of reaction,

(a) Reaction with Lewis bases such as a chloride or bromide ion

result in replacement



(b) Coupling occurs when the diazonium compound is reacted with certain aromatic compounds



G is a strongly electron releasing group such as OH, NR₂, NH₂, etc.

The Van Slyke determination of amino nitrogen is based on the replacement reaction. Primary amines react with nitrous acid to yield nitrogen. The reaction is quantitative and holds for amino acids.

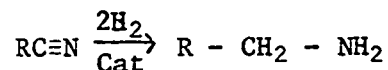
The coupling reaction is the basis of a common colourimetric test for nitrite ion. Sulfanilamide will couple with amine such as N(1 Naphthyl) ethylene diamine dihydrochloride to yield a coloured dye in the presence of nitrite.

Nitriles: Alkyl halides will react with sodium or potassium cyanide to form an organic salt called a nitrile.

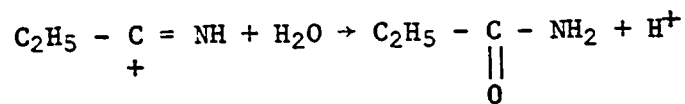
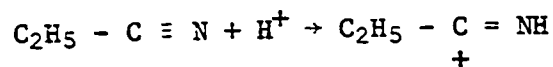


These compounds have two important reactions.

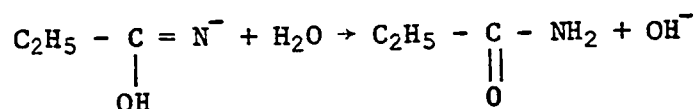
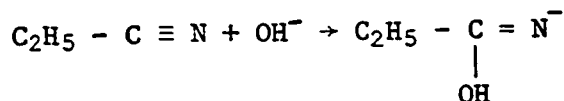
(1) They can be reduced to yield amines.



(2) They undergo hydrolysis to amides.



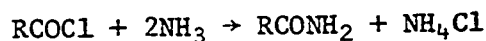
or



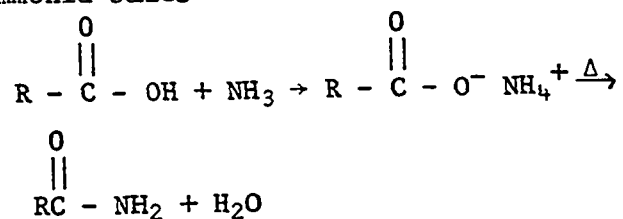
Since amides also undergo hydrolysis, this is primarily a means of preparing carboxylic acids rather than amides.

Amides: Amides are prepared as follows:

(1) From acid chlorides

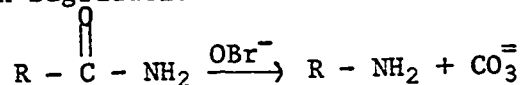


(2) From ammonia salts

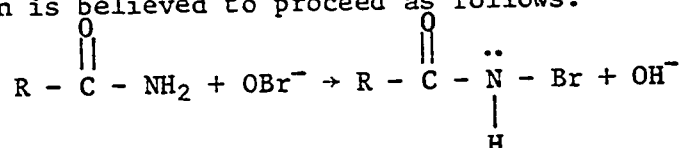


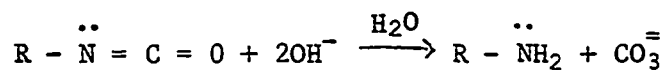
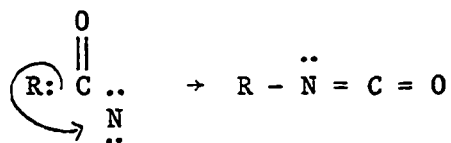
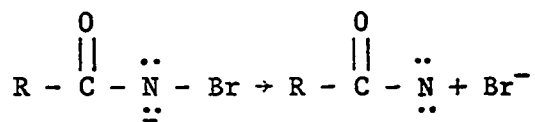
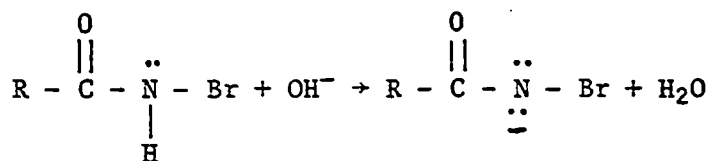
Reactions -

(1) Hoffman Degradation

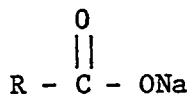
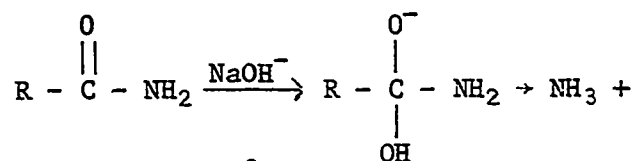
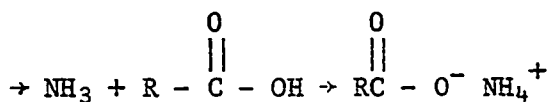
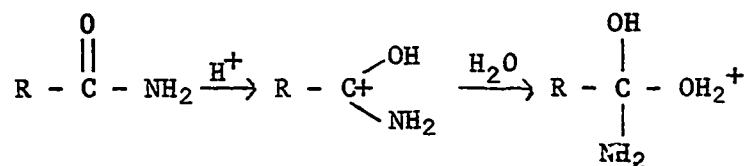


the reaction is believed to proceed as follows:

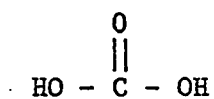




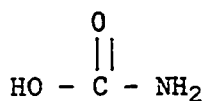
(2) Hydrolysis



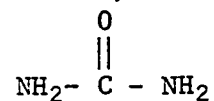
Urea and Related Compounds: Urea and certain other compounds can formally be considered as derivatives of carbon dioxide (carbonic acid).



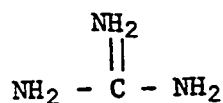
Carbonic Acid



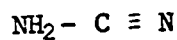
Carbamic Acid



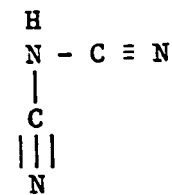
Urea



Guanidine



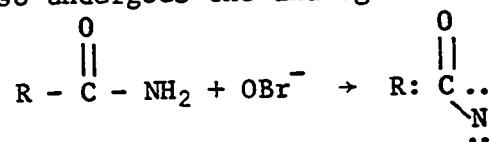
Cyanamide



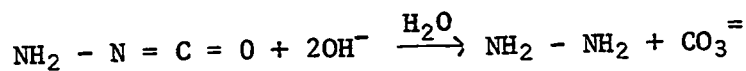
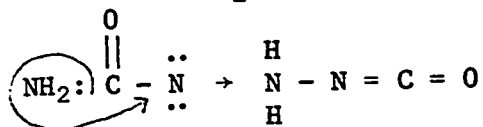
di-Cyanimide

Urea is an amide and undergoes characteristic reactions:

(1) It also undergoes the analog of the Hoffman reaction

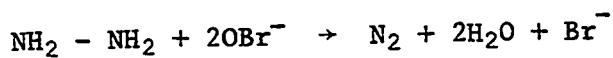


where R is now NH_2

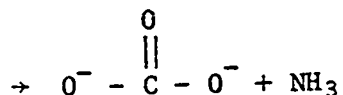
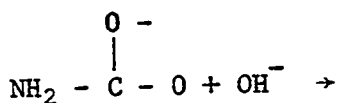
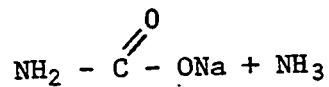
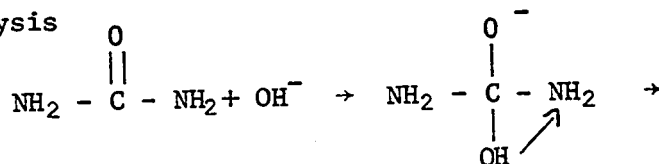


Hydrazine

Hydrazine is oxidized by hypobromite to nitrogen

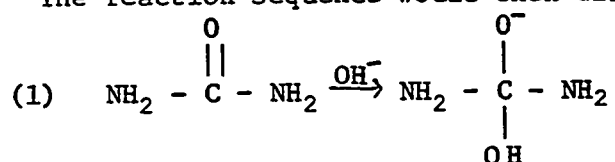


(2) Hydrolysis

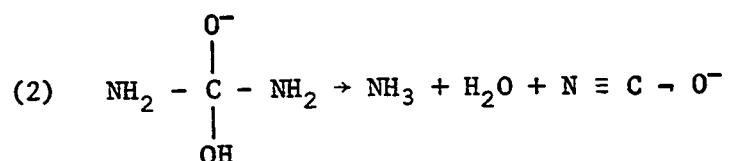


This particular reaction deserves special comment. Work by LYNN (1965) and WARNER (1942) indicates that cyanate rather than carbamate may be formed as the principle intermediate during basic hydrolysis.

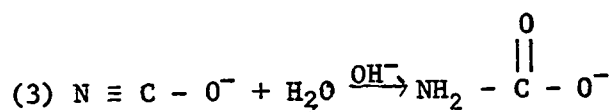
The reaction sequence would then differ in the following way.



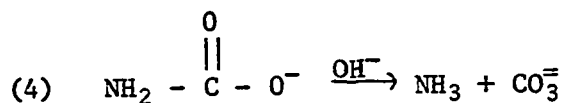
Loss of water concurrent with the elimination of ammonia would result in the formation of a cyanate ion.



The cyanate must then undergo subsequent hydrolysis,



to form a carbamate intermediate which decomposes.



CHAPTER 4

REVIEW OF NITROGEN ISOTOPE FRACTIONATION STUDIES

4.1 Terrestrial Variations

Two stable isotopes of nitrogen are found in nature; N^{14} and N^{15} . The heavy isotope N^{15} was discovered by NAUDE (1929) and has a natural relative abundance of about 0.37%. NIER (1950) reported a value of 0.365% for the relative N^{15} atmospheric abundance. DOLE et al (1954) have examined air samples collected by various locations and altitudes by balloon and rockets and has found the Nier abundance in all cases, contrary to the results of MCQUEEN (1950) who found separations of up to 3.9% at altitudes between 41 and 58 km.

Variations in the terrestrial abundance of N^{15} occur, however, within the lithosphere and the biosphere. It is conventional to express these variations in a parts per thousand difference scale as follows:

$$\delta N^{15} = \frac{\left(\frac{N^{15}}{N^{14}}\right)_x - \left(\frac{N^{15}}{N^{14}}\right)_{Atm}}{\left(\frac{N^{15}}{N^{14}}\right)_{Atm}} \times 1000$$

in $\frac{0}{00}$

where the atmospheric abundance is taken as a reference value. A positive δN^{15} value means that the sample is enriched in N^{15} with respect to the reference.

HOERING (1957) found that ammonium ion in rain water was enriched in N^{15} with respect to co-existent nitrate ion. This was attributed to a

kinetic isotope effect during the oxidation of ammonia to nitrate.

MAYNE (1957) reported variations of over 4 percent in the isotopic composition of nitrogen in igneous rocks. No age effect was discernable, but the δN^{15} value increased with decreasing total nitrogen. Mayne suggested that this was consistent with escape of N^{14} enriched nitrogen into the atmosphere. However, results of HOERING (1956) suggest that the isotopic composition of nitrogen in igneous rocks is constant.

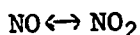
HOERING and MOORE (1957) found large variations in the N^{15}/N^{14} ratio in nitrogen in natural gas and crude oils. Since systematic variations existed in going across a natural gas field, some experiments were conducted on the diffusion of nitrogen through porous limestone. These experiments indicated that the fractionation of nitrogen isotopes in natural gas could arise in two processes - effusive flow and surface diffusion. CRAIG (1968), however, has re-interpreted these experiments in terms of "carrier diffusion" in the gas phase rather than to surface interaction.

HOERING (1955) and PARWEL et al (1957) reported on variations in the N^{15}/N^{14} ratio for a number of natural materials. The abundances ranged from $\delta N^{15} = + 12$ to $- 13$ % with respect to atmospheric nitrogen. Since differences in isotopic composition were found even among various parts of a given plant or animal species, no generalizations are possible. CHENG et al (1964) found that different forms of soil nitrogen vary in N^{15} abundance by some 30 %. 70 determinations were made and there is some evidence of trends. The hydrolyzable material was more enriched in N^{15} than the non-hydrolyzable, but this difference varied from 21 % down to 5 % for different locations and soil types. HOERING and FORD (1960)

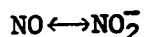
observed no isotope effect in the fixation of nitrogen by Azotobacter. Since nitrogen fixation represents the chief means by which atmospheric nitrogen enters the biosphere, HOERING suggests that any departure of the average N^{14}/N^{15} content of the biosphere from atmosphere abundance results during the return of N_2 to the atmosphere or in losses such as the formation of sediments. To date, the large N^{14}/N^{15} variations within the biosphere have not been adequately accounted for.

4.2 Equilibrium Isotope Exchange Reactions

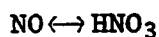
The following isotopic exchange reactions have been examined in the laboratory. In most cases, the motive was to investigate the feasibility of using the reaction in commercial N^{15} separation. Therefore, some of the separation factors quoted do not represent true equilibrium exchange constants but are dependent upon the actual refluxing conditions.



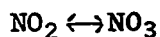
LEIFER (1940) showed exchange to be rapid and determined a value for the exchange equilibrium constant. (used optical methods). TAYLOR and SPINDEL (1948) obtained effective isotope separation using a column. BEGUN and MELTON (1956) redetermined the exchange constant. [K = 1.023 favouring N^{15} in NO_2 .] KLEIN et al (1963) showed that NO_2 is a necessary catalyst for exchange to occur.



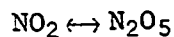
BROWN and DRURY (1968) investigated this exchange in an indirect manner and determined a value of 1.024 for the equilibrium exchange constant favouring N^{15} in NO_2^- .



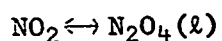
SPINDEL and TAYLOR (1955) (1956) did a preliminary study on the use of this system to obtain enriched N^{15} . KAUDER et al (1959) found values of α , from 1.065 to 1.045 at 25°C favouring N^{15} in HNO_3 . These were HNO_3 concentration dependent. BROWN and BEGUN (1959) obtained effective separation factors and recognized that many species exist in equilibrium. STERN et al (1961) investigated the temperature dependence of the exchange. TAYLOR (1962) (1963) reported the results of different exchange columns and certain reflux improvements.



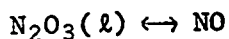
BROWN and DRURY (1968) reported a value of $\alpha = 1.057$ for this exchange favouring N^{15} enrichment in NO_3 .



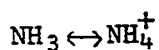
OGG (1947) reported on spectroscopic and tracer experiments which show exchange in the gas and liquid phase.



BEGUN (1956) measured this exchange by investigating isotope effects in the distillation of N_2O_4 .



MONSE et al (1960) (1963) report a higher rate of exchange for this system than with the $\text{NO} \leftrightarrow \text{HNO}_3$ system. They found effective separation factors of 1.035 at -14°C and 1.016 at $+14^\circ\text{C}$. TAYLOR (1963) discusses the results of reflux experiments using this system.



THODE and UREY (1939) obtained N^{15} enriched samples using a reflux system. KIRCHENBAUM et al (1955) investigated the dependence of exchange on the concentration of ammonium ion. ISHIMORI (1960); SPEDDING et al (1955) (1963); ROTH (1965) investigated N^{15} separation by means of ion exchange techniques.

NO \leftrightarrow NOCl

YEATTS (1958) investigated this exchange at $\sim -50^{\circ}\text{C}$ and found α to be about 1.01 favouring NOCl.

NO \leftrightarrow NOBr

NARTEN (1962) found the equilibrium constant for this exchange to be too small (1.0046 at -20°C , 1.0024 at $+8^{\circ}\text{C}$) for effective use in the concentration of N^{15} .

The results of NORRIS et al (1941) and JORIS (1941) deserve special mention since N_2 is often used as a purging gas. They investigated the possibility of exchange between N_2 and NO_2^- , NO_3^- and NH_2OH . In all cases, exchange was not evident in 360 hours at room temperature.

4.3 Kinetic Nitrogen Isotope Studies

Few studies involving kinetic isotope effects have been reported for nitrogen compounds.

FRIEDMAN and BIGELEISEN (1950) reported on oxygen and nitrogen isotope effects in the thermal decomposition of ammonium nitrate. BROWN and DRURY (1966) have reported on nitrogen isotope effects in the reduction of nitrate,

nitrite, and hydroxylamine to ammonia with Fe(II) in sodium hydroxide. TIKHOMIROV and VERGUN (1963) investigated the isotope effect in the reduction of nitric acid to nitric oxide in the presence of Hg. BROWN and DRURY (1965) investigated isotope effects in the decomposition of diazonium salts.

Studies involving fractionation during the formation of inorganic complexes have been reported. GREEN et al (1965); ISHIMORI (1960); MURMANN et al (1957); WIESENDANGER et al (1957).

HOERING (1960) reported no isotope effect during the catalytic hydrogenation of azo-benzene.

CHAPTER 5

EXPERIMENTAL PROCEDURES

5.1 Introduction

As indicated in previous chapters, research in nitrogen isotope fractionation lags behind that on other elements of importance e.g. sulfur, carbon. Part of this lag is no doubt due to the difficulty of converting samples into gases suitable for isotopic analyses. To date, only nitrogen gas and nitrous oxide have been successfully analyzed in a mass spectrometer. Part of our experimental work has been the investigation of chemical techniques for sample preparation. Another aspect of our work has been an investigation of fractionations occurring in biological systems or in chemical systems of biological interest. The second aspect has naturally been limited by the first.

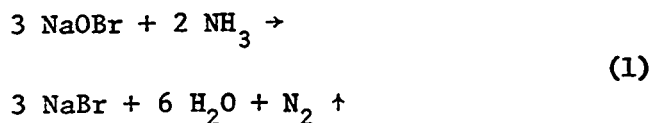
In some of our microbiological experiments, nitrous oxide was believed to be the main product. However, traces of carbon dioxide, which were also produced, were difficult to remove despite trapping in alkaline solutions. These gases cannot be separated by distillation because of similarities in vapour pressure. They mutually interfere mass spectrometrically (e.g. $C^{12}O^{16}O^{16}$ and $N^{14}N^{14}O^{16}$ at mass 44). This problem can no doubt be resolved to permit both nitrogen and carbon isotope fractionation studies in biological systems. However, this was not pursued and all determinations in this thesis were done on nitrogen gas.

Where nitrogen gas is not the direct product of a process, additional chemical reactions are necessary for sample conversion. Ammonia can be converted to N_2 by a hypobromite oxidation technique as developed by SPRINSON and RITTENBERG (1949). This makes possible the analysis of Kjeldahl digestions and also kinetic studies on systems which yield ammonia as a product. As an example of the later, we have examined isotopic fractionation during the alkaline hydrolysis of urea. This material is produced as an end product in protein metabolism and its hydrolysis is a representative reaction for a large class of organic compounds. Our results in this case were surprising and will be discussed in detail later.

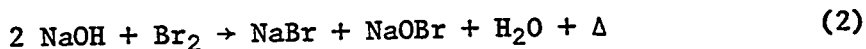
Nitrates, nitrites, and hydroxylamine can be reduced to ammonia and converted by hypobromite oxidation to nitrogen gas. BROWN and DRURY (1967) have investigated the kinetic isotope effects in these reductions. We have also examined these reductions in the cases of nitrate and nitrite to determine their suitability for routine analysis. A number of factors not discussed by the above authors were found to influence the isotope effects.

5.2 Hypobromite Oxidation

Our hypobromite oxidation of ammonia to nitrogen gas is based on that given by SCHOENHEIMER and RITTENBERG (1938); SPRINSON and RITTENBERG (1949). The reaction is represented as



The hypobromite solution was prepared by adding 50 ml of reagent grade bromine to 150 ml of 30% NaOH solution in an ice bath over a 10 minute period. A second portion of 150 ml of 30% NaOH is then added along with a trace of potassium iodide. The solution is lemon yellow in colour when fresh, but fades as the solution ages. It only retains its strength at room temperature for about 4-5 days, but if refrigerated does not deteriorate as quickly. A considerable amount of sodium bromide forms as a precipitate during and after preparation. This was not removed in our experiments and did not interfere with the oxidation. The deterioration involves oxygen production which sometimes does interfere with mass spectrometric analysis. Equation (2) shows the preparation while equation (3) represents the decomposition.



The decomposition (3) is accelerated by traces of copper. It probably takes place through a free radical mechanism since iodide is an inhibitor and copper is known to be a catalyst (PRYOR, 1965).

The reduction is carried out in an inverted Y-tube as shown in figure 5.1. The hypobromite solution was normally introduced into the bottom of the tube, with the ammonia sample solution in the side arm. The system was then evacuated by means of a fore-pump and mercury diffusion pump. The hypobromite solution out-gases and may froth. The ammonia solution will boil. The water vapour pulled off tends to

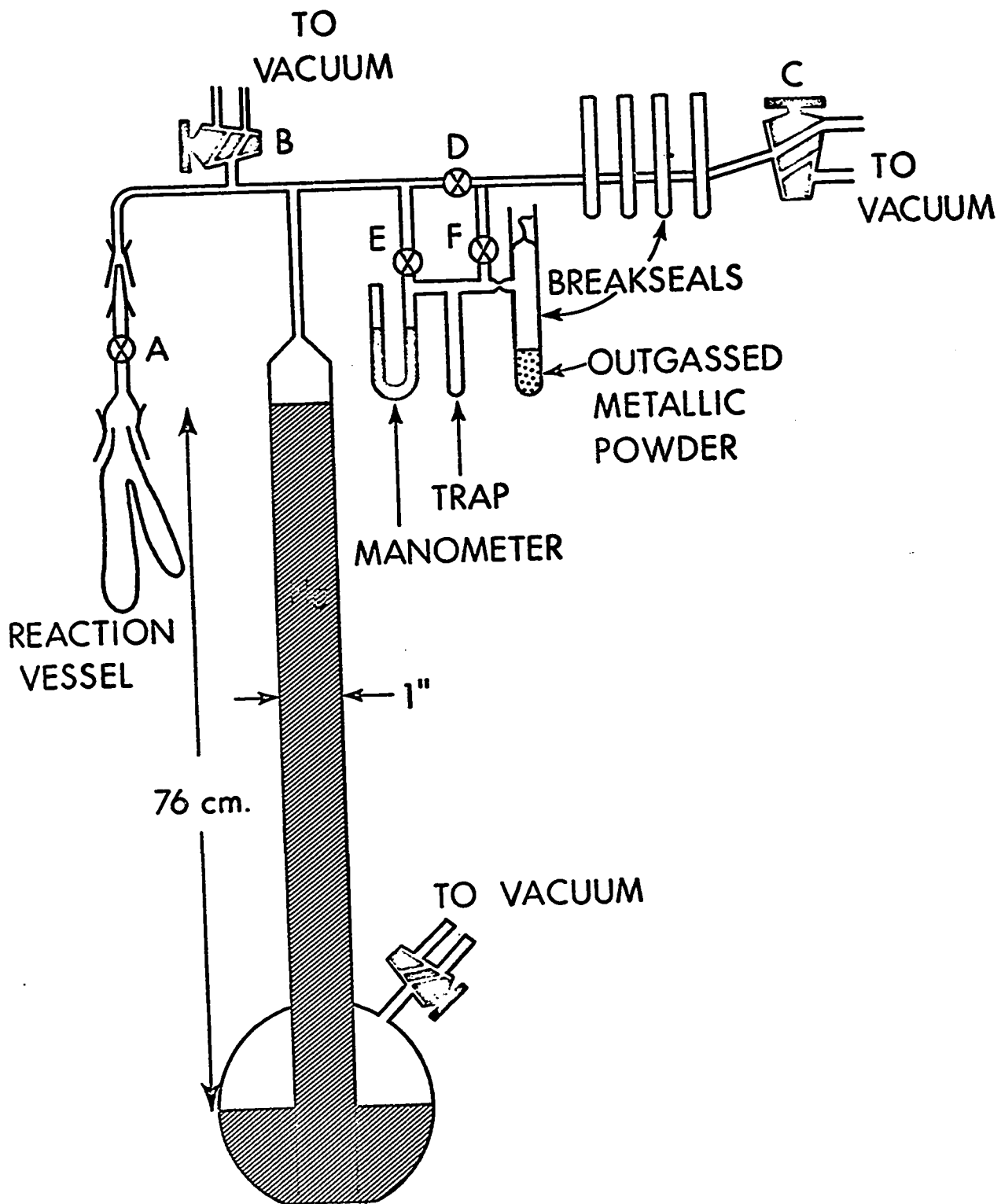


Figure 5.1 Schematic drawing of sample transfer line

sweep residual atmospheric nitrogen from the system quite rapidly so extensive pumping does not seem to be needed. After evacuation is complete, the stopcocks are closed. The reaction vessel is removed and the two solutions are mixed. A certain amount of heat is evolved and most but not all of the yellow colour due to the hypobromite should disappear. The reactor is then connected to the transfer line of figure 5.1 and the ground joint is pumped out. The system is then isolated from the vacuum line and the sample gas is admitted by opening the valve on the reaction vessel. All valves are then closed and the gas is forced into the breakseal by means of the mercury ram. The cold finger-trap was cooled by liquid nitrogen to remove water vapour. The break seal is then removed and the system is prepared for the next sample.

An incomplete reaction may result in considerable error. The final solution after reaction should have a distinct yellow colour showing the presence of an excess of hypobromite. This is especially important since the strength of the hypobromite deteriorates with age.

It was observed that a slight heating of the reaction vessel after mixing resulted in a larger "apparent" sample size. This was, however, due entirely to water vapour and oxygen evolved from the excess hypobromite since no difference in nitrogen quantity was observed on the mass spectrometer. Therefore, heating of the reaction tube serves no useful purposes and is in fact detrimental.

Oxygen from the hypobromite may react with carbon in the mass spectrometer source to form CO. Since C^{13} has a higher abundance (1%) than N^{15} (0.3%), CO contamination yields a false enrichment of N^{15} . Samples badly contaminated with oxygen cause the mass spectrometer to behave erratically and the observed isotope ratio appears to change with time.

In later work, we introduced a few grams of copper powder into the breakseals prior to filling. The sample with the copper was then baked in an oven at about $450^{\circ}C$ for a few hours. The copper usually blackens because of traces of O_2 in the hypobromite procedure. Samples treated in this way definitely behave better in the mass spectrometer and are more reproducible.

5.3 Nitrate and Nitrite Reduction (Chemical)

Nitrate is reduced to ammonia by ferrous iron in alkaline media with a silver catalyst. The reaction presumably goes by stages, first to nitrite and finally to hydroxylamine and ammonia. The first step, nitrate to nitrite is slower than succeeding steps (BROWN and DRURY, 1967).

Stock solutions of the following reagent grade chemicals were prepared: 30% NaOH (wt/wt); 0.1M. HCl; 0.1M. KNO₃; 1.0M FeSO₄; saturated Ag₂SO₄ was added, along with water to yield a final volume at 175ml. A nitrogen purge, a splash trap and condenser, and a pressure equalizing separatory funnel (75ml) was fitted to the flask along with a heating mantle as shown in figure 5.2. The mixture was heated to boiling at which time 50ml of the ferrous sulfate solution were added by means of the separatory funnel. Nitrogen purging was started when the mantle was first heated.

The product ammonia from a delivery tube at the upper end of the condenser was collected in sample vials containing 5.00ml of 0.1M HCl solution, or in 0.05M H₂SO₄ in a sealed trap as discussed later. In earlier runs, phenolphthalein was used as an indicator while in later experiments, methyl red was used. A Texas Instrument chart recorder was used to record times for kinetic studies. The input to the recorder was connected to a voltage divider, dry cell battery and push button switch. Closing the switch produced a blip on the chart paper which served as a time marker.

The recorder was turned on when the ferrous sulfate solution was added. When the first colouration was observed in the indicator, the solution was removed and the time was marked on the recorder by pushing

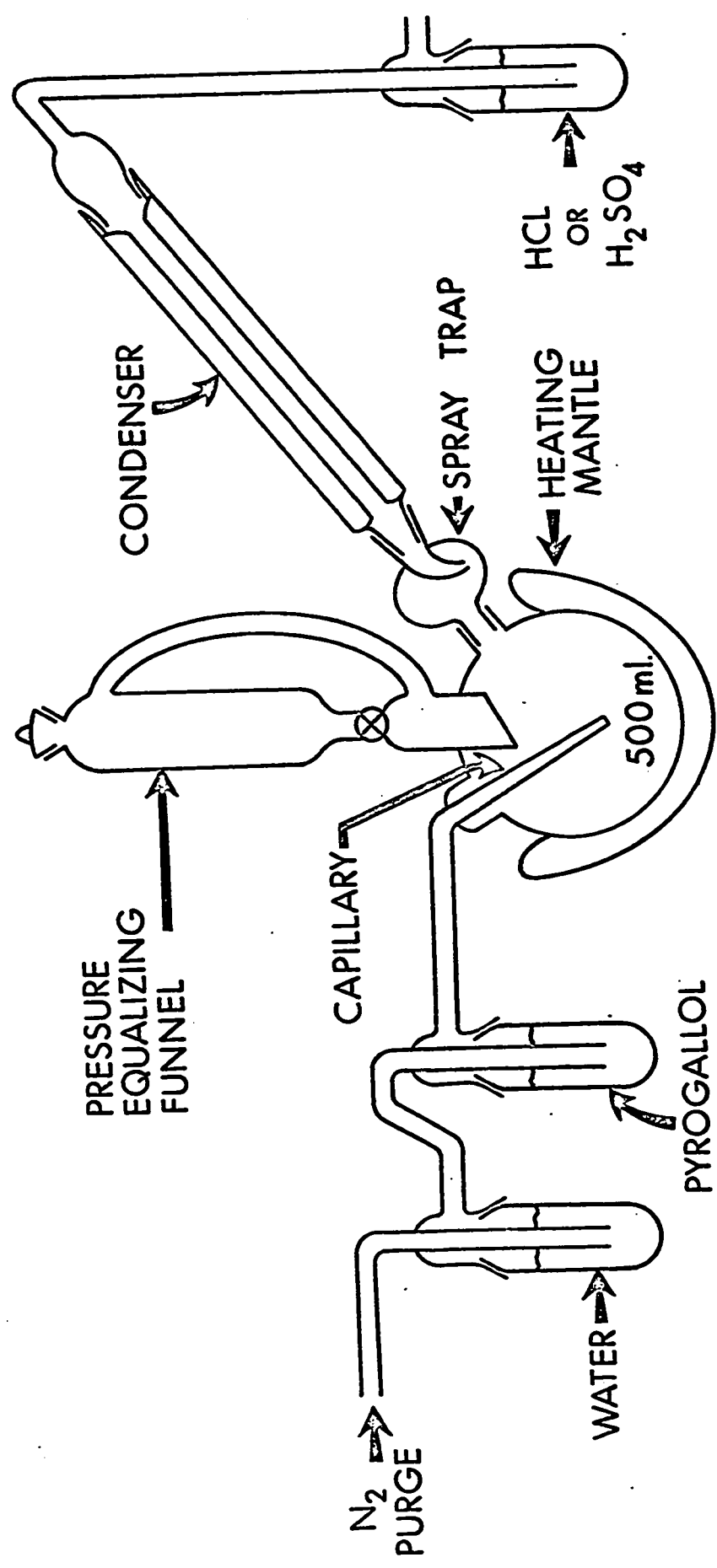


Figure 5.2 Experimental setup for sample reduction and urea hydrolysis

the button. When nine such samples were collected, the quantity of HCl was reduced to 2.5ml, and finally to 1.0ml.

After reduction, the samples were back-titrated with the standard HCl, using an eye-dropper which delivered 0.04ml/ drop. The last sample was titrated with standard 0.1M. NaOH using the same eye dropper. In all experiments, the volume of the initial nitrate solution was controlled by use of a pipet or a buret. Other volumes were approximate (graduated cylinder).

The reaction was assumed to go to completion when the last sample failed to change colour in a half hour time period. The quantities of reagents used were such that the calculations are simple. In principle, 50ml of HCl should correspond to 10% reaction.

Similar reductions were carried out with nitrite, 50.0ml of 0.1M KNO_2 being used in place of the nitrate. The remaining procedure was the same as that for the nitrate.

5.4 Nitrate and Nitrite Reduction (Microbiological)

Two categories of nitrate and nitrite reduction are distinguished by microbiologists. The first, assimilation of nitrate or assimilatory nitrate/nitrite reduction, in which either anion is reduced for synthesis of cell material. The second, denitrification, the process whereby certain aerobic microorganisms are able to grow anaerobically with nitrate as the terminal electron acceptor. This process is often termed nitrate or nitrite respiration or dissimilatory nitrate/nitrite reduction. The products of reduction

in this process are either N_2 or N_2O or a mixture of both gases.

The bacteria selected for these experiments are of the second type and are commonly called "denitrifiers". Details about the organisms are listed in Table 5.1.

Preparation of media: For growth experiments, Penassay Broth (Difco) was used for all organisms except Bacillus I₂. For growth of this organism (run D.) a medium with the following composition was used: K_2HPO_4 0.08; KH_2PO_4 0.02; $Mg SO_4$ 0.02; $NaCl$ 0.02; $Fe SO_4$ 0.0002; $Na MoO_4$ 0.0002; Yeast extract (Difco) 0.5 and peptone (Difco) 0.5. All figures are percentage w/v. An additional amount of crystalline vitamin B_{12} (2 $\mu g/ml$) was added to this medium because Bacillus I₂ was shown to require large amounts of this vitamin for growth. Sterile KNO_3 or KNO_2 was added aseptically to all media at the concentrations required.

Resting cell experiment: Resting cells of P. stutzeri were prepared by growing organisms in appropriate nitrate broth for 48 hours at 28°C. The cells were harvested by centrifugation and washed twice in cold sterile 0.1M phosphate buffer at pH 7.0. Freshly prepared cells were added to the following reaction mixture:

TABLE 5.1

DENITRIFIERS SELECTED FOR REDUCTION EXPERIMENTS

Bacteria	Run	Major Product of Denitrification	Source
<u>Pseudomonas stutzeri</u>	A,B,G	N ₂	isolated from soil
<u>Alcaligenes faecalis</u> 4456	F	N ₂	isolated from bovine rumen
<u>Bacillus</u> 625	C,E	N ₂	isolated from soil
<u>Bacillus</u> I ₂	D	N ₂	isolated from soil
<u>Agrobacterium radiobacter</u> 500	H	N ₂ O	isolated from soil
<u>Agrobacterium tumefaciens</u> SU 586	I	N ₂ O	isolated from soil
<u>Agrobacterium tumefaciens</u> 1025	J	N ₂ O	isolated from tomato galls.

Runs A, B, C, and D are growth experiments in which nitrate was reduced. E and F are growth experiments in which nitrite was reduced. G was a resting cell experiment in nitrate reduction. Samples from H, I, J have not been analyzed as yet isotopically because of CO₂ contamination.

0.01 M phosphate buffer pH 7.3
0.002 M Na succinate
0.01 M KNO_3

Washed cells constituted 40 mg dry wt/liter. Reaction temperature was 30°C. For this experiment, 2 liters of the mixture were used for collection of the gas.

Sample collection: Figure 5.3 is a schematic representation of the sample collection system used. Inoculated medium was placed in the two liter flask, which had been previously sterilized. The ground joints were carefully flamed, greased and the flask mounted in position. Liquid nitrogen was placed on the trap and the system including the 500 cc sample bulb was evacuated. The media froths and boils so that a certain amount of water collects in the trap. The valves were turned so that the flask was continuous with the sample bulb but isolated from the vacuum line. When sufficient sample had been collected in the bulb, the time was recorded and the flask was isolated from the system. Liquid nitrogen was placed around the bulb to freeze down water vapor and carbon dioxide. The pressure was then measured with the manometer. The bulb was removed for analysis and another evacuated bulb installed. The system was evacuated for about 5 minutes prior to each sample collection to remove previously produced residual nitrogen. The loss of product N_2 represents a cumulative error of about 5 percent.

The samples were analysed immediately following a run or concurrent with it in order to minimize the chance of air leakage into the storage bulbs.

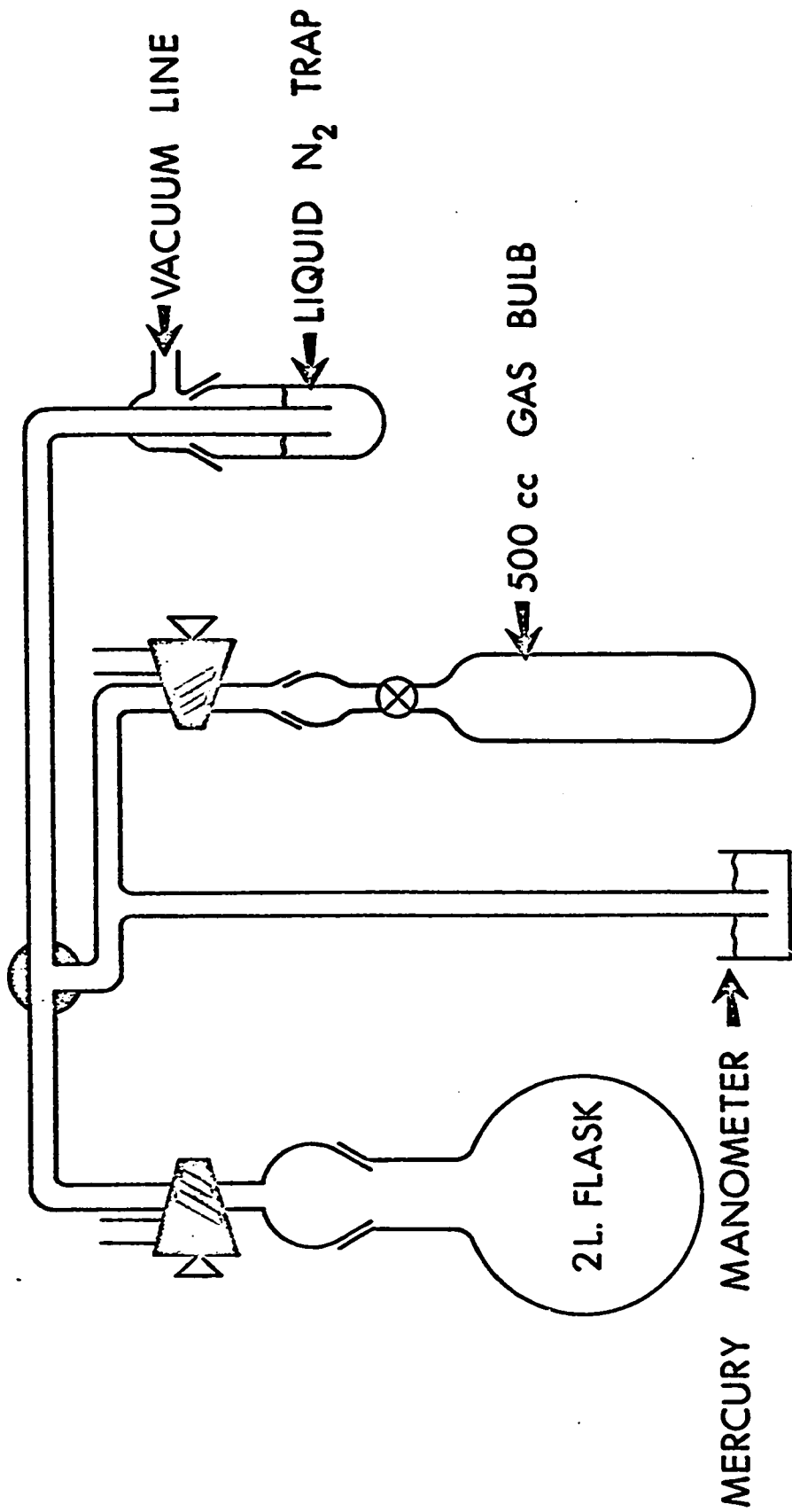


Figure 5.3 Sample line for microbiological reduction

The samples were frozen with liquid nitrogen prior to analysis to freeze out CO₂ which gives abnormal results as previously mentioned.

The same nitrate and nitrite was used in all microbiological work, so all samples have the same isotopic reference, + 5.0 for the nitrate and + 10.0_‰ for the nitrite.

In certain cases, samples of the media were removed and analysed for residual nitrate and nitrite. No attempt was made to do isotopic analysis on these since procedures and techniques had not yet been developed.

5.5 Hydrolysis of Urea

Urea was hydrolysed in alkaline solution. The product ammonia was collected in HCl or in H₂SO₄ and was converted to nitrogen by the hypobromite oxidation previously described. The experimental set-up is shown in figure 5.2. Kinetic data was obtained in the same manner as that described under nitrate reduction.

Run #1 (8.0M NaOH; 0.25M Urea)

100ml of 30% NaOH (wt/wt) were heated to boiling in the 3-neck flask. 25ml of 1.005M urea were added by means of the separatory funnel. Product ammonia was collected sequentially in 5ml samples of 1.07M HCl in 10ml sample vials, using phenolphthalein as an indicator.

Run #2 (16.0M NaOH; 0.20M Urea)

64.0g NaOH were added to 62.5ml of water in the 3-neck flask and heated to boiling. 1.5g of urea were dissolved in

20ml of water and added via the separatory funnel. Methyl red was used as an indicator (0.1g of the indicator was dissolved in 60ml of ethanol. Water was then added to give a final volume of 100ml.) Product ammonia was collected in sample vials as above.

Run #3 (5.0M NaOH; 0.8M Urea)

50g of NaOH were added to 1.50ml of water in the 3-neck flask. 12g of urea were dissolved in 100ml of water which was added to the sodium hydroxide. The resulting mixture was brought to a boil and product ammonia was collected in 10.0ml quantities of 2.06M HCl in small demountable traps.

Run #4 (8.0M NaOH; 1.92M Urea)

80g of NaOH was dissolved in 200ml of water and brought to a boil in the 3-neck flask. 50ml of 1.0M urea was added through the separatory funnel. Samples were collected in 5ml quantities of 1.03N H₂SO₄ containing methyl red. The acid was placed in the first trap. The second trap contained 0.04ml of 1.03N H₂SO₄ and methyl red indicator and served as a check on the first trap.

5.6 Mass Spectrometry

The mass spectrometer used is a 12 inch radius, 90° magnetic analyzer featuring simultaneous collection of masses 28 and 29 and digital recording, as described by MCCULLOUGH and KROUSE (1965).

The standard and unknown were alternately introduced into the mass spectrometer through a magnetic valve system. The mass 28 ion current of the unknown was matched with that of the standard prior to print out by control

of sample pressure. In the absence of contaminants, (CO, O₂, CO₂) comparisons are valid to within 0.2 %.

The sample inlet system is conventional, featuring dual sample and standard lines, capillary leaks, adjustable gas storage bulbs and associated atmospheric cut-offs.

CHAPTER 6

EXPERIMENTAL DATA

6.1 Calculation of Reservoir Nitrogen

In any physical or chemical process, the law of conservation of mass applies to each isotopic species. If the isotopic composition of the whole system, called the reference is known relative to some standard, and if samples n_i are sequentially removed, then assuming $N^{15}/N^{14} + N^{15} \approx N^{15}/N^{14}$,

$$N \left(\frac{N^{15}}{N^{14}} \right)_{\text{ref}} = \sum_{i=1}^K n_i \left(\frac{N^{15}}{N^{14}} \right)_i$$

$(N^{15}/N^{14})_i$ is the isotopic composition of the i^{th} sample; n_i is its mole number, (or any number proportional to it). N is the total moles; $(N^{15}/N^{14})_{\text{ref}}$ is the isotopic composition of the whole system.

In any process, the composition of the "unreacted" part which we have called the reservoir can be calculated if the reference, initial amount, and product are known. After Q samples have been collected, the reservoir composition is given by.

$$\left(\frac{N^{15}}{N^{14}} \right)_{\text{res.}} = \frac{N \left(\frac{N^{15}}{N^{14}} \right)_{\text{ref}} - \sum_{i=1}^Q n_i \left(\frac{N^{15}}{N^{14}} \right)_i}{N - \sum_{i=1}^Q n_i}$$

The instantaneous fractionation is the difference at any point in a process between the isotopic composition of the product and the reservoir. A slight complication occurs in that the isotopic composition of the reservoir is known for a specific point while the value obtained for the product is the mean value over some interval. For this reason, graphical methods offer a distinct advantage. We have obtained instantaneous fractionation values at the mid-point of the sample collection interval. It is possible to obtain meaningful values up to about 75% of the reaction. Several factors limit the calculations beyond this. In these regions

$$N - \sum_i n_i$$

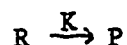
becomes small and uncertainties in n_i introduce progressively larger errors into the calculated value for the reservoir. Further, $(\delta N^{15})_{n_i}$ usually has a sizable positive value and these large isotopic differences cannot be measured as accurately with the mass spectrometer. Further $(\delta N^{15})_{n_i}$ is increasing very rapidly with the amount of reaction in these regions. Another problem arises in that errors accumulate during the course of the reaction i.e. a calculation based on a sample collected at say 90% reaction is subject to the errors of all the previously collected samples.

6.2 Experimental Data

The following section contains both tabulated and graphical data. In the chemical experiments, isotopic analysis was not performed on all reductions carried out. In certain cases, the reactions were known to be

incomplete, or the experiment was known to be deficient in some respect. In these cases, the whole experiment was repeated, but certain of these experiments yielded, for example, good kinetic data.

A first order rate process can be represented as



with rate equations

$$\frac{-dR}{dt} = KR$$

(a)

$$\frac{dP}{dt} = K(P_{\max} - P)$$

(b)

In general, if intermediates exist, $-dR/dt$ need not equal dP/dt . This is often the case in heterogeneous catalysis where there may be a significant time lag between the disappearance of reactant and the appearance of product.

Kinetic data was obtained in this thesis on the basis of product collected. Integration of equation (b) above yields

$$\ln \left(1 - \frac{P}{P_{\max}} \right) = \ln \frac{1}{P_{\max}} + K(t-t_0)$$

(c)

The quantity P/P_{\max} represents the fractional degree of the reaction and for convenience has been expressed as percent reaction/100. The quantity

$1 - \frac{\%R}{100}$ has been plotted on semilog paper so that figures 6.1 - 6.3, 6.8 - 6.10, 6.14 - 6.15 display the linear relation implied by equation (c) above.

An ambiguity exists in the choice of the initial time t_0 . With two exceptions, the zero point in time was taken at the instant that reactants

were mixed. A time lag occurs between this time and the first appearance of product. In two of the nitrate reduction experiments, (table 6.4, 6.6 and figure 6.3) the zero point of time was established on the basis of product formation by inserting a drop of indicator in the top of the condenser. The recorder was operated in the normal fashion except that t_0 was taken as the time at which the indicator changed colour.

TABLE 6.1

KINETIC DATA

NO₃⁻ Reduction (Chemical) 4.0M NaOH*

Sample	Time (sec)	Millimoles NH ₃	% Reaction	$1 - \frac{\%}{100}$
(1)	262	.50	13.0	.87
(2)	357	.54	27.0	.73
(3)	389	.532	40.9	.591
(4)	453	.536	55.0	.45
(5)	537	.528	68.5	.315
(6)	657	.512	82.0	.18
(7)	921	.516	95.4	.046

* 90mls 30% NaOH

TABLE 6.2

ISOTOPE FRACTIONATION

NO₃⁻ Reduction - 4.0M NaOH

Sample	% Reaction	δ N ¹⁵ (NH ₃)	δ N ¹⁵ Reservoir	I.F.
1	0 - 10.8	- 49.7	+ 11.6	1.058
2	10.8 - 21.4	- 39.7	+ 18.5	1.055
3	21.4 - 32.2	- 32.2	+ 26.6	1.053
4	32.2 - 42.8	- 24.0	+ 36.0	1.055
5	42.8 - 53.0	- 17.0	+ 47.5	1.058
6	53.0 - 63.4	- 12.0	+ 64.5	1.067
7	63.4 - 73.5	- 10.0	+ 94.0	--
8	73.5 - 77.0	- .2	-	--

NO₃⁻ Reference = + 5.0

TABLE 6.3

KINETIC DATA

NO₃⁻ Reduction (Chemical) 2.66M NaOH*

Sample	Time (sec)	Millimoles NH ₃	% Reaction	$1 - \frac{\%}{100}$
(1)	261	.50	13.9	.861
(2)	353	.66	32.9	.671
(3)	420	.52	46.5	.536
(4)	545	.576	62.4	.370
(5)	758	.516	76.7	.233
(6)	816	.172	81.6	.184
(7)	893	.116	85.0	.15
(8)	∞	.536	--	--
(1)	195	.56	15	.85
(2)	296	.56	33	.67
(3)	338	.604	48.4	.516
(4)	424	.58	64.4	.356
(5)	632	.556	79.5	.205
(6)	908	.556	94.5	.055

* 60mls 30% NaOH

TABLE 6.4

KINETIC DATA

NO₃⁻ Reduction (Chemical) 1.08M NaOH*

Sample	Time (sec)	Millimoles NH ₃	% Reaction	$1 - \frac{\%}{100}$
(1)	27	.50	9.6	.904
(2)	113	.52	19.6	.804
(3)	172	.536	30	.70
(4)	247	.528	40	.60
(5)	357	.548	50.6	.49
(6)	475	.536	61.0	.39
(7)	645	.540	71.4	.29
(8)	915	.532	81.5	.19
(9)	1395	.532	91.9	.08
(10)	1960	.278	97	.027
(11)	2755	.104	99.1	.01

* Total volume 275mls; 30mls 30% NaOH

TABLE 6.5

ISOTOPE FRACTIONATION

NO₃⁻ Reduction - 1.08M NaOH

Sample	% Reaction	δ N ¹⁵ (NH ₃)	δ N ¹⁵ Reservoir	I.F.
1	0 - 9.6	- 25.1	+ 7.4	1.032
2	9.6 - 19.6	- 16.2	+ 11.6	1.026
3	19.6 - 30.0	- 6.8	+ 14.3	1.020
4	30.0 - 40.0	- .16	+ 17.0	1.016
5	40.0 - 50.6	+ 3.0	+ 20.0	1.016
6	50.6 - 61.0	+ 8.1	+ 23.4	1.014
7	61.0 - 71.4	+ 9.3	+ 28.8	1.016
8	71.4 - 81.5	+ 14.7	+ 37.4	1.017
9	81.5 - 91.9	+ 16.5	--	--
10	91.9 - 97.0	+ 22.8	--	--
11	97.0 - 99.1	--	--	--
12	> 99.1	+ 103.7	--	--

TABLE 6.6

KINETIC DATA

NO₃⁻ Reduction (Chemical) 0.667M NaOH*

Sample	Time (sec)	Millimoles NH ₃	% Reaction	$1 - \frac{\%}{100}$
(1)	73	.50	8.95	.91
(2)	163	.528	18.4	.816
(3)	241	.52	27.6	.724
4	319	.516	36.9	.631
5	401	.516	46.0	.54
6	496	.516	55.4	.496
7	609	.516	64.5	.355
8	763	.516	73.9	.263
9	998	.52	83.1	.169
10	1194	.262	88.9	.111
11	1437	.262	93.4	.066
12	1775	.258	97.0	.032
13	2209	.108	98.7	.013

* 15mls 30% NaOH

TABLE 6.7

ISOTOPE FRACTIONATION

NO₃⁻ Reduction - 0.67M NaOH

Sample	% Reaction	δ N ¹⁵ (NH ₃)	δ N ¹⁵ Reservoir	I.F.
1	0 - 9.12	- 44.2	+ 10.1	1.052
2	9.12 - 18.7	- 35.5	+ 15.3	1.048
3	18.7 - 28.3	- 32.5	+ 21.5	1.051
4	28.3 - 37.8	--	+ 28.8	--
5	37.8 - 47.3	- 20.0	+ 37.2	1.053
6	47.3 - 56.8	--	+ 49.2	--
7	56.8 - 66.4	- 18.5	+ 67.0	--
8	66.4 - 75.9	- 16.0	--	--
9	75.9 - 85.0	- 3.8	--	--

NO₃⁻ Reference = +5.0

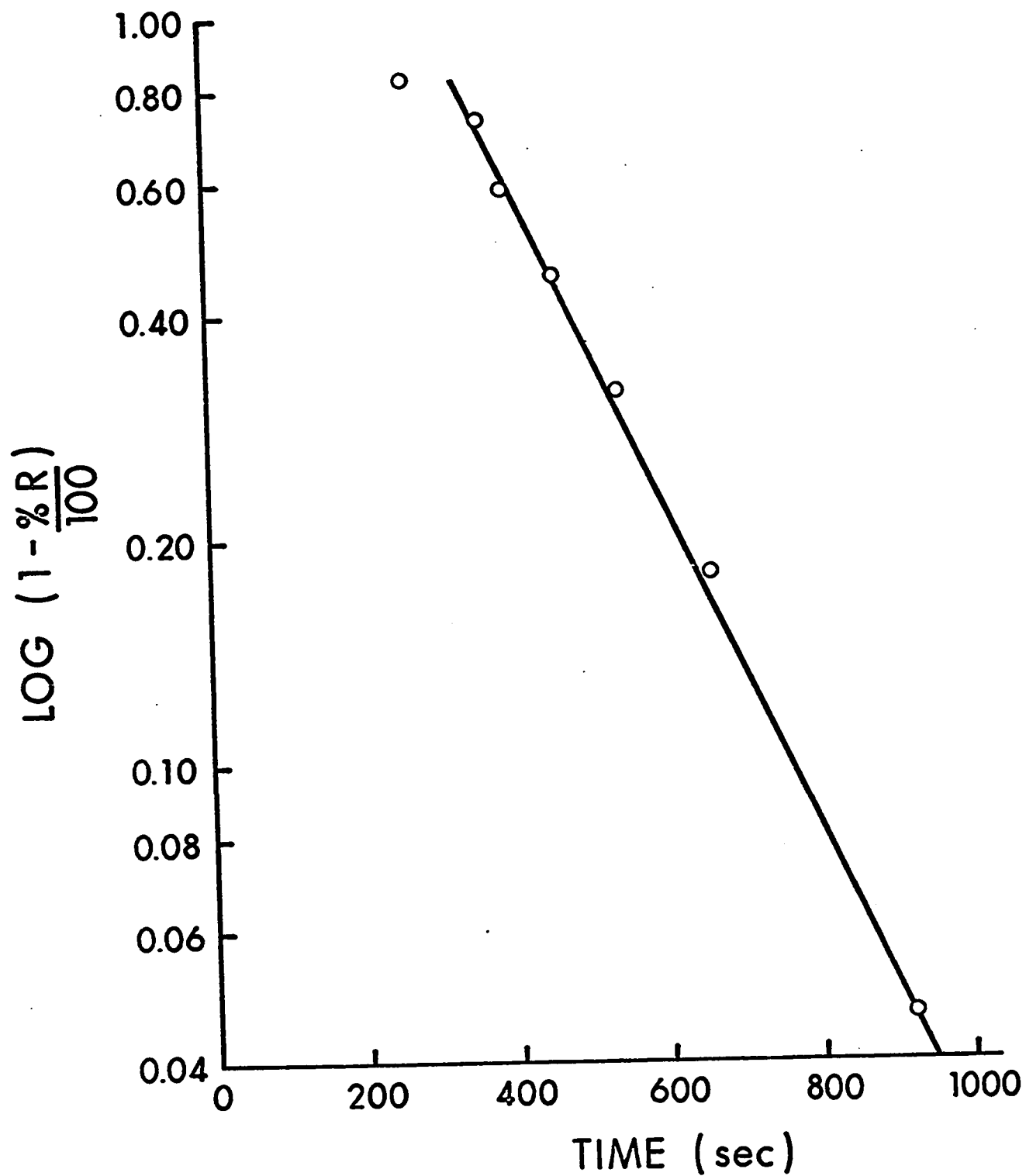


Figure 6.1 Kinetic graph for NO_3^- reduction in 4.0M NaOH

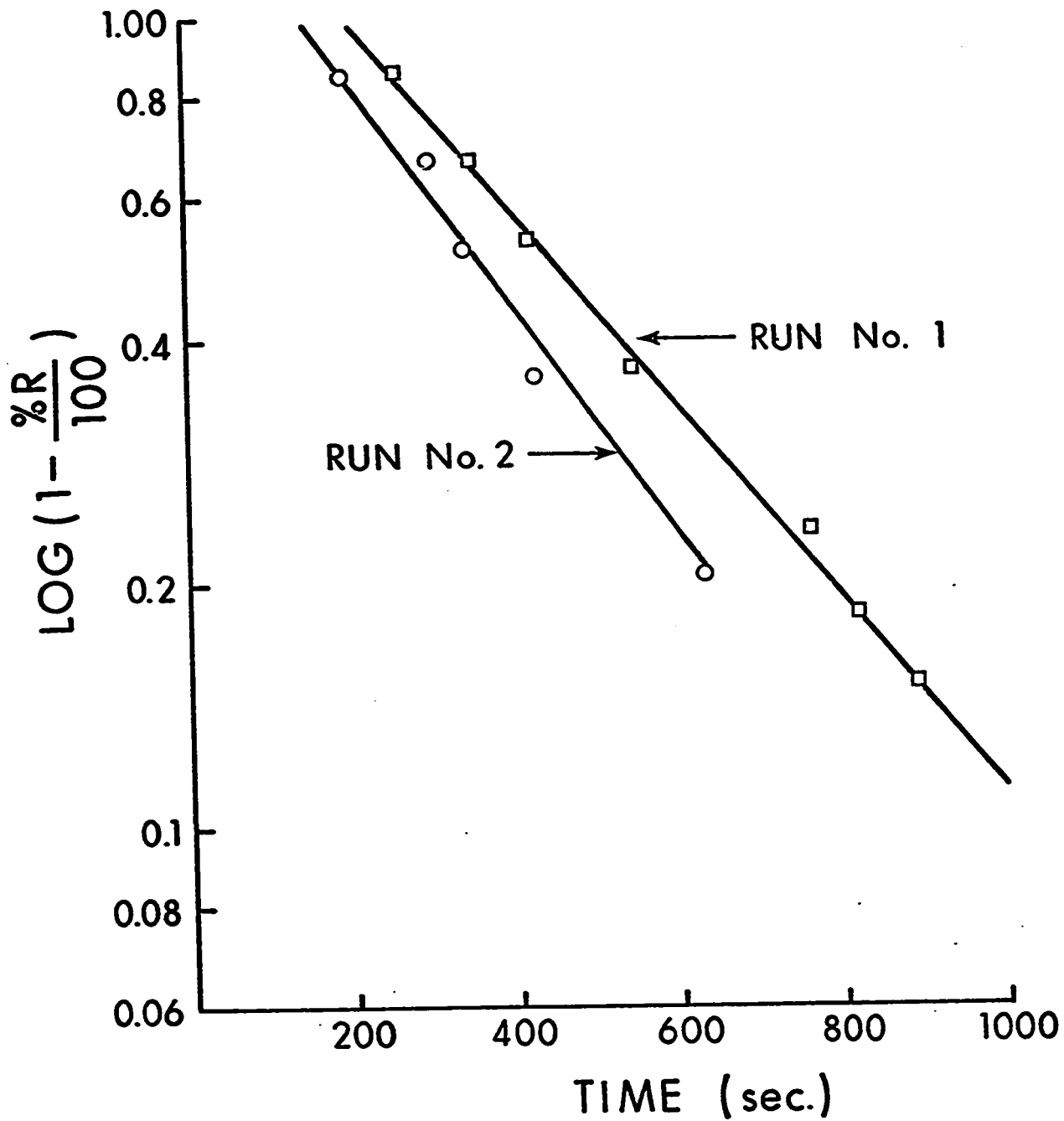


Figure 6.2 Kinetic graph for NO_3^- reduction in 2.66M NaOH

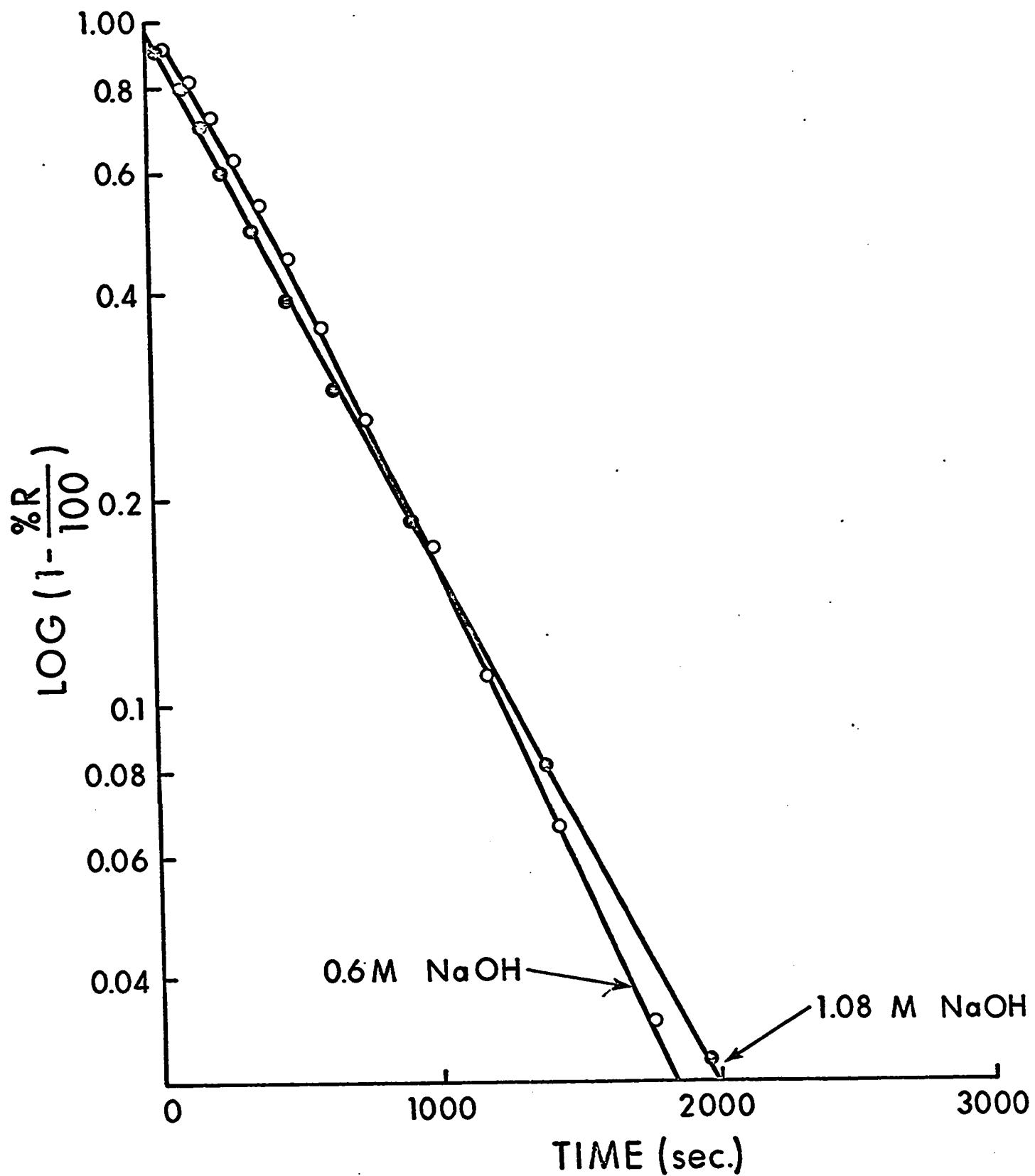


Figure 6.3 Kinetic graph for NO_3^- reduction in 1.08M and 0.67M NaOH

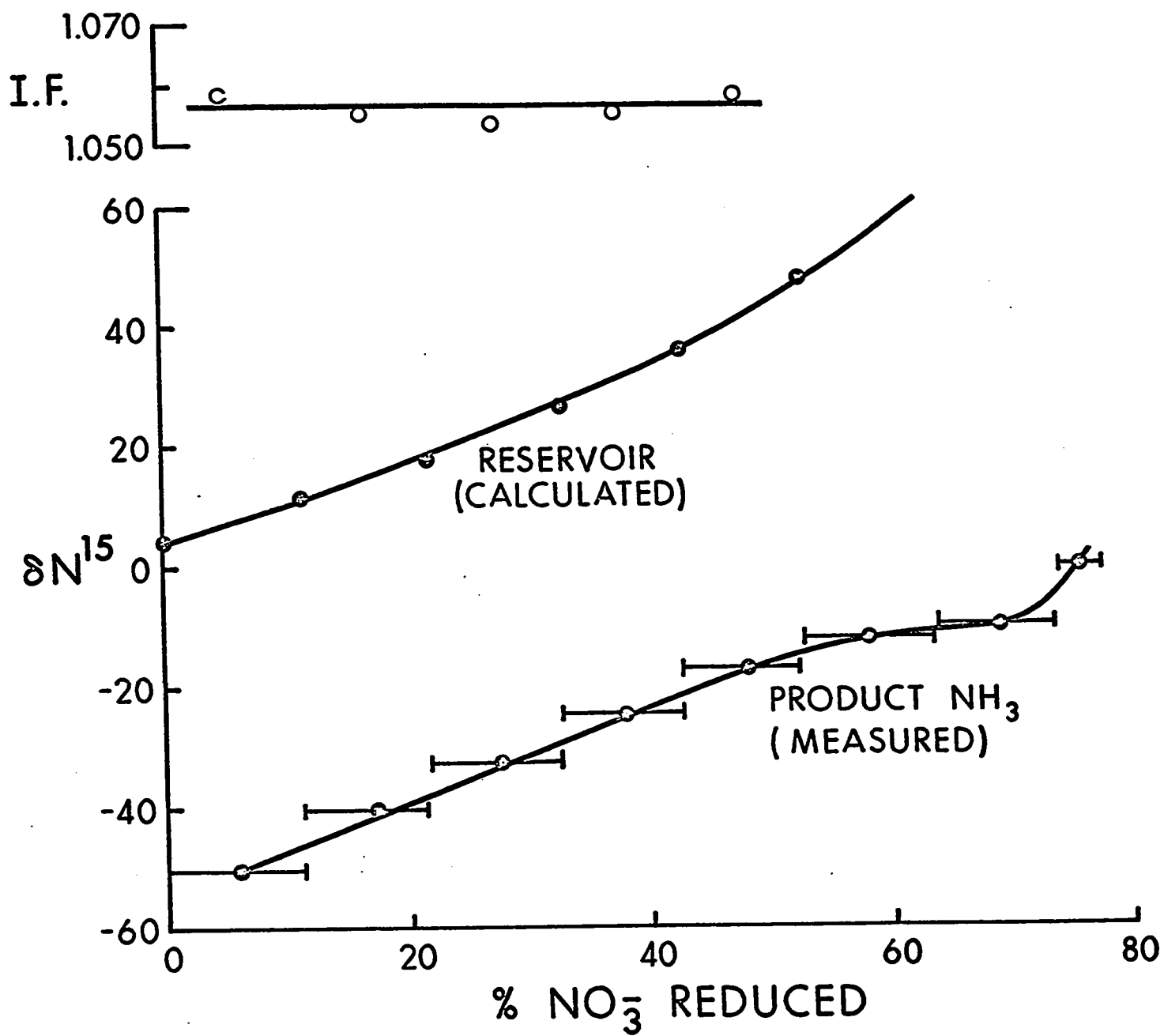


Figure 6.4 Isotope fractionation during NO₃⁻ reduction in 4.0M NaOH

Figure 6.5 Isotope fractionation during NO_3^- reduction in 1.34M NaOH

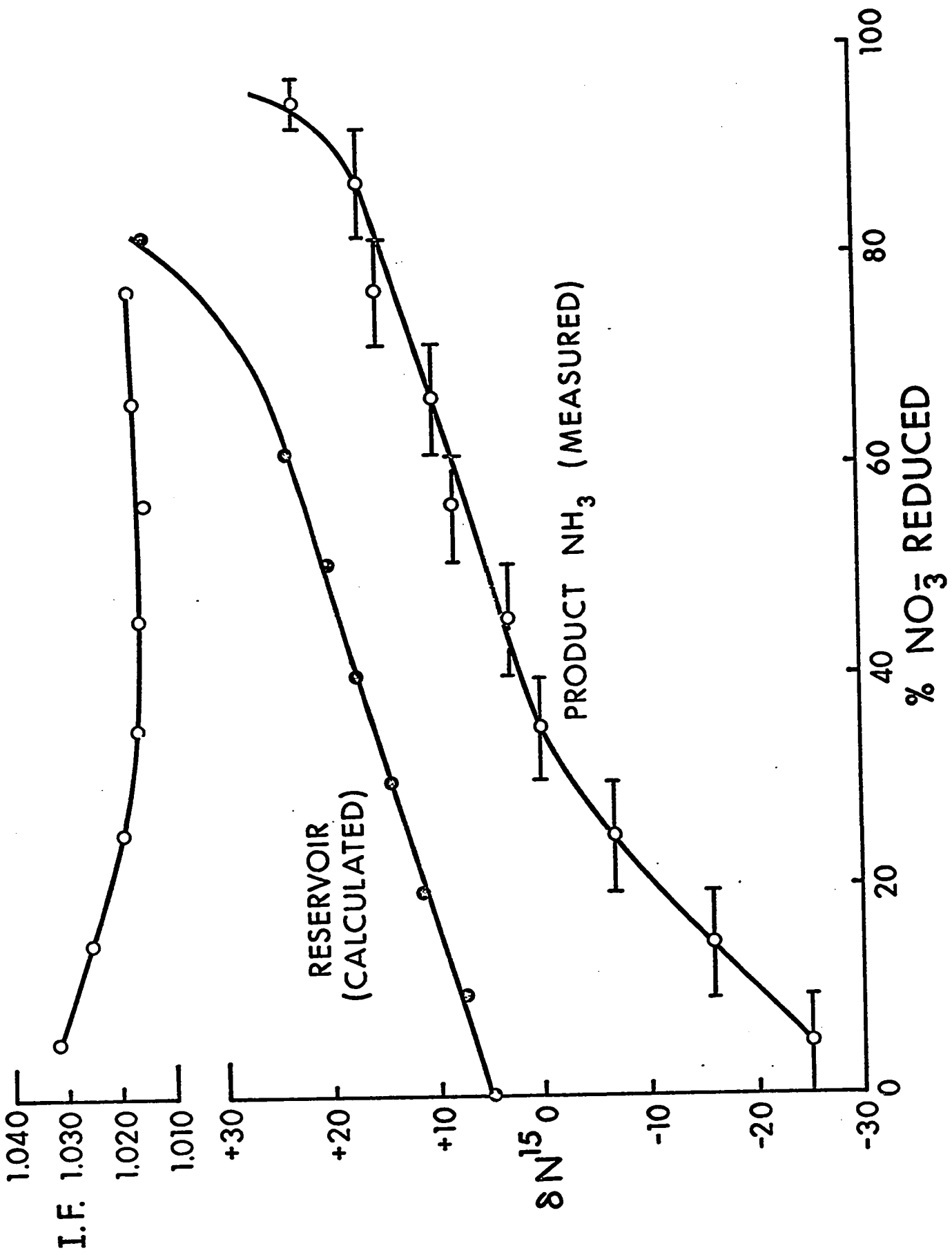
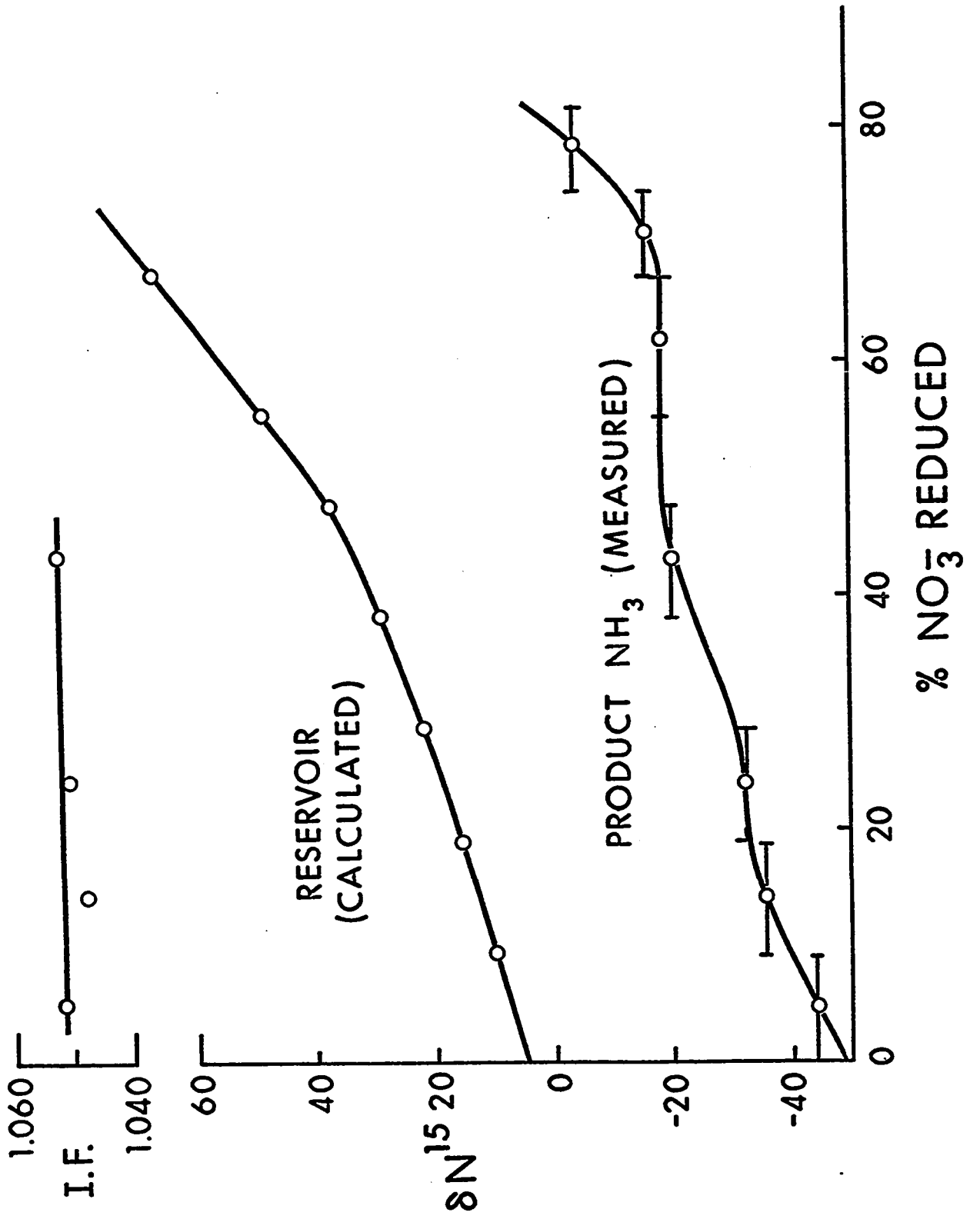


Figure 6.6 Isotope fractionation during NO_3^- reduction in 0.67M NaOH



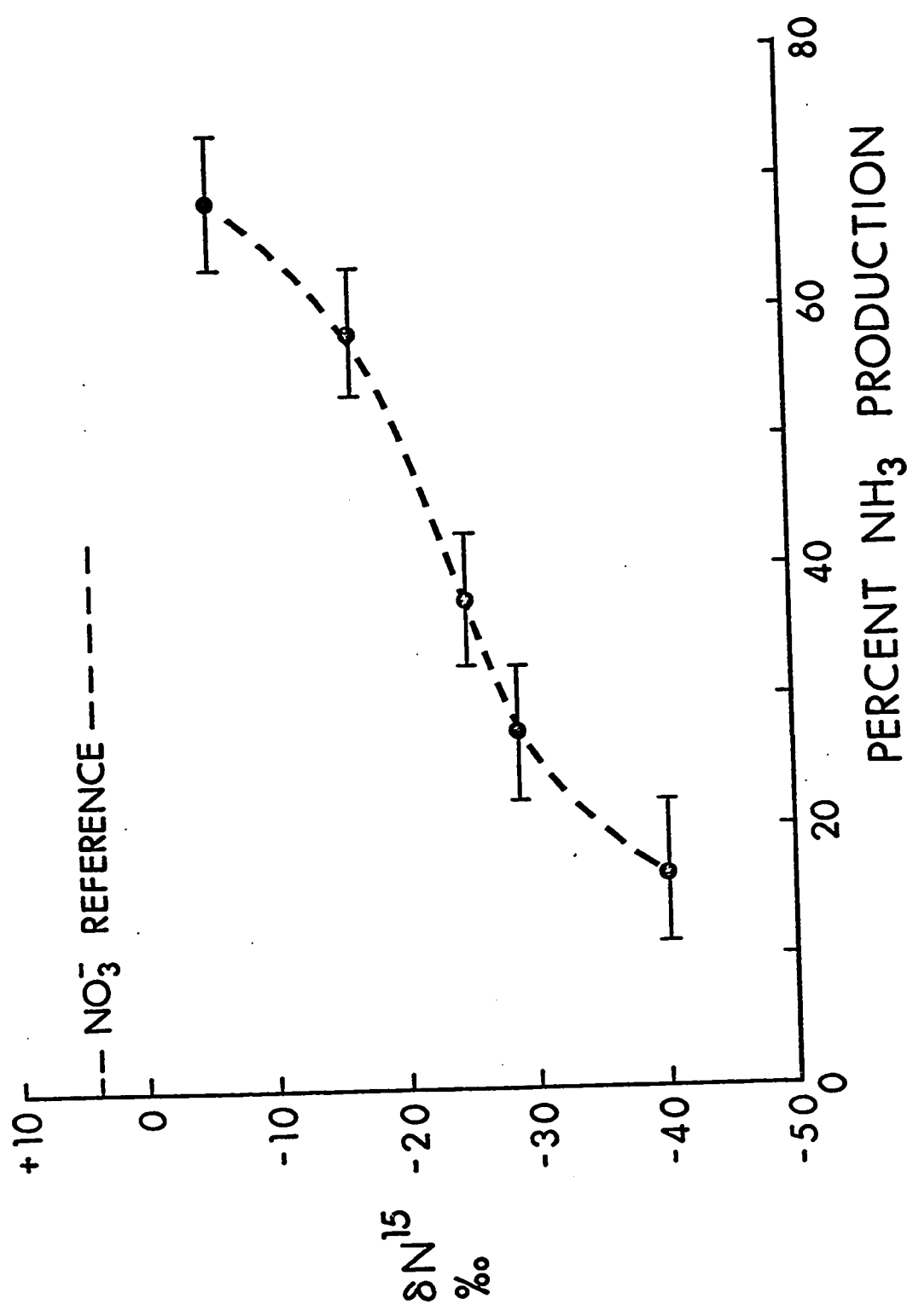


Figure 6.7 Isotope fractionation during NO_3^- reduction in 1.34M NaOH with modified procedure

TABLE 6.8

KINETIC DATA

NO₂⁻ Reduction (Chemical) 4.0M NaOH*

Sample	Time	Millimoles NH ₃	% Reaction	$1 - \frac{\%}{100}$
1	291	.50	7.16	.928
2	332	.52	14.6	.854
3	364	.508	21.9	.781
4	396	.524	29.4	.706
5	426	.516	36.9	.631
6	459	.516	44.3	.557
7	493	.528	51.7	.483
8	534	.528	59.3	.407
9	578	.52	66.7	.333
10	656	.658	76.5	.24
11	765	.628	84.8	.152
12	927	.544	93.0	.07
13	982	.120	94.7	.053
14	1048	.118	96.5	.035
15	1135	.108	98.0	.0189

* 90mls 30% NaOH

TABLE 6.9

KINETIC DATA

NO₂⁻ Reduction (Chemical) 2.66M NaOH*

Sample	Time	Millimoles NH ₃	% Reaction	$1 - \frac{\%}{100}$
(1)	248	.50	11.4	.886
(2)	292	.592	24.9	.751
(3)	350	5.38	37.2	.628
(4)	425	5.20	49.0	.51
(5)	520	5.16	60.8	.392
(6)	659	5.20	72.5	.275
(7)	870	5.08	84.1	.259
(8)	1394	5.12	95.7	.043
(9)	1738	5.08	98.4	.016
(10)	∞	.76	-	

* 60mls 30% NaOH

TABLE 6.10

ISOTOPE FRACTIONATION
 NO_2^- Reduction 2.66M NaOH

Sample	% Reaction	$\delta \text{N}^{15}(\text{NH}_3)$	δN^{15} Reservoir	I.F.
1	0 - 13.5	- 15.1	+ 12.7	1.025
2	13.5 - 25.7	- 6.8	+ 15.9	1.019
3	25.7 - 37.4	- .8	+ 19.1	1.018
4	37.4 - 49.1	+ 5.7	+ 22.2	1.015
5	49.1 - 61.0	+ 6.6	+ 27.0	1.017
6	61.0 - 73.0	+ 12.6	+ 33.0	1.018
7	73.0 - 84.2	+ 22.7	+ 41.0	1.014
8	84.2 - 96.0	+ 30.7	+ 73	--
9	96.0 - 98.5	37.0	--	--
10	> 98.5	41.0	--	--

TABLE 6.11

KINETIC DATA

NO₂⁻ Reduction (Chemical) 1.33M NaOH^{*}; Run 1

Sample	Time	% Reaction	$1 - \frac{\%}{100}$
1	238	8.70	.913
2	298	17.7	.823
3	355	29.1	.709
4	406	38.7	.613
5	464	46.9	.531
6	518	56.5	.435
7	584	65.6	.344
8	665	75.0	.25
9	785	84.4	.156
10	860	89.5	.105
11	910	91.2	.088
12	965	93.4	.066
13	1025	95.4	.046
14	1100	97.0	.03
15	1850		

* 30mls 30% NaOH

TABLE 6.12

KINETIC DATA

NO₂⁻ Reduction (Chemical) 1.33M NaOH*; Run 2

Sample	Time	Millimoles NH ₃	% Reaction	$1 - \frac{\%}{100}$
(1)	191	.50	12.3	.877
(2)	270	.52	25.2	.748
(3)	351	.516	38.0	.62
(4)	453	.508	50.5	.495
(5)	565	.536	63.7	.363
(6)	711	.512	76.5	.235
(7)	968	.512	89.0	.11
(8)	-	.444		

* 30mls 30% NaOH

TABLE 6.13

ISOTOPE FRACTIONATION

NO₂⁻ Reduction - 1.33M NaOH

Sample	% Reaction	δ N ¹⁵ (NH ₃)	δ N ¹⁵ Reservoir	I.F.
1	0 - 12.7	- 16.6	11.1	1.025
2	12.7 - 25.2	- 5.7	14.1	1.017
3	25.2 - 37.6	+ 2.7	16.2	1.014
4	37.6 - 50.7	5.4	18.9	1.014
5	50.7 - 63.0	11.0	21.7	1.009
6	63.0 - 75.6	15.8	24.8	1.008
7	75.6 - 87.9	20.7	29.2	-
8	87.9 - 98.8	31.4	-	-
9	> 98.8	37.8	-	-

NO₂⁻ Reference = + 8.5

TABLE 6.14

KINETIC DATA

NO₂⁻ Reduction (Chemical) 0.667M NaOH*

Sample	Time	Millimoles NH ₃	% Reaction	$1 - \frac{\%}{100}$
(1)	218	.70	10.8	.892
(2)	297	.728	22.2	.778
(3)	376	.728	33.3	.667
(4)	466	.736	44.8	.552
(5)	573	.740	56.2	.438
(6)	715	.724	67.5	.325
(7)	920	.728	78.5	.215
(8)	1283	.740	90.2	.098
(9)	1470	.228	93.5	.065
(10)	1773	.212	97.0	.03

*15mls 30% NaOH

TABLE 6.15

ISOTOPE FRACTIONATION

NO₂⁻ Reduction - 0.67M NaOH

Sample	% Reaction	δ N ¹⁵ (NH ₃)	δ N ¹⁵ Reservoir	I.F.
1	0 - 11.3	- 16.5	11.8	1.027
2	11.3 - 22.6	--	14.4	-
3	22.6 - 34.0	- 6.0	17.0	1.022
4	34.0 - 45.5	+ 2.0	19.1	1.016
5	45.5 - 56.7	+ 6.0	21.0	1.014
6	56.7 - 68.0	+ 13.5	22.4	1.009
7	68.0 - 79.3	+ 15.8	22.8	-
8	79.3 - 90.8	+ 17.0	--	-
9	90.8 - 94.0	+ 22.0	--	-
10	94.0 - 97.5	+ 35.2	--	-

NO₂⁻ = +9.0

Figure 6.8 Kinetic graph for NO_2^- reduction in 2.66M NaOH

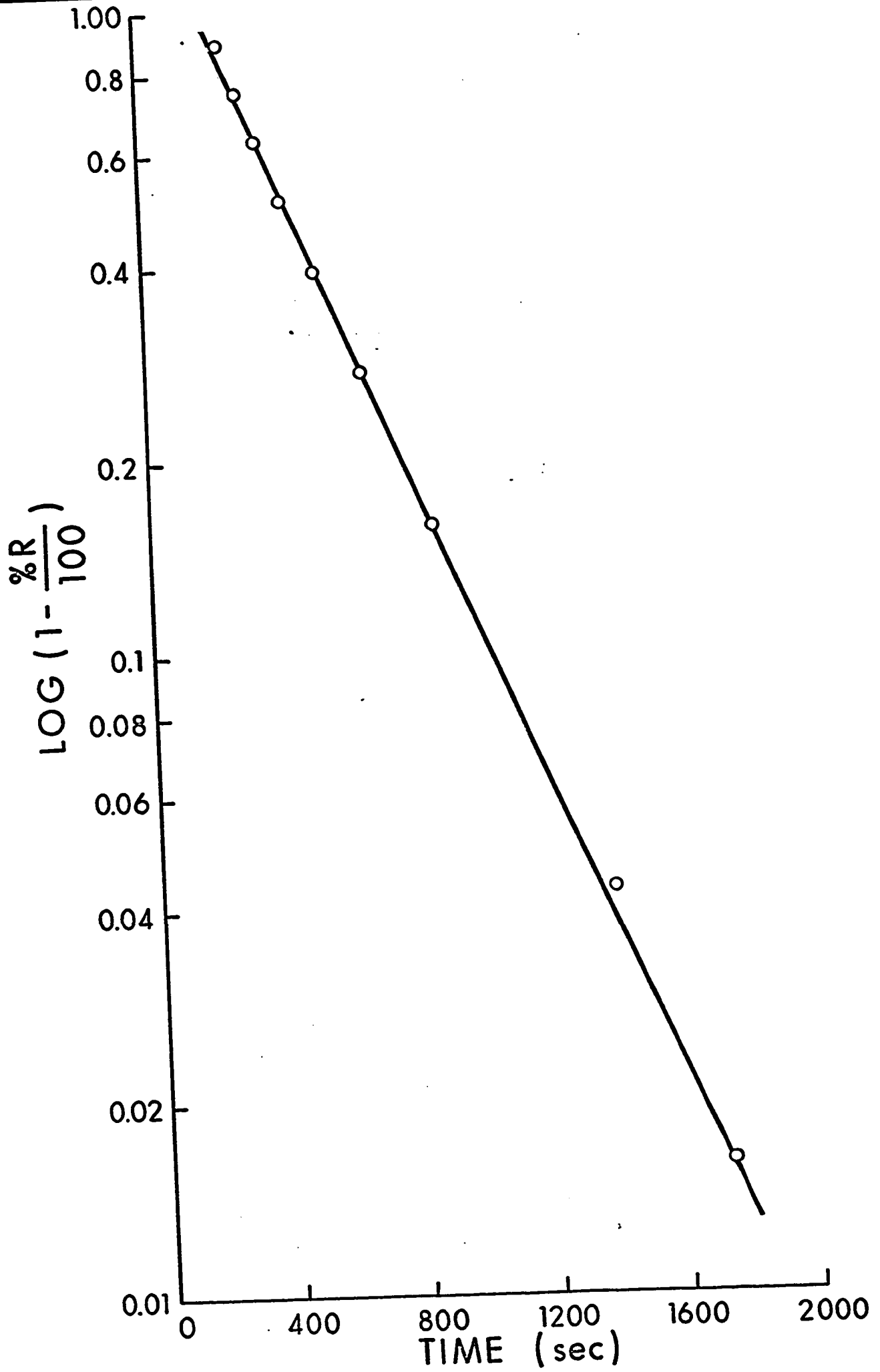
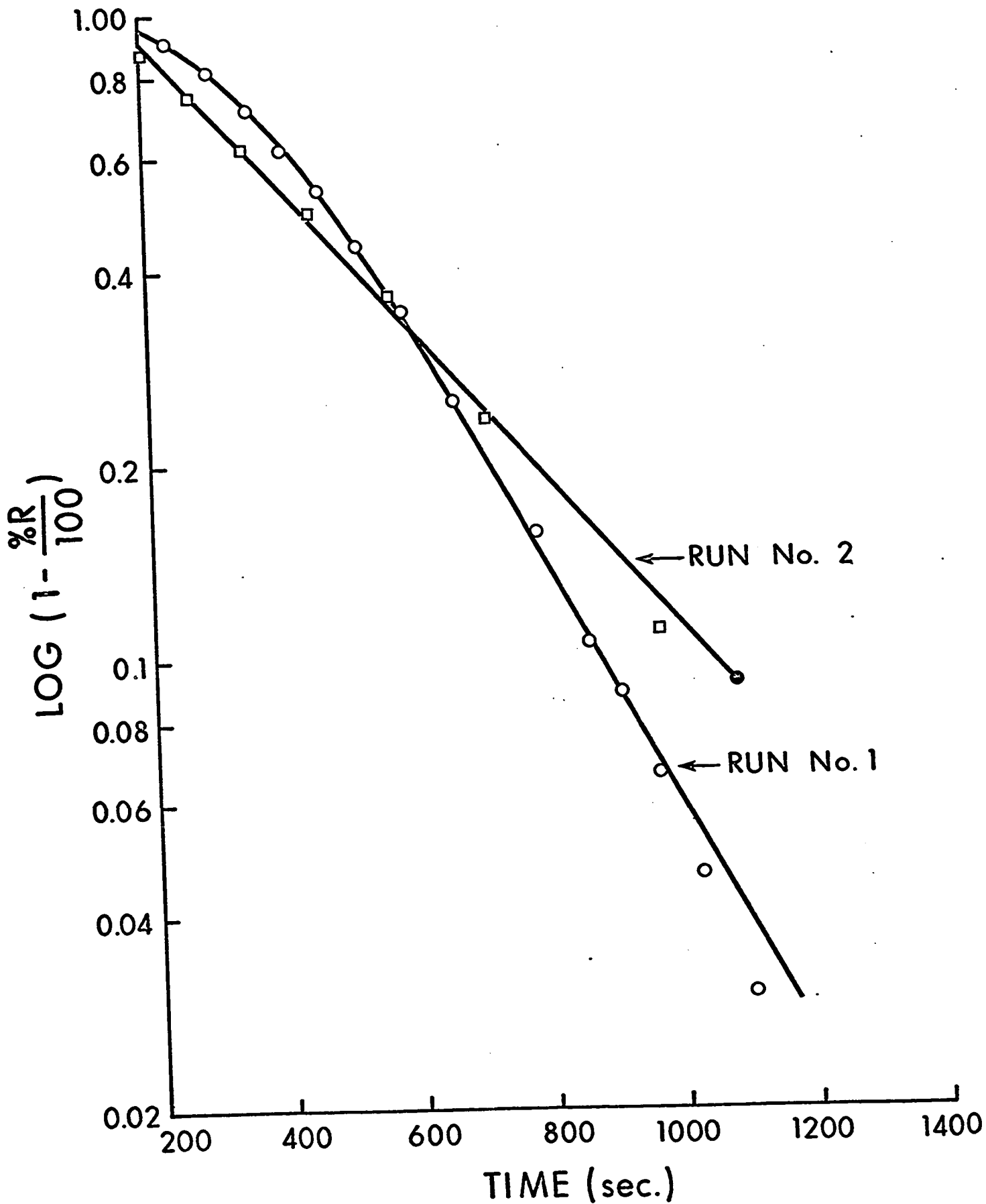
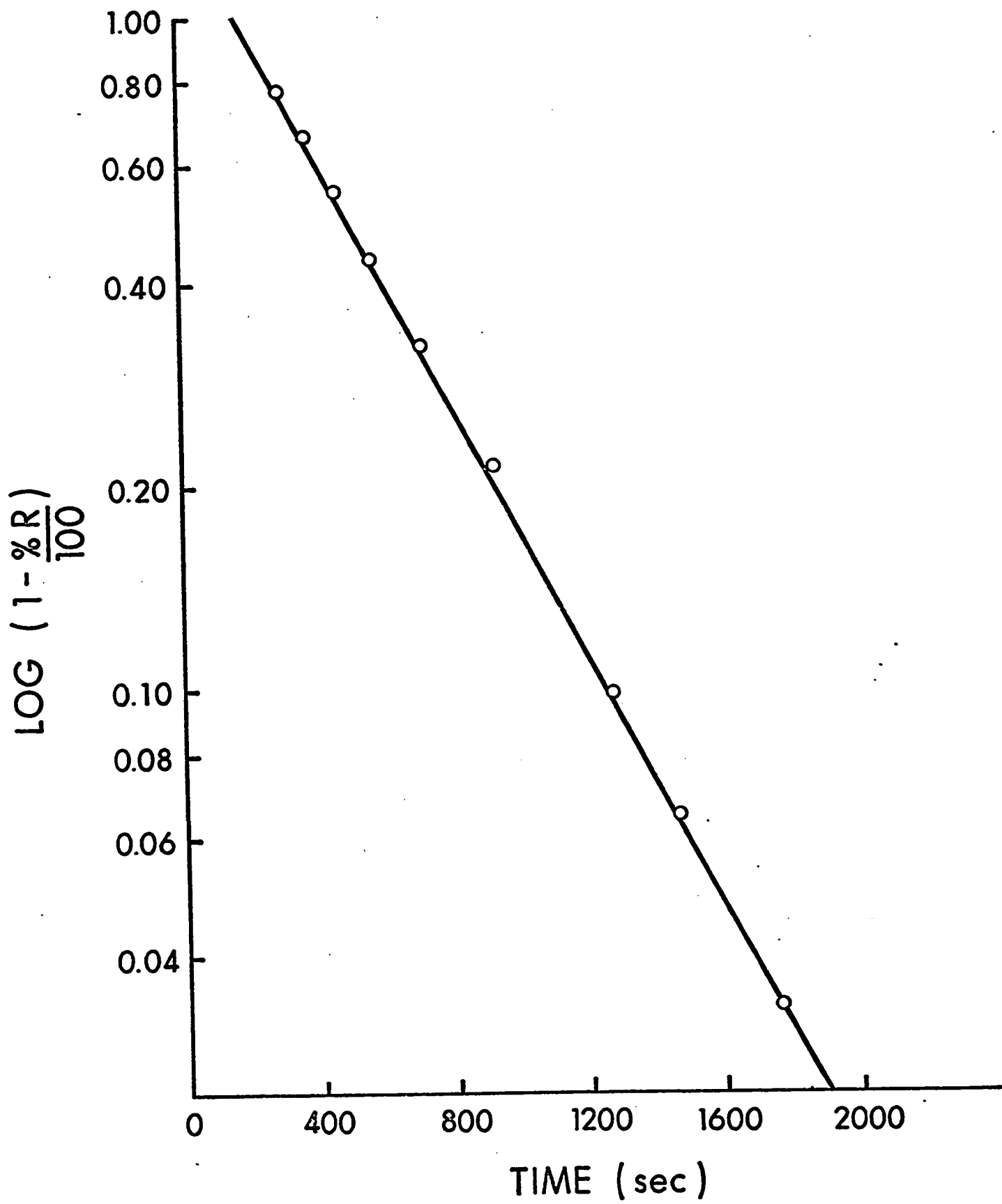


Figure 6.9 Kinetic graph for NO_2^- reduction in 1.33M NaOH

Figure 6.10 Kinetic graph for NO_2^- reduction in 0.67M NaOH





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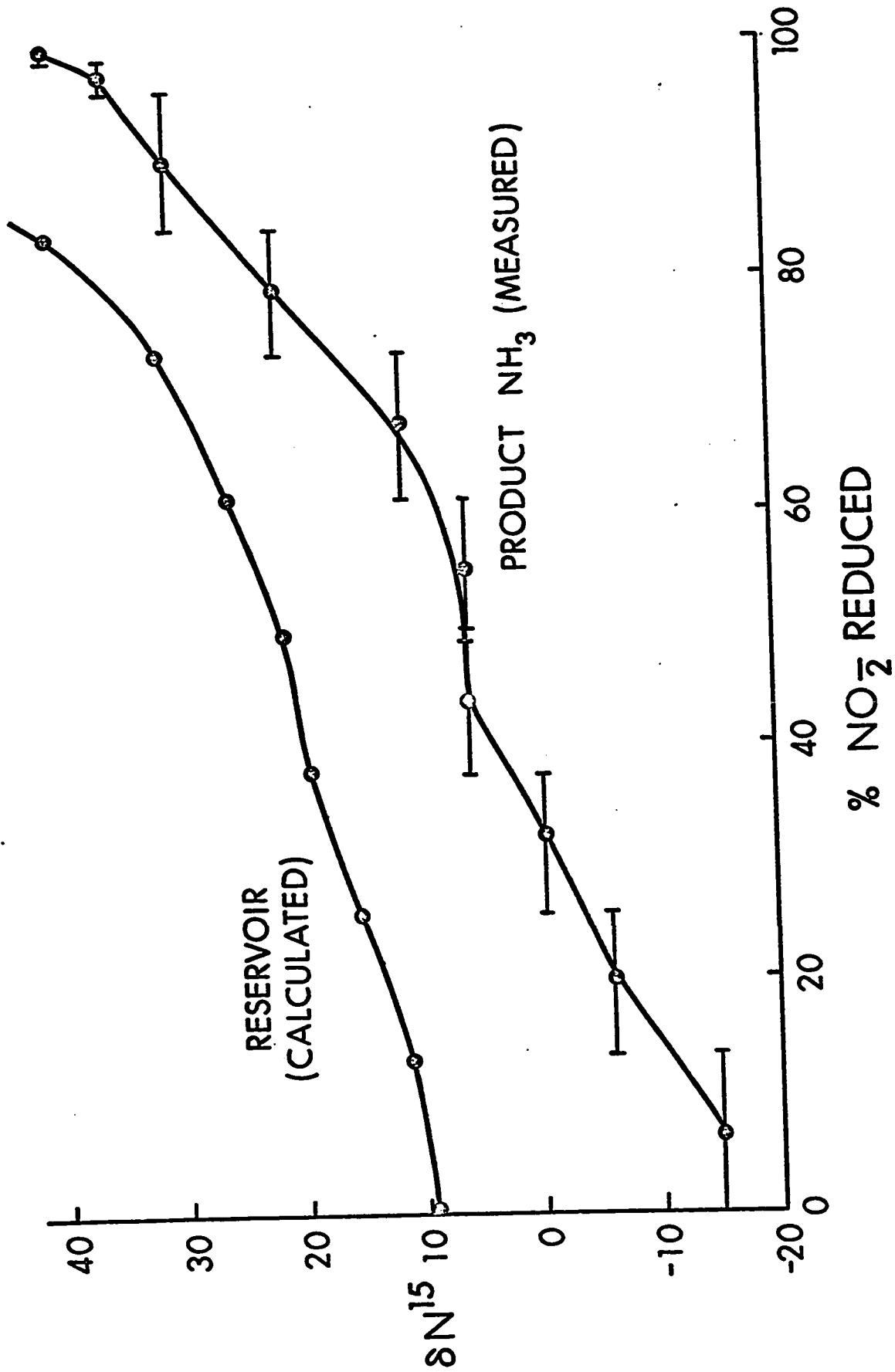


Figure 6.11 Isotope fractionation during NO_2 reduction in 2.66M NaOH

Figure 6.12 Isotope fractionation during NO_2^- reduction in 1.33M NaOH

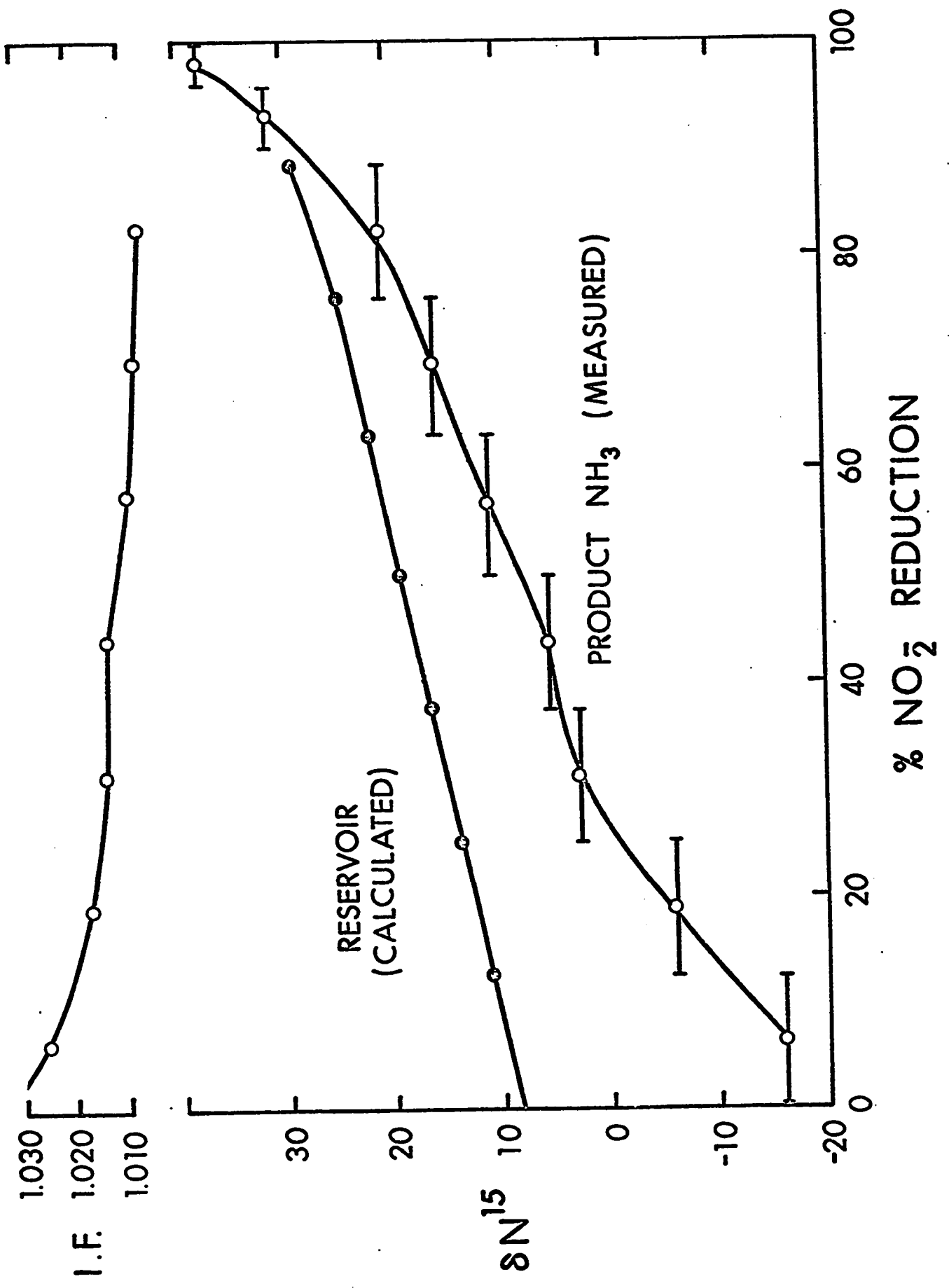


Figure 6.13 Isotope fractionation during NO_2^- reduction in 0.67M NaOH

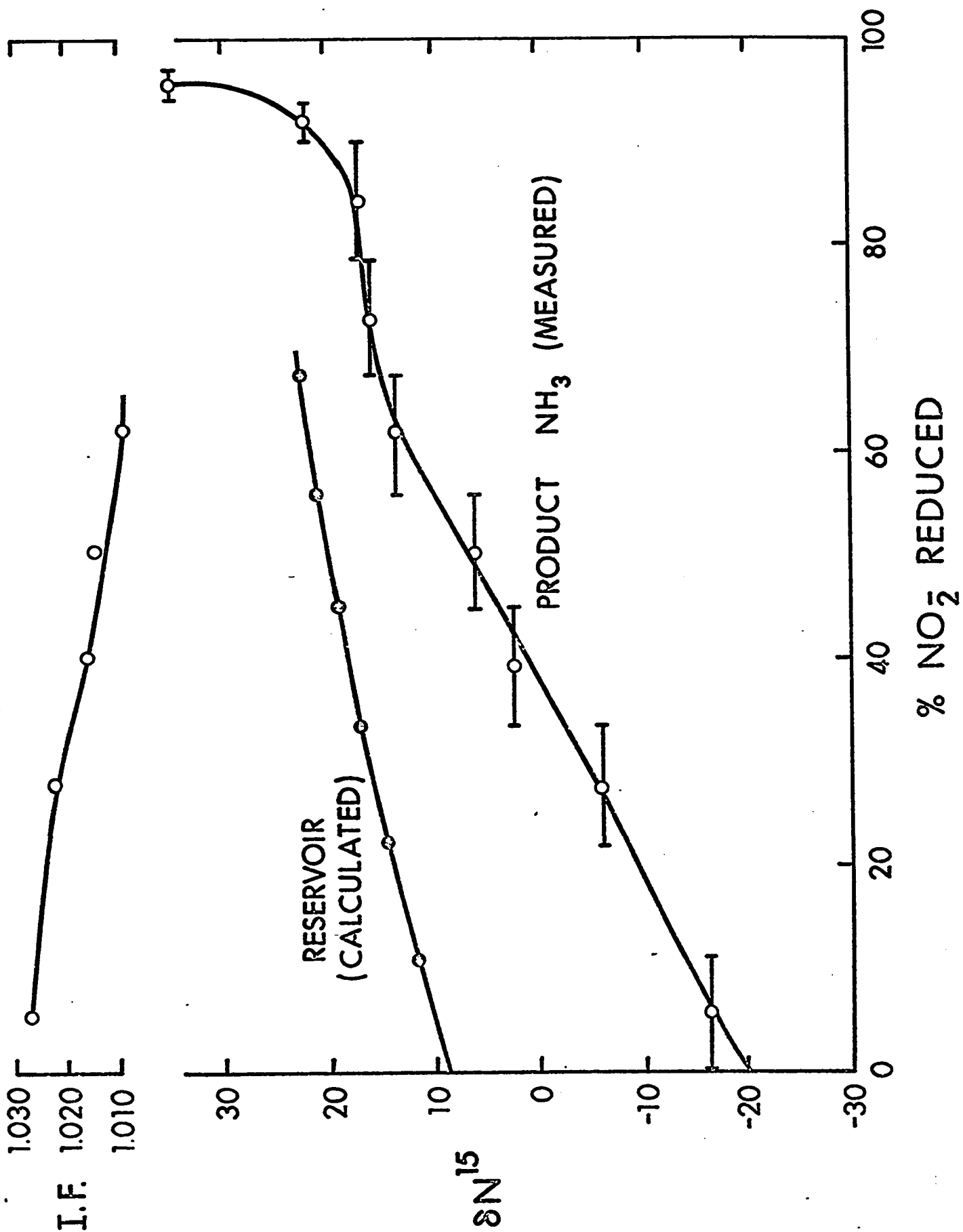


TABLE 6.16

KINETIC DATA, UREA HYDROLYSIS, RUN #1

Sample	Time (sec)	% Reaction	$(1 - \frac{\% R}{100})$
(1)	438	0 - 12.2	0.878
(2)	629	12.2 - 28.8	0.712
(3)	835	28.8 - 44.6	0.554
(4)*	1006	44.6 - 58.7	0.413
(5)	1512	58.7 - 72.7	0.273
(6)	2493	72.7 - 86.7	0.133
(7)	--	86.7 - 99.0	0.01

* Nitrogen purge rate increased.

TABLE 6.17

KINETIC DATA, UREA HYDROLYSIS, RUN #2

Sample	Time (sec)	% Reaction	$(1 - \frac{\% R}{100})$
1	403	0 - 11.4	0.886
2	585	11.4 - 23.1	0.769
3	797	23.1 - 35.0	0.65
4	1044	35.0 - 46.5	0.535
5	1337	46.5 - 58.6	0.414
6	1716	58.6 - 70.5	0.295
7	2263	70.5 - 82.0	0.18
8	3330	82.0 - 94.0	0.06
9	4950	> 94.0	-

TABLE 6.18

KINETIC DATA, UREA HYDROLYSIS, RUN #3

Sample	Time (min)	% Reaction	$(1 - \frac{\% R}{100})$
1	16.5	5.50	0.945
2	21.3	11.1	0.889
3	25.3	16.7	0.833
4	30.4	22.1	0.779
5	36.0	27.6	0.724
6	41.7	33.3	0.667
7	49.4	39.0	0.610
8	57.4	44.9	0.551
9	65.7	50.3	0.497
10	75.4	56.0	0.440
11	86.5	61.0	0.390
12	99.4	67.0	0.330
13	115	72.7	0.273
14	134	78.3	0.217
15	161	84.0	0.160
16	256	94.6	0.054
17	321	97.7	0.023
18	390	--	--

TABLE 6.19

KINETIC DATA, UREA HYDROLYSIS, RUN #4

Sample	Time (sec)	% Reaction	$(1 - \frac{\% R}{100})$
(1)	250	5.2	0.948
(2)	385	10.5	0.895
(3)	520	16.1	0.839
(4)	644	21.3	0.787
(5)	769	26.7	0.733
(6)	898	32.2	0.678
(7)	1028	37.5	0.625
(8)	1165	43.0	0.570
(9)	1315	48.2	0.518
(10)	1470	53.7	0.463
(11)	1641	59.0	0.410
(12)	--	64.1	0.359
(13)	--	69.5	0.305
(14)	--	75.0	0.250
(15)	--	80.0	0.200
(16)	--	85.5	0.145
(17)	--	90.7	0.093
(18)	--	96.6	--

TABLE 6.20

ISOTOPIC DATA FOR UREA HYDROLYSIS, RUN #1

Sample	% Reaction	$\delta \text{N}^{15}(\text{NH}_3)$
1	0 - 16.6	- 6.9
2	16.6 - 32.4	+ 10.0
3	32.4 - 46.4	+ 31
4	46.4 - 60.7	+ 2
5	60.7 - 74.5	+ 2
6	74.5 - 87.1	- 2
7	87.1 - 98	+ 15.6
8	> 98	+ 12.4

TABLE 6.21

ISOTOPIC DATA FOR UREA HYDROLYSIS, RUN #2

Sample	% Reaction	$\delta \text{N}^{15}(\text{NH}_3)$
2	11.2 - 23.5	+ 2.9
3	23.5 - 35.2	+ 7.2
4	35.2 - 47.1	+ 24.8
5	47.1 - 58.9	+ 8.0
6	58.9 - 70.6	+ 2.5
7	70.6 - 82.4	+ 25
8	82.4 - 94.0	+ 25

TABLE 6.22

ISOTOPIC DATA FOR UREA HYDROLYSIS, RUN #3

Sample	% Reaction	$\delta \text{N}^{15}(\text{NH}_3)$	δN^{15} Reservoir	I.F.
1	0 - 5.54	- 12.2	+ 3.27	1.015
2	5.54 - 11.08	- 8.0	3.98	1.012
3	11.08 - 16.4	- 5.6	4.62	1.010
4	16.4 - 23.0	- 3.0	5.19	1.008
5	23.0 - 28.6	- 1.0	5.66	1.007
6	28.6 - 34.2	- .5	6.17	1.007
7	34.2 - 40.0	-	6.78	-
8	40.0 - 45.7	+ 0.0	7.46	1.007
9	45.7 - 51.3	+ 1.2	8.23	1.007
10	51.3 - 57.0	-	9.08	-
11	57.0 - 62.4	+ 3.2	9.95	1.007
12	62.4 - 68.0	+ 4.5	10.9	1.006
13	68.0 - 73.6	+ 6.2	11.9	-
14	73.6 - 79.4	+ 8.0	12.8	1.005
15	79.4 - 84.9	+ 10.0	13.9	-
16	84.9 - 95.5	+ 12.5	18.8	1.004
17	95.5 - 99.0	+ 15	-	-
18	> 99.0	+ 18	-	-

Reference = + 2.42

TABLE 6.22a

ISOTOPIC DATA FOR UREA HYDROLYSIS #4

Sample	% Reaction	$\delta \text{N}^{15}(\text{NH}_3)$
1	0 - 5.3	- 9.9
2	5.3 - 10.8	+ 0.9
3	10.8 - 16.1	
4	16.1 - 21.5	+ 6.9
5	21.5 - 26.9	
6	26.9 - 32.2	- 1.8
7	32.2 - 37.6	- 3.5
8	37.6 - 42.9	- 2.8
9	42.9 - 48.2	- 1.1
10	48.2 - 53.6	+ 1.1
11	53.6 - 58.9	+ 1.6
12	58.9 - 64.2	+ 4.7
13	64.2 - 69.5	
14	69.5 - 74.7	+ 10.7
15	74.7 - 80.1	
16	80.1 - 85.4	
17	85.4 - 90.8	
18	90.8 - 99.6	+ 34.4

Figure 6.14 Kinetic graph for the hydrolysis of urea #3

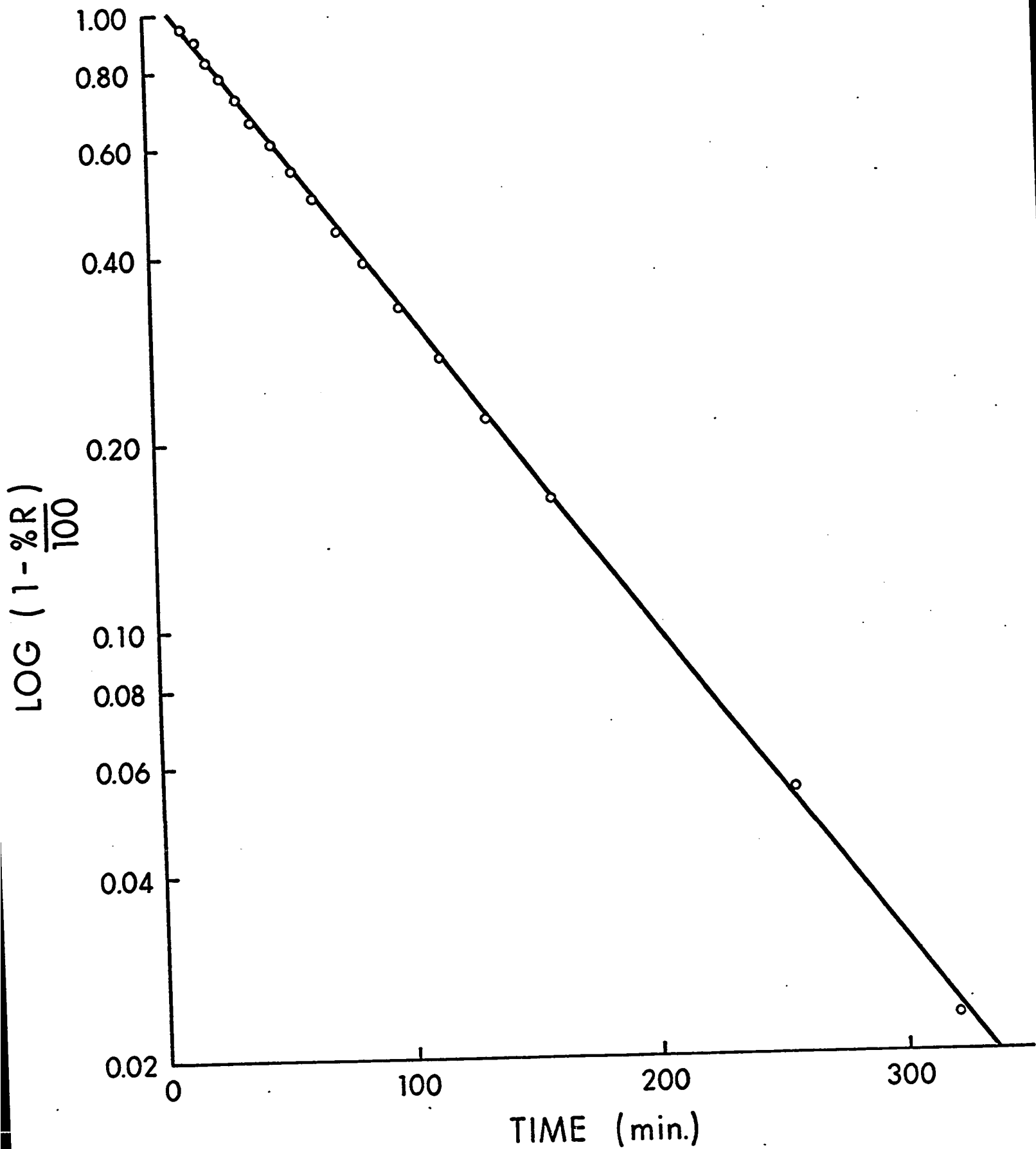
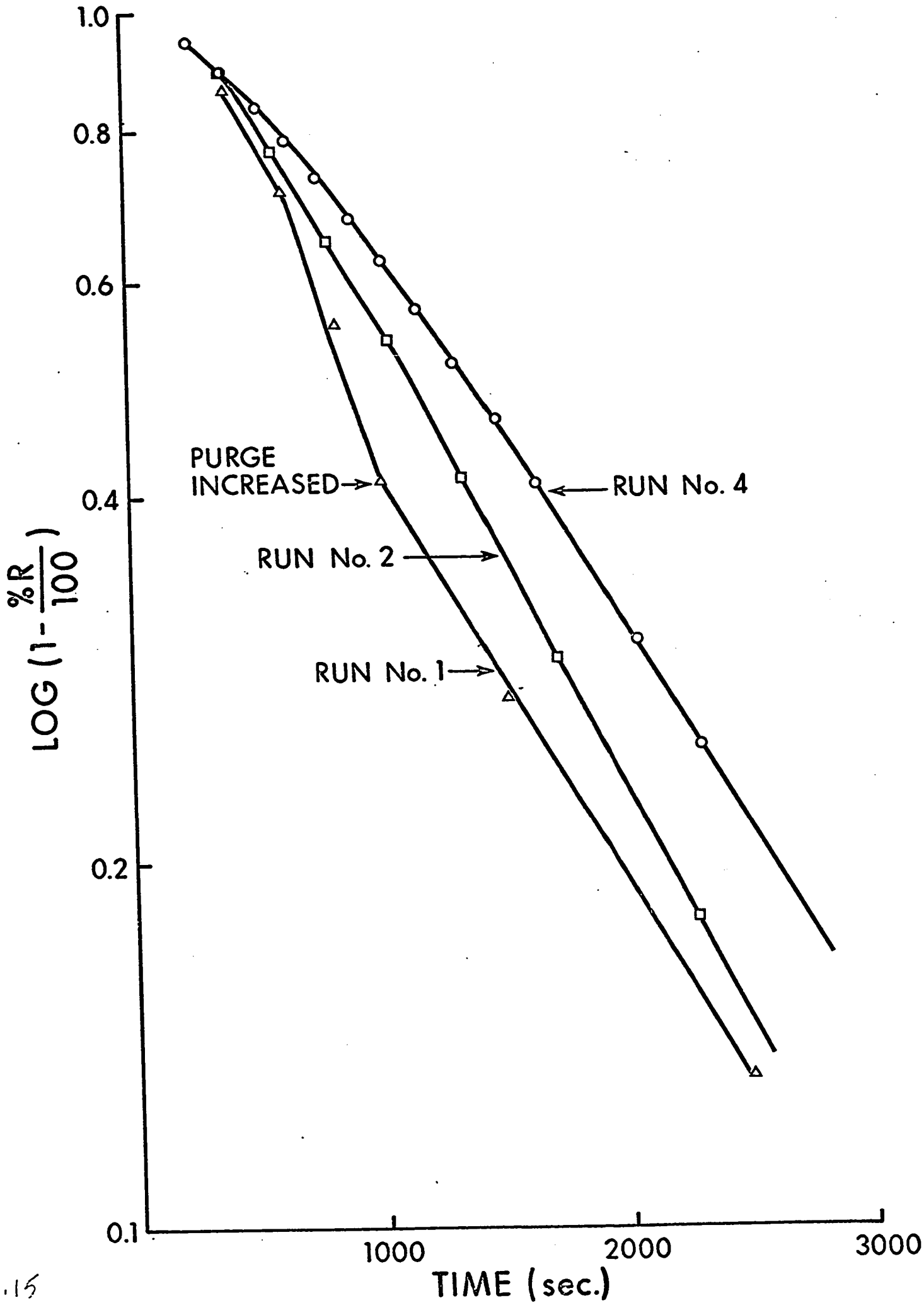
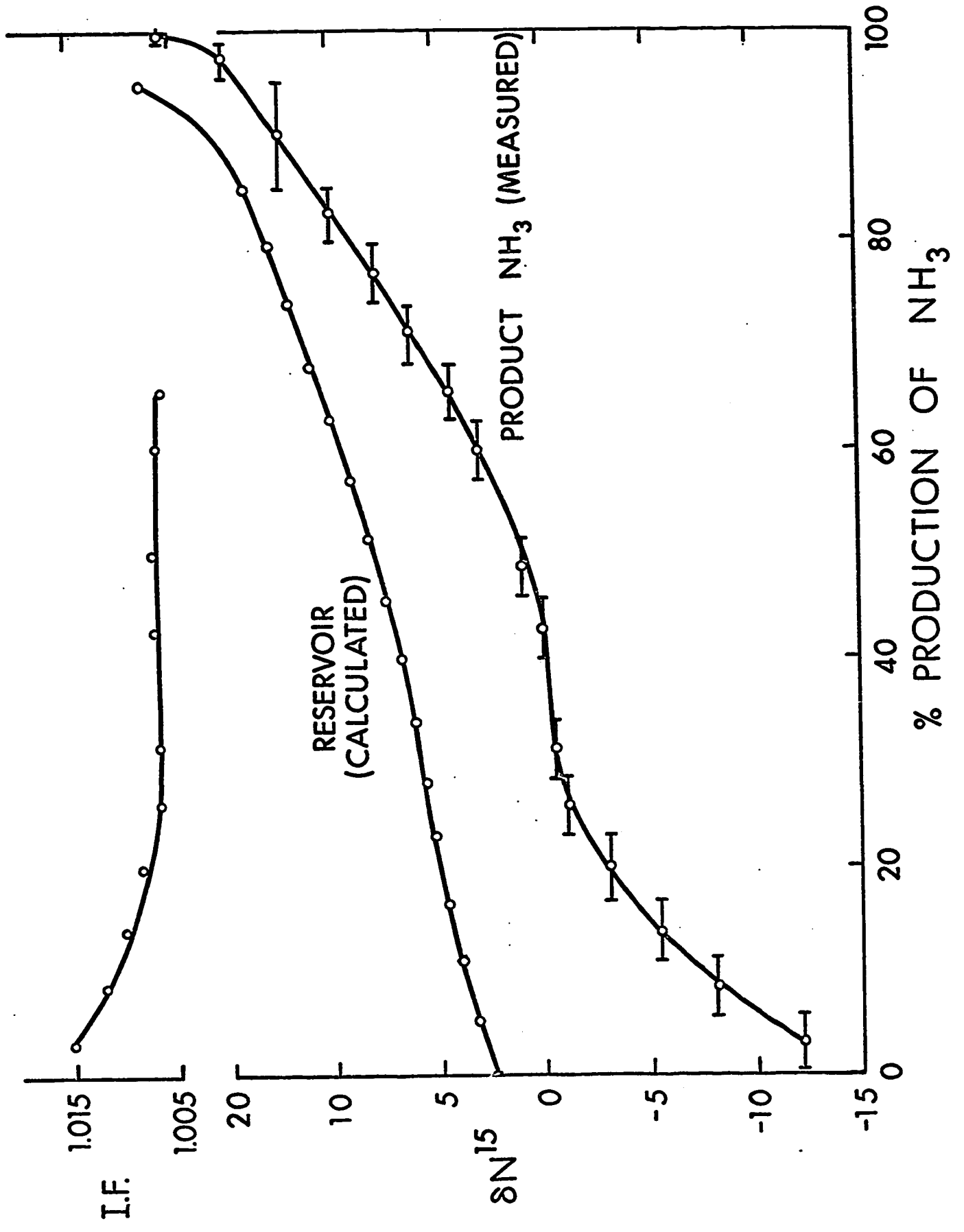


Figure 6.15 Kinetic graphs for the hydrolysis of urea #1, 2 and 4



6.15

Figure 6.16 Isotopic fractionation in the hydrolysis of urea; run #3



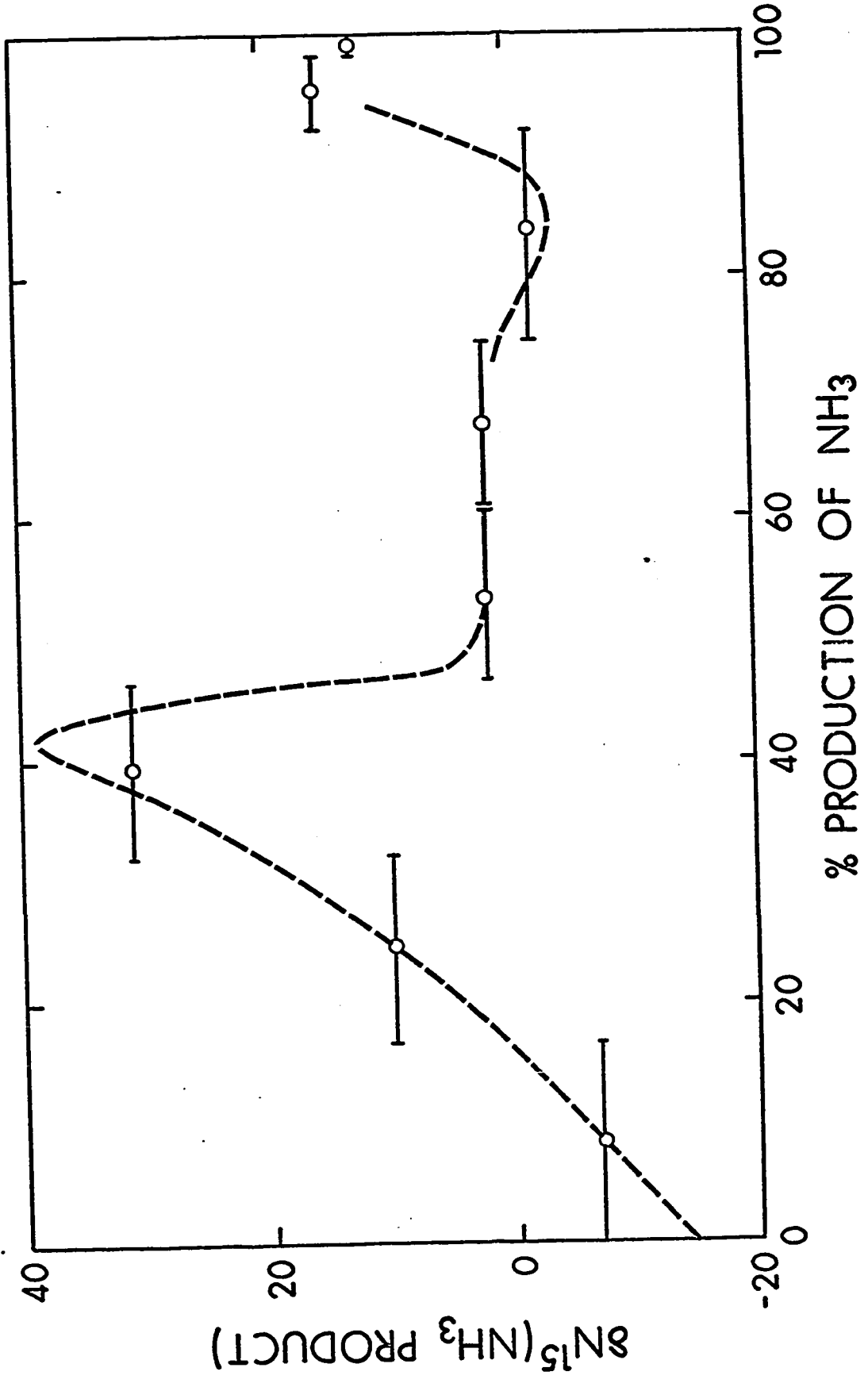


Figure 6.17 Isotopic fractionation in the hydrolysis of urea; run #1

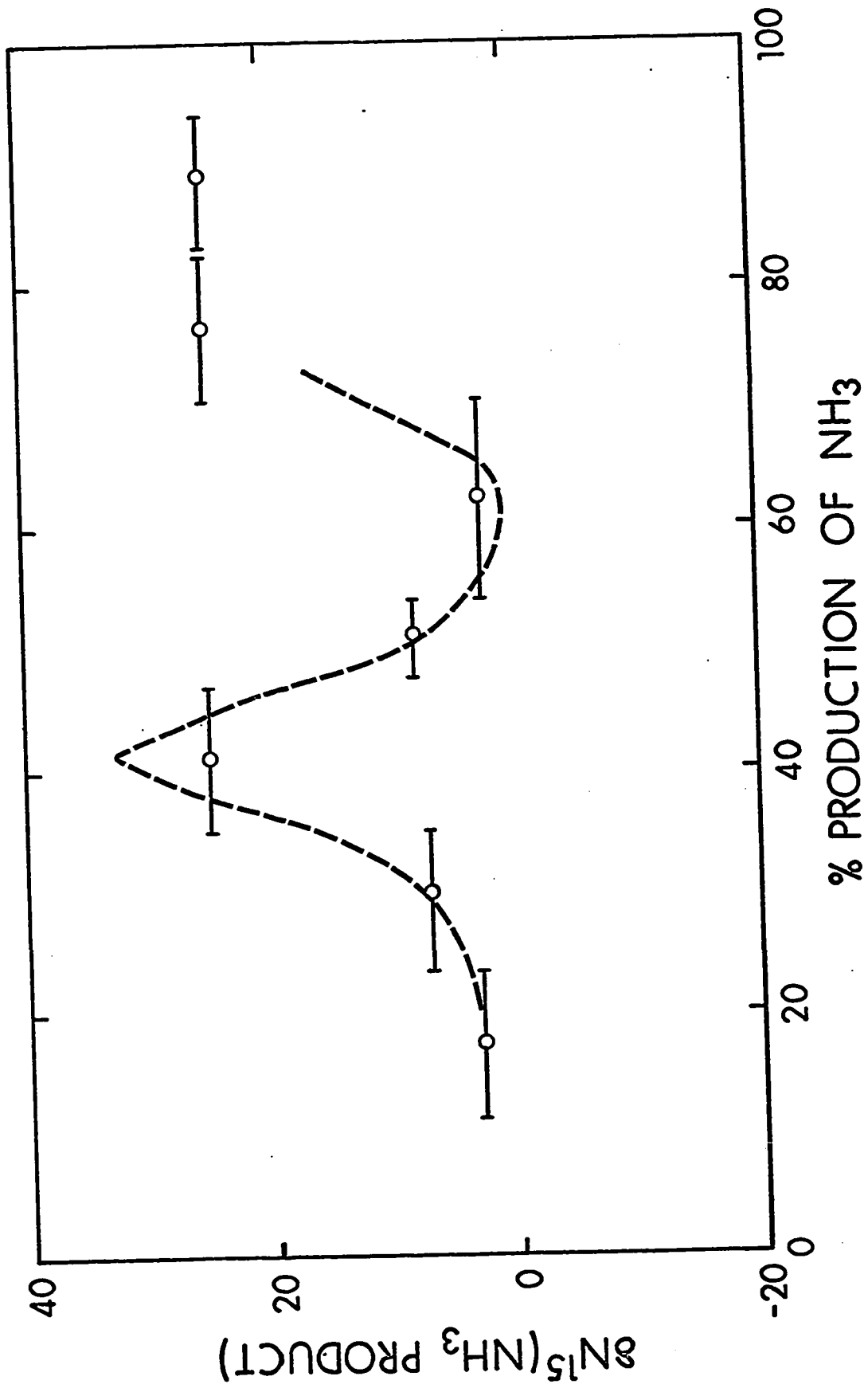


Figure 6.17a Nitrogen isotope fractionation in the hydrolysis of urea; run #2

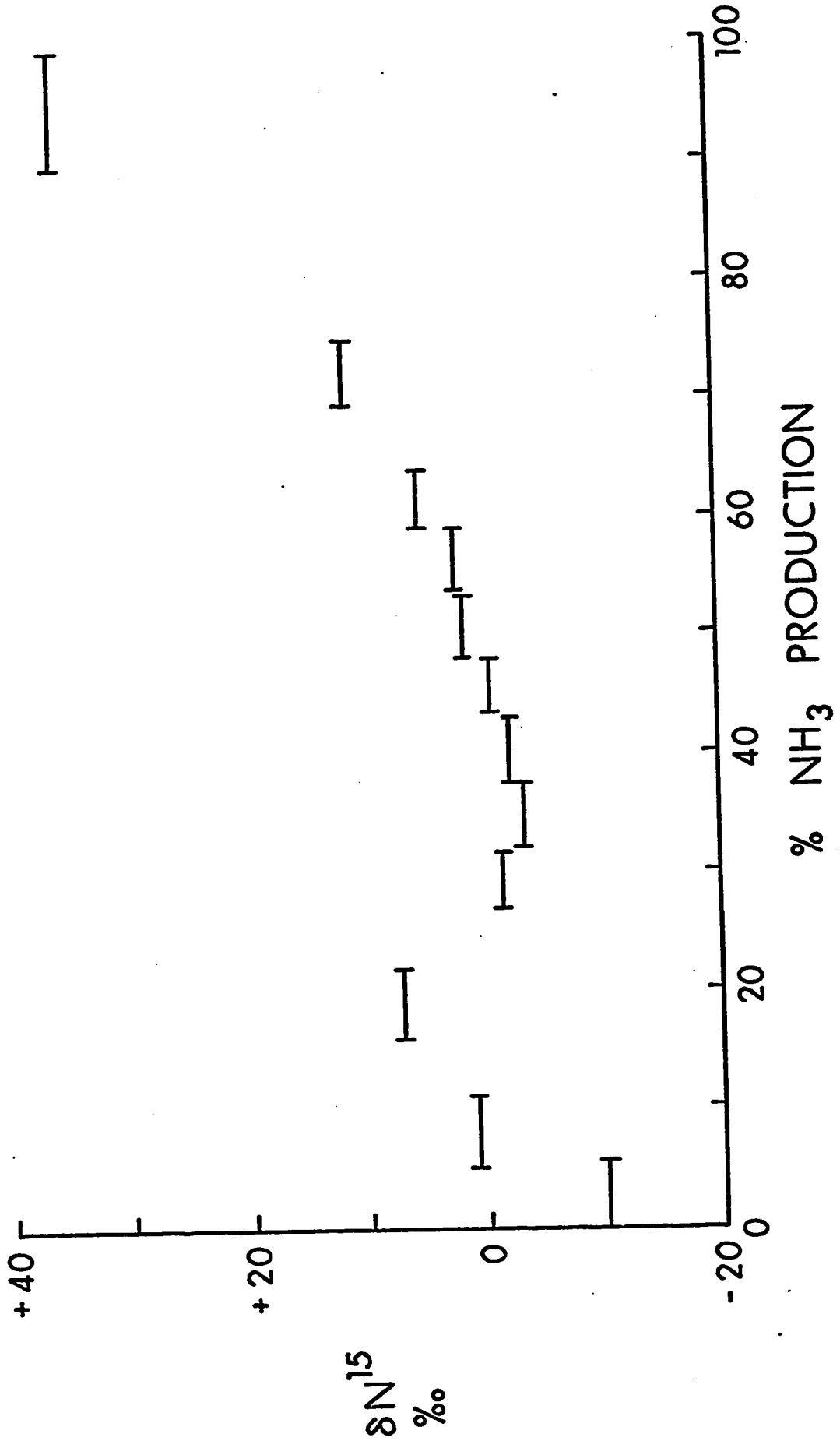


Figure 6.18 Isotopic fractionation in the hydrolysis of urea; run #4

TABLE 6.23

ISOTOPIC FRACTIONATION
 NO₃⁻ Reduction (Microbiological)
 (A) Pseudomonas stutzeri

Sample	% Reduction	$\delta \text{N}^{15}(\text{N}_2)$ Product	δN^{15} Reservoir	I.F.
1	0 - 5.7	- 17.2	+ 8.0	1.024
2	7.0 - 11.7	- 23.3	+ 11.5	1.033
3	11.7 - 17.9	- 20.5	15.0	1.033
4	17.9 - 28.8	- 16.2	21.0	1.034
5	28.8 - 33.4	- 9.4	24.3	1.032
6	33.4 - 52.6	+ 2.0	+ 33.6	1.027
7	58.1 - 91.6	+ 31.3	+ 63	1.017
8	97.0 - 100	+ 51.3		

Reference = 10.6

TABLE 6.24

ISOTOPIC FRACTIONATION
 NO₃⁻ Reduction (Microbiological)
 (B) Pseudomonas stutzeri

Sample	% Reaction	δ N ¹⁵ (N ₂) Product	δ N ¹⁵ Reservoir	I.F.
1	0 - 5.1	- 2.9	+ 1	
2	5.1 - 11.0	- 14.0	+ 1.5	1.016
3	11.0 - 14.3	- 17.7	+ 2.2	1.020
4	14.3 - 19.5	- 16.2	+ 4.0	1.020
5	19.5 - 27.4	- 15.1	+ 6.5	1.020
6	27.4 - 40.0	- 11.9	+ 10.5	1.022
7	40.0 - 52.0	- 10.1	+ 17	1.025
8	52.0 - 62.0	+ 4.8	+ 21	1.014
9	62.0 - 79.4	+ 18.2	-	-
10	79.4 - 83.0	+ 25.1	-	-
11	88.0 - 92.0	+ 28.0	-	-
12	92.0 - 95.0	+ 31.7	-	-
13	95.0 - 99.5	+ 18.8	-	-

TABLE 6.25

ISOTOPIC FRACTIONATION

NO₃⁻ Reduction (Microbiological)(C) Bacillus sp

Sample	% Reaction	δ N ¹⁵ (N ₂) Product	δ N ¹⁵ Reservoir	I.F.
1	0 - 22.4	- 9.5	+ 9.15	1.017
2	30.0 - 46.3	- 16.1	+ 20.5	1.031
3	47.5 - 64.5	- 10.0	+ 36.0	1.037
4	64.5 - 72.4	- 6.3	+ 48.0	1.050
5	76.2 - 81.5	- 4.5	+ 103	-
6	81.5 - 95.0	- 2.5	+ 600	-
7	95.0 - 97.5	+ 89.7	-	-
8	97.5 - 100	+ 1.3	-	-

TABLE 6.26

ISOTOPIC FRACTIONATION
 NO₃ Reduction (Microbiological)
 (D) Bacillus I₂

Sample	% Reaction	δ N ¹⁵ (N ₂) Product	δ N ¹⁵ Reservoir	I.F.
1	0 - 6.96	- 15.5	+ 6.6	1.022
2	6.96 - 13.2	- 20.0	+ 8.4	1.028
3	13.2 - 14.7	- 19.0	+ 8.5	1.028
4	15.3 - 16.8	- 18.3	+ 9.6	1.028
5	17.2 - 25.6	- 15.0	+ 12.4	1.026
6	26.2 - 39.5	- 4.0	+ 16.3	1.018
7	41.0 - 49.7	+ .1	+ 19.2	1.018
8	50.6 - 67.0	+ 5.0	+ 29.3	1.018
9	67.0 - 78.0	+ 7.5	+ 42.0	1.026
10	78.0 - 81.5	+ 8.0		
11	81.5 - 83.4	+ 8.0		
12	83.4 - 84.5	+ 8.0		
13	84.5 - 88.0	+ 8.2		
14	88.0 - 90.0	+ 8.0		
15	90.0 - 91.4	+ 11.8		
16	91.4 - 97.0	+ 23.6		
17	97.0 - 100	+ 12.5		

TABLE 6.27

ISOTOPIC FRACTIONATION
 NO_3^- Reduction - Resting Cell
 (G) P. stutzeri

Sample	% Reaction	$\delta \text{N}^{15}(\text{NH}_3)$ Product	I.F.
1	5.76	- 9.7	1.010
2	11.6	- 10.5	1.011
3	13.1	- 13.5	1.014
4	17.4	- 6.6	1.007
5	35.8	+ 1.5	1.002
6	44.2	- 2.5	1.003
7	47.2	- 3.0	1.005
8	53.0	- 4.5	1.006
9	64.0	- 5.7	1.005
10	69.5	- 5.4	1.000
11	89.0	0	1.006
12	92.0	- 5.5	--
13	93.0	-	1.006
14	94.4	- 6.1	1.005
15	96.0	- 5	1.003
16	> 96	- 2.6	

TABLE 6.28

ISOTOPIC FRACTIONATION

NO₂⁻ Reduction (Microbiological)(E) Bacillus sp 625

Sample	% Reaction	δ N ¹⁵ (N ₂) Product	δ N ¹⁵ Reservoir	I.F.
1	0 - 9.0	[+ 21.2]	8.9	
2	9.0 - 18.2	- 8.0	10.8	1.019
3	21.0 - 26.9	- 7.7	13.1	1.020
4	26.9 - 36.5	- .7	13.8	1.020
5	-	-	15.7	-
6	46.4 - 63.0	+ 3.5	20.4	1.015
7	63.0 - 70.6	+ 7.6	23.2	1.014
8	70.6 - 80.0	+ 14.1	26.4	1.011
9	80.0 - 87.7	+ 16.0	30.4	1.013
10	87.7 - 92.2	+ 23.2	32.8	1.009
11	92.2 - 98.5	+ 25.7	61.0	
12	> 98.5	+ 28.3		

TABLE 6.29

ISOTOPIC FRACTIONATION

NO₂⁻ Reduction (Microbiological)(F) Alcaligenes faecalis 4456

Sample	% Reaction	δ N ¹⁵ (N ₂) Product	δ N ¹⁵ Reservoir	I.F.
1	0 - 11.6	- 8.2	+ 12.2	1.019
2	11.6 - 12.8	- 12.5	-	1.025
3	12.8 - 25.8	- 10.1	+ 16.9	1.025
4	25.8 - 46.5	- 5.0	+ 25.2	1.026
5	46.5 - 59.2	-	+ 33.2	-
6	59.2 - 74.6	+ 17.4	-	-
7	74.6 - 88.0	+ 21.4	-	-
8	88.0 - 93.0	+ 21.5	-	-
9	93.0 - 98.0	+ 17.2	-	-
10	> 98.0	+ 21.7	-	-

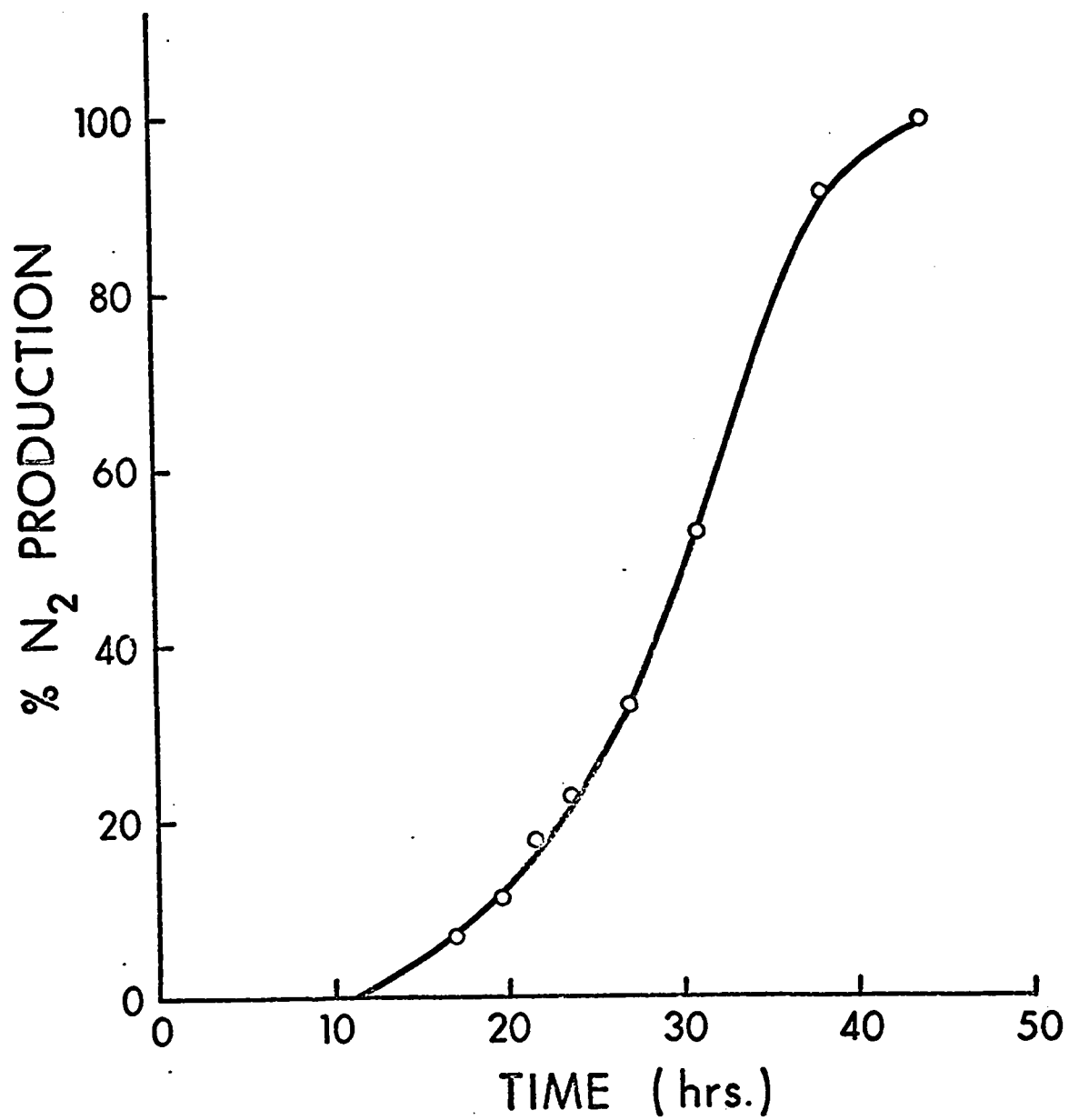


Figure 6.19

Percent N_2 production vs time; microbiological nitrate reduction "A"

Figure 6.20 Relative rate of N_2 production vs time;microbiological
nitrate reduction "A"

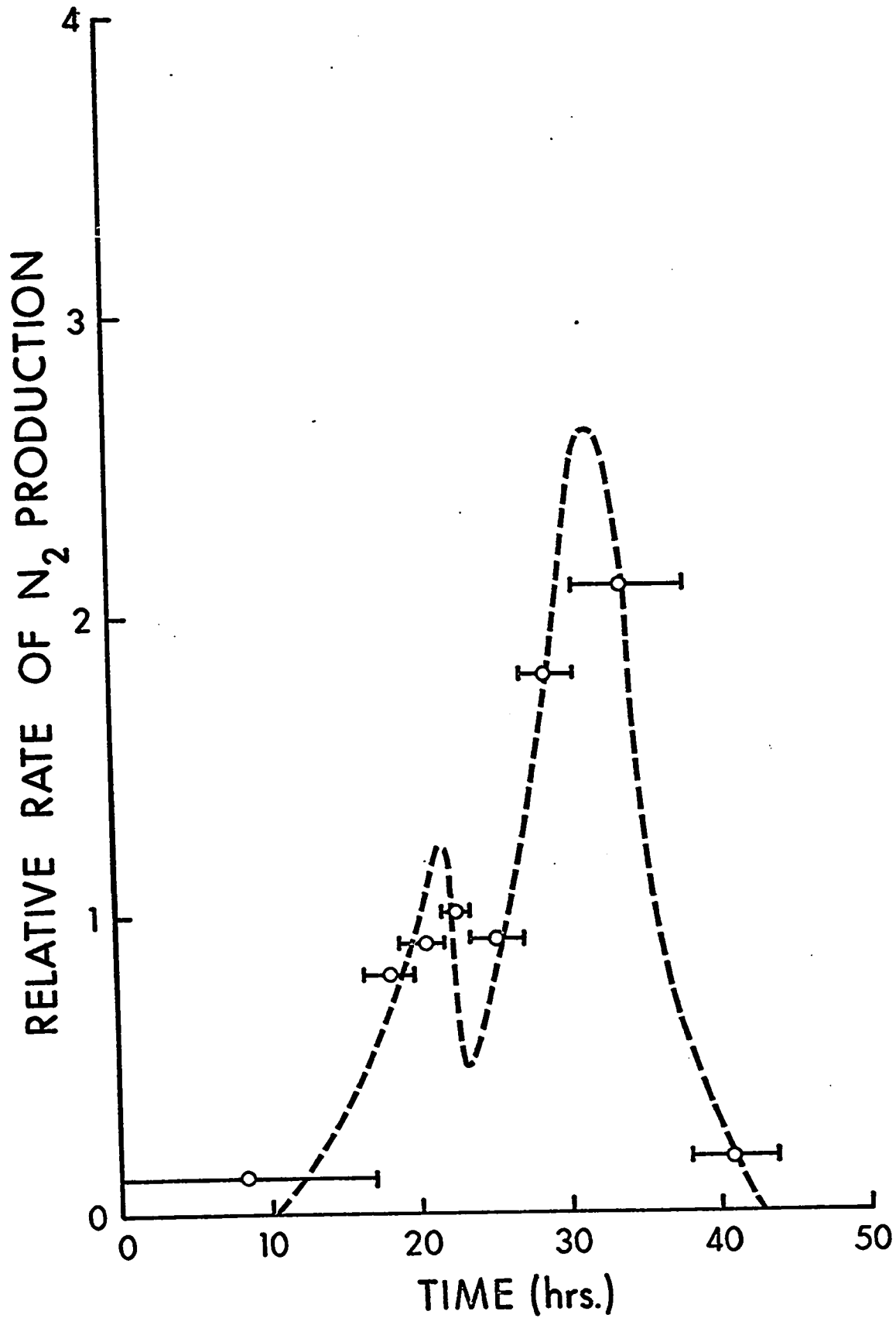


Figure 6.21 Isotopic fractionation in microbiological nitrate reduction "A"

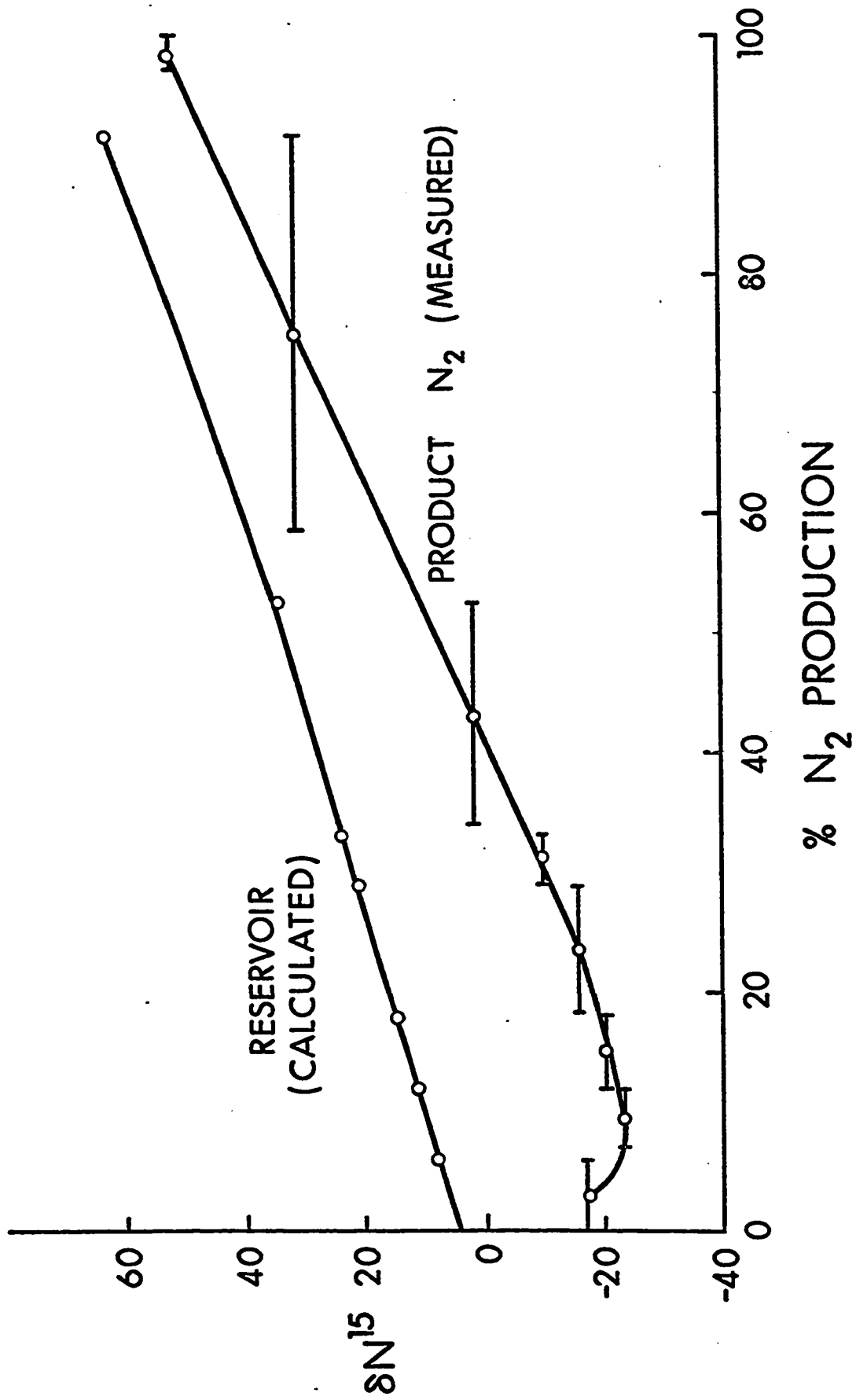


Figure 6.22 Relative rate of N_2 production vs time; microbiological nitrate reduction "B"

RELATIVE PRODUCTION RATE OF N₂ (arbitrary units)

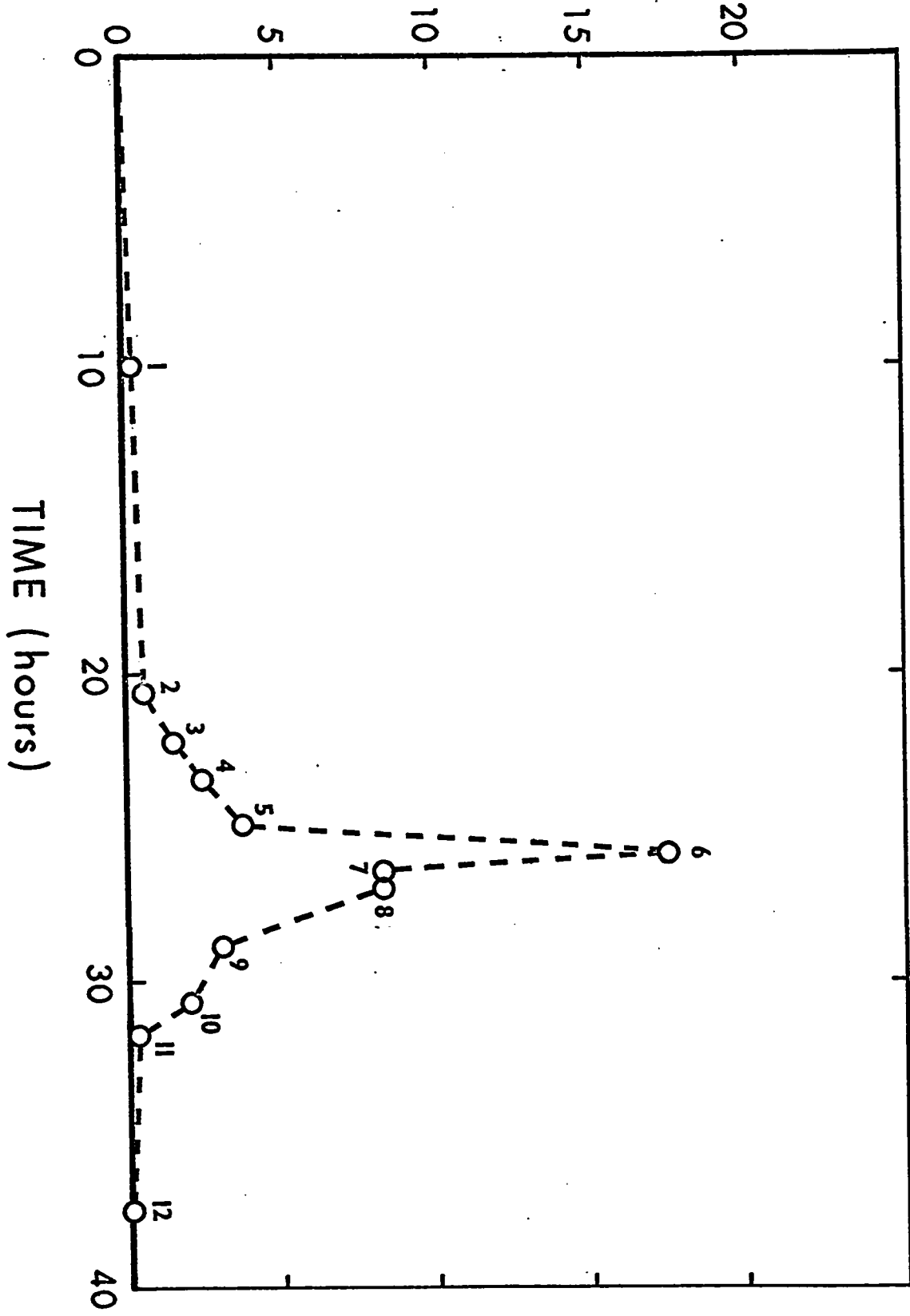
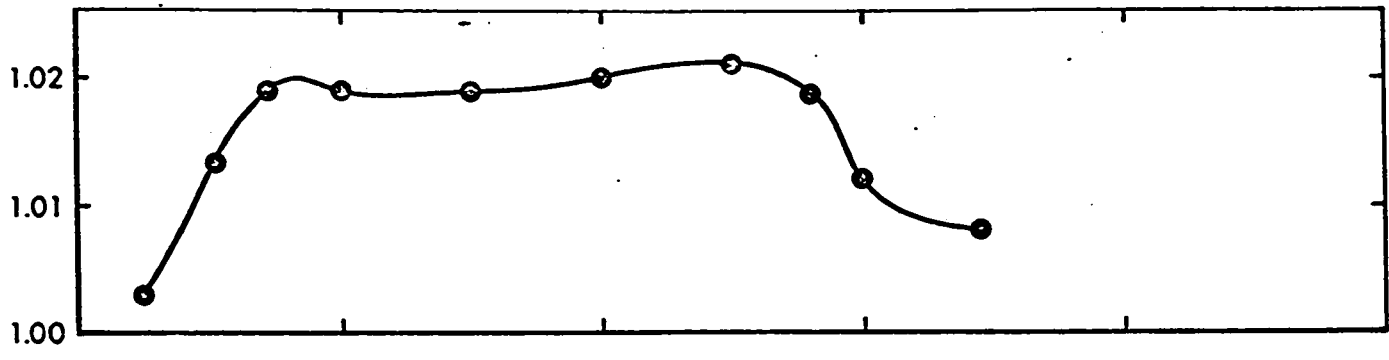
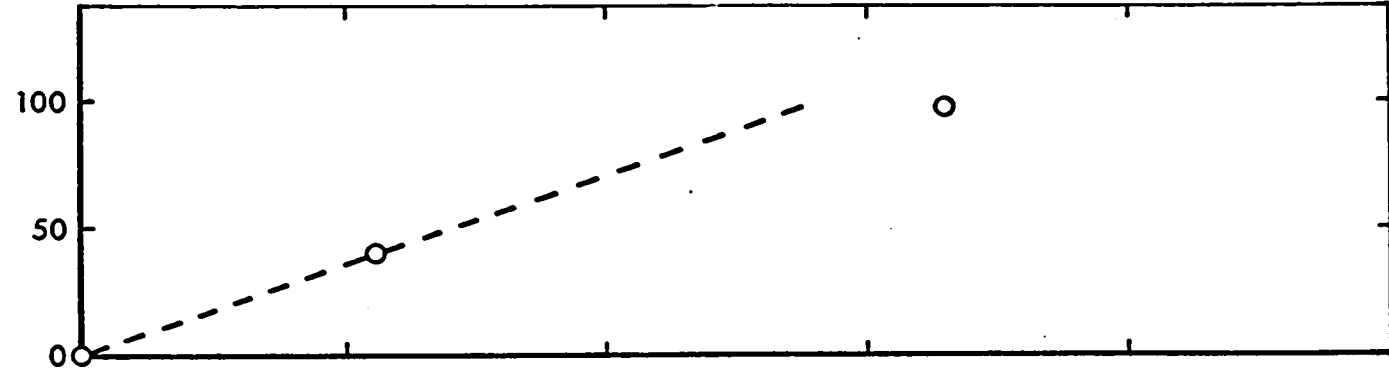


Figure 6.23 Isotopic fractionation in microbiological nitrate
reduction "B"

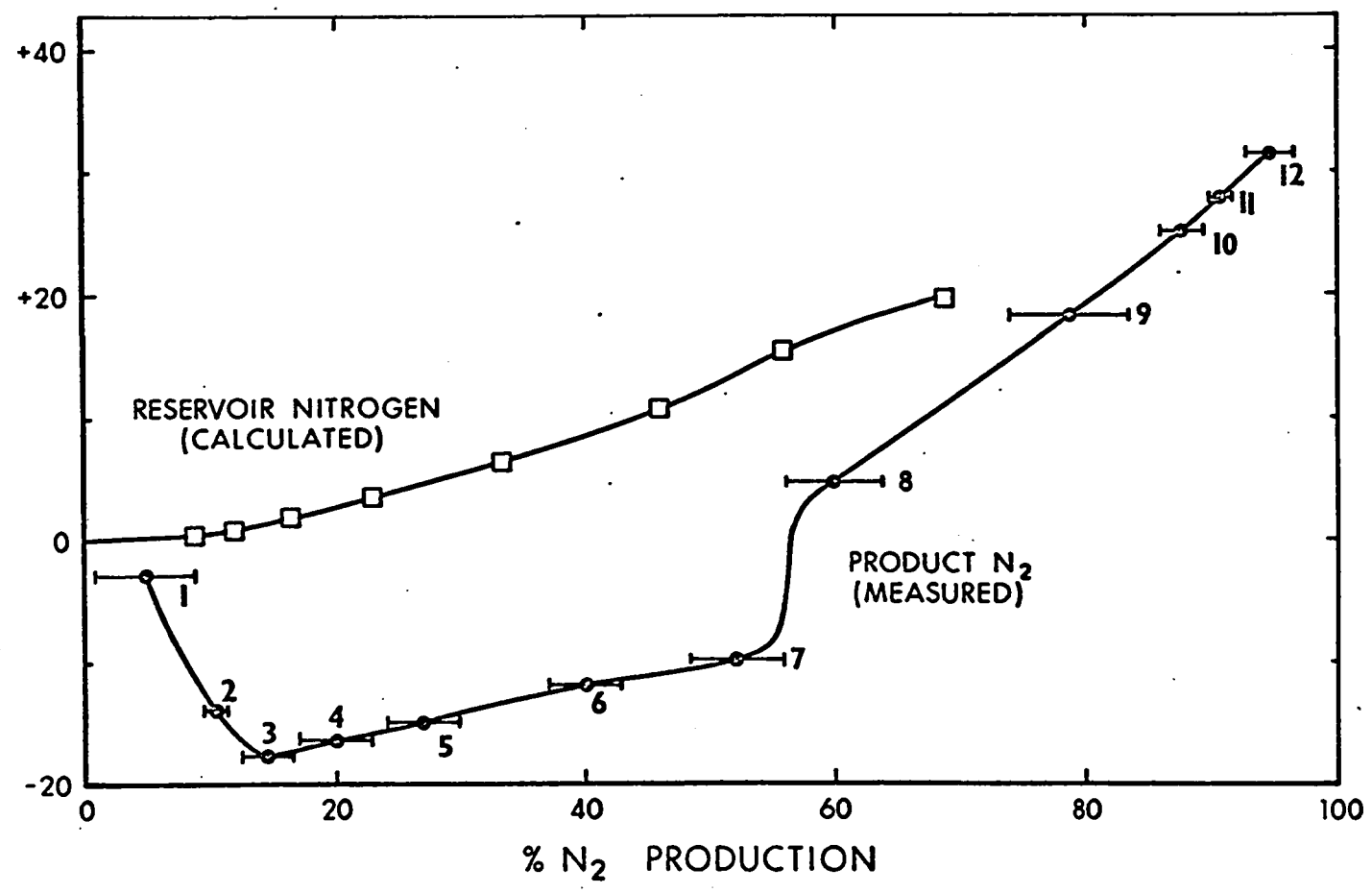
INSTANTANEOUS ISOTOPE FRACTIONATION FACTOR



% NO₃ REACTED



δN¹⁵



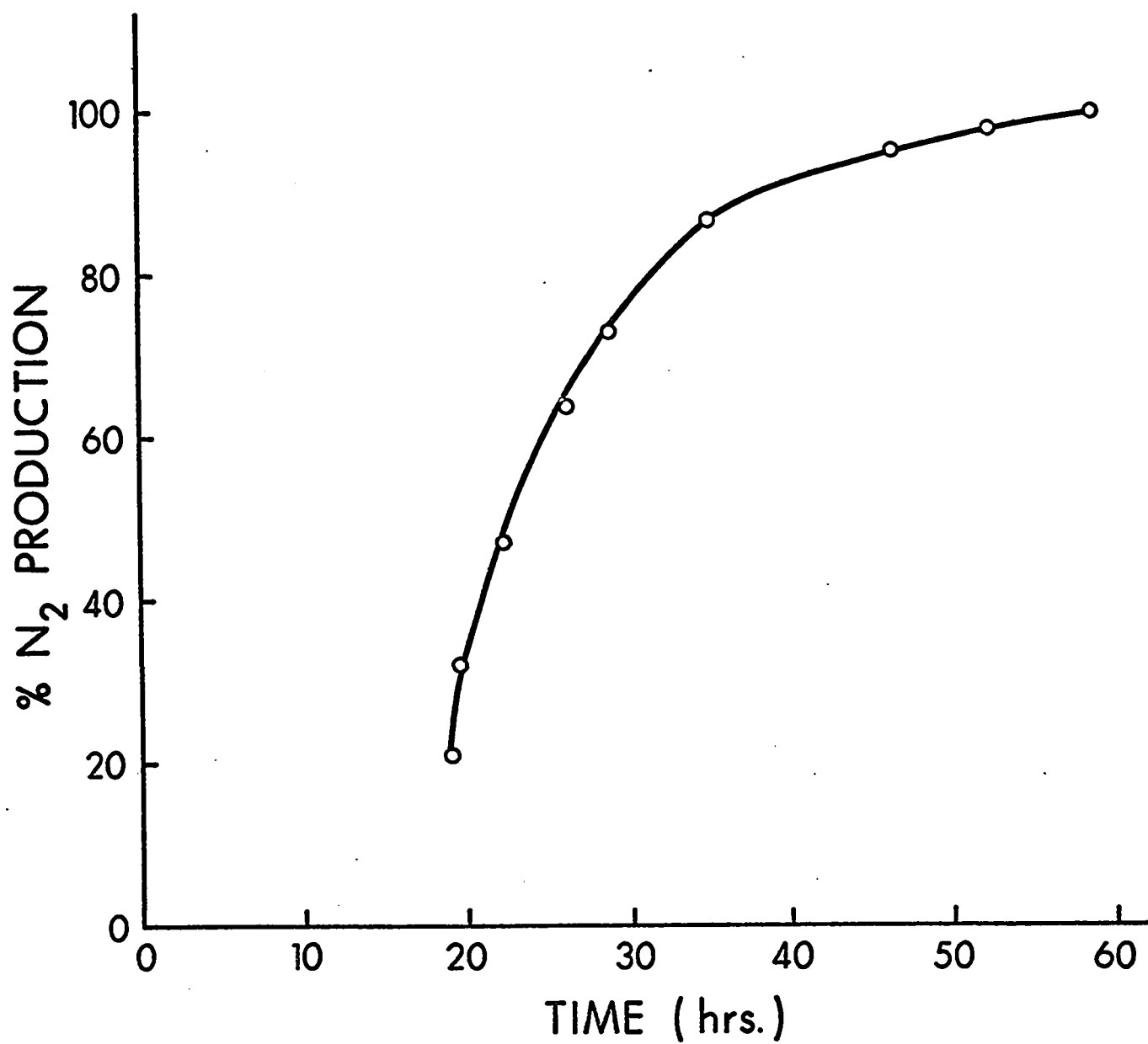


Figure 6.24 Percent N_2 production vs time; microbiological nitrate reduction "C"

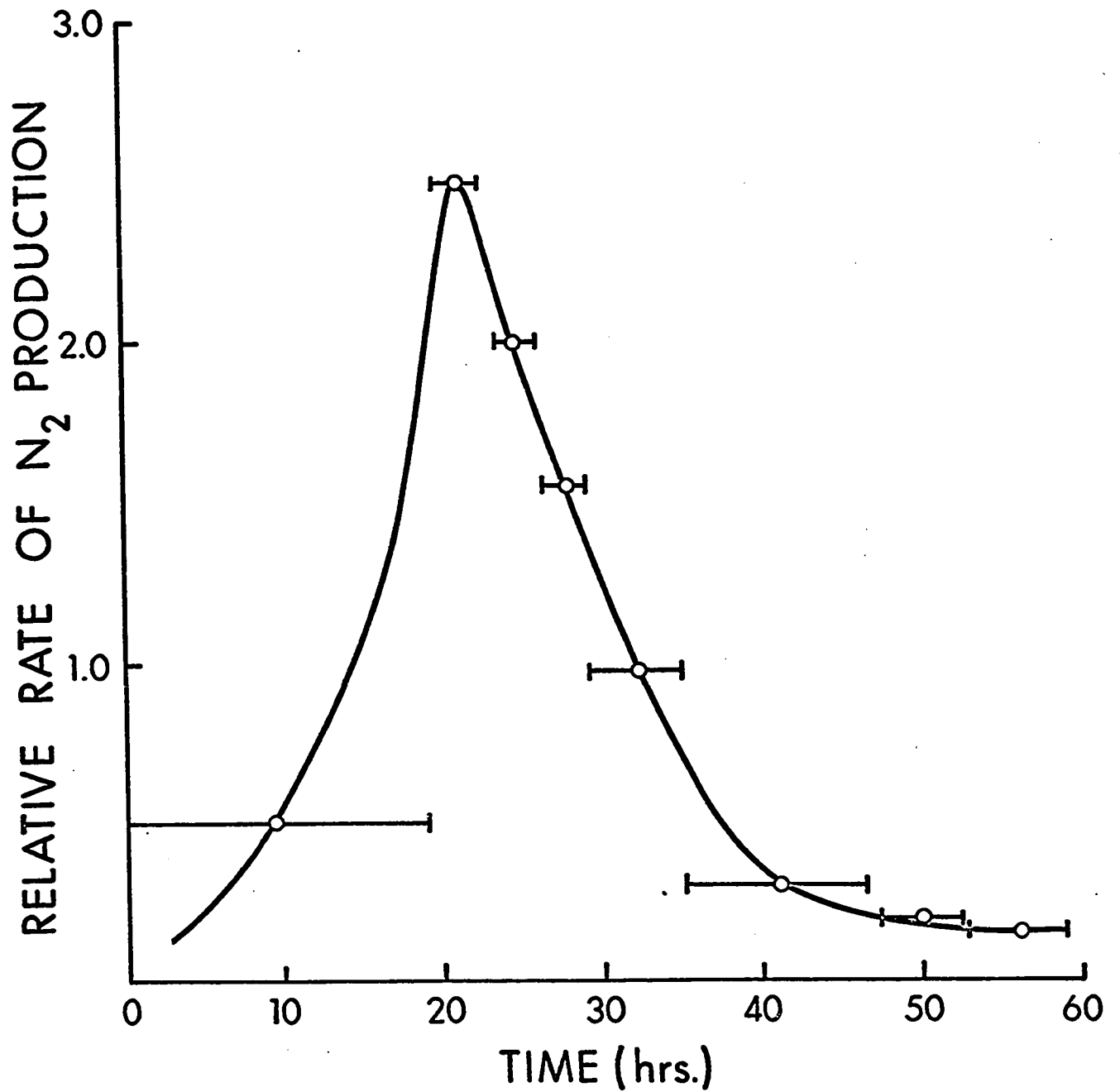


Figure 6.25 Relative rate of N₂ vs time microbiological nitrate reduction "C"

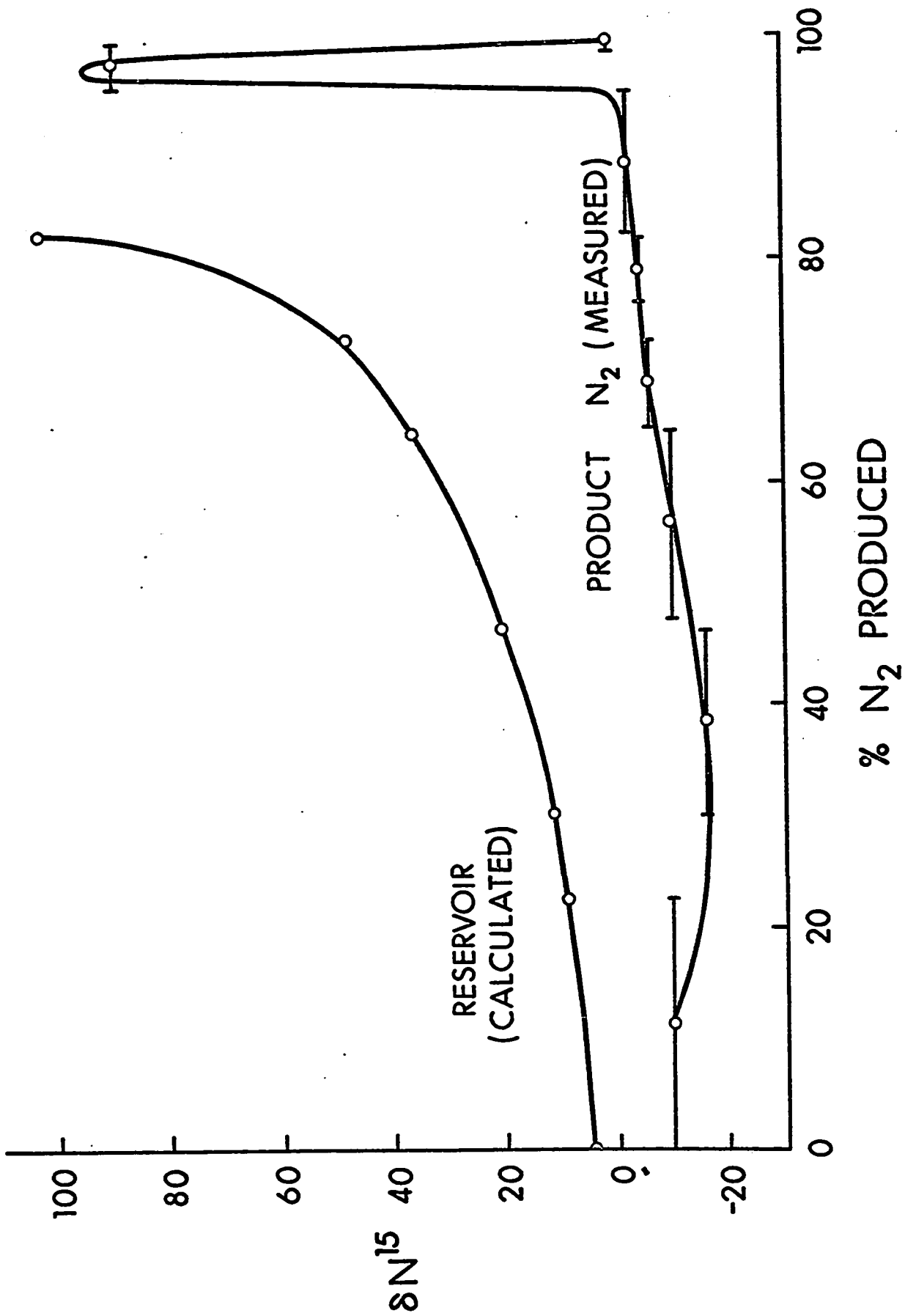
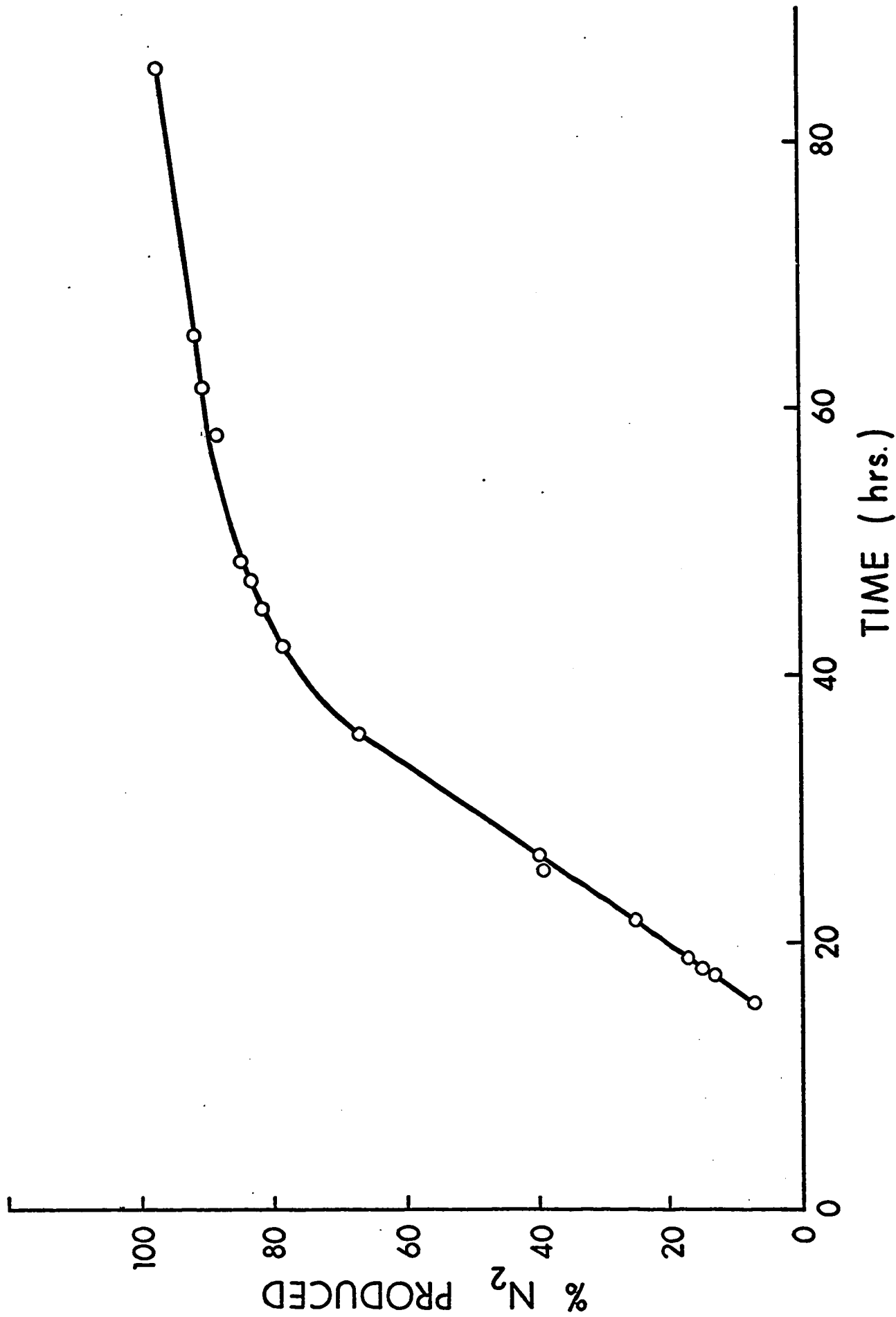


Figure 6.26 Isotopic fractionation in microbiological nitrate reduction "C"

Figure 6.27 Percent N₂ production vs time; microbiological nitrate reduction "D"



107

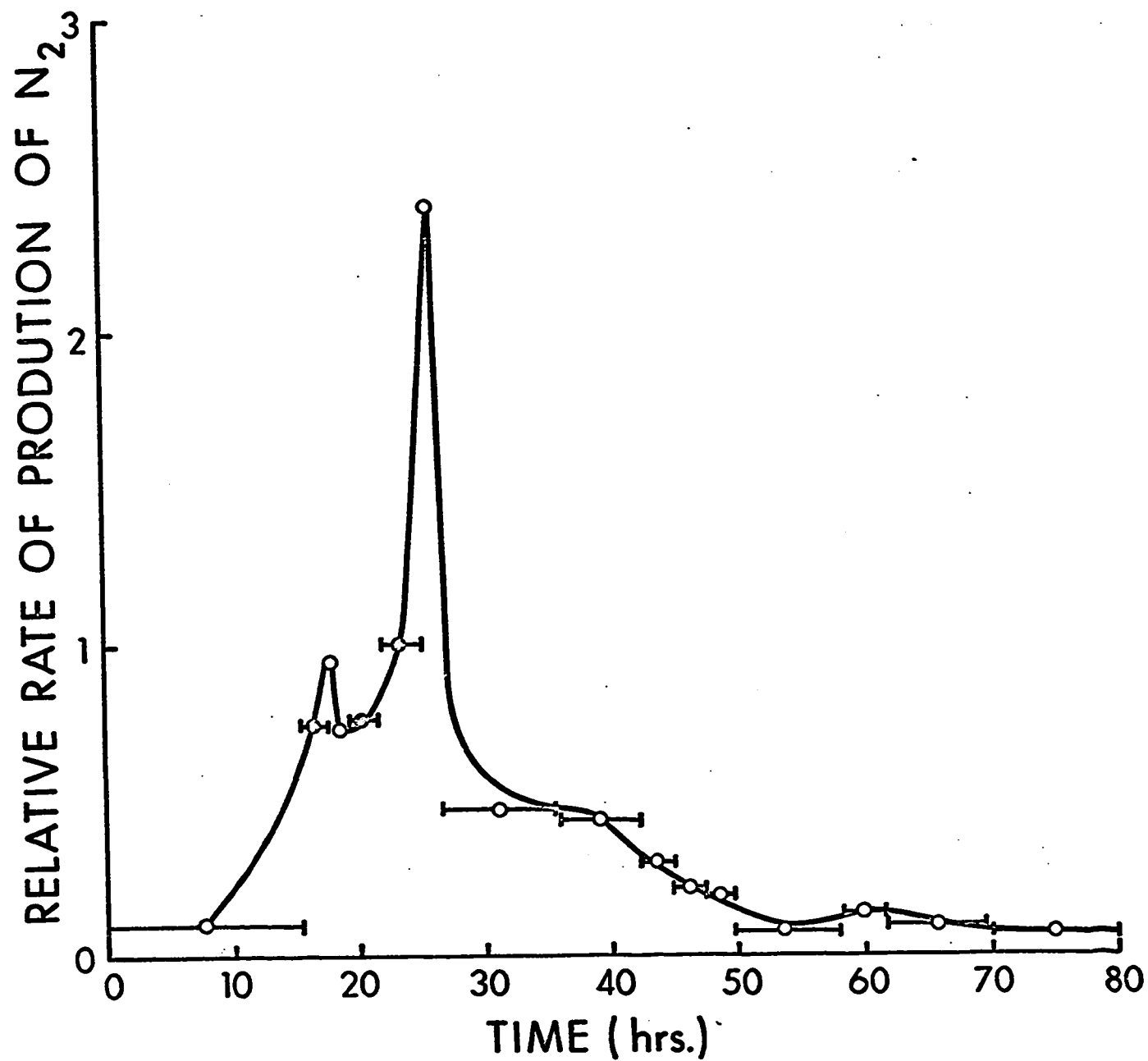
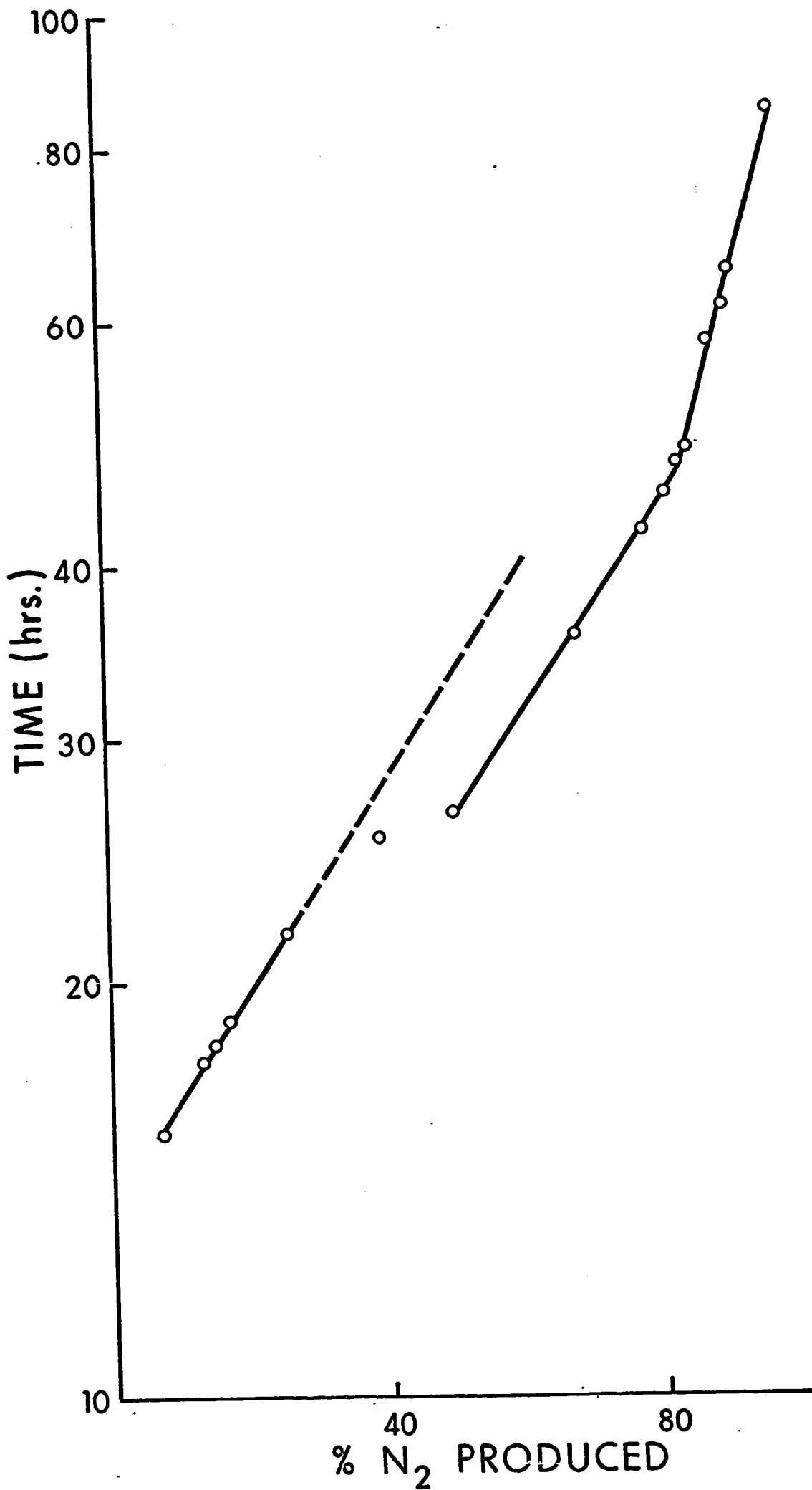


Figure 6.28 Relative rate of N_2 production vs time; microbiological nitrate reduction "D"

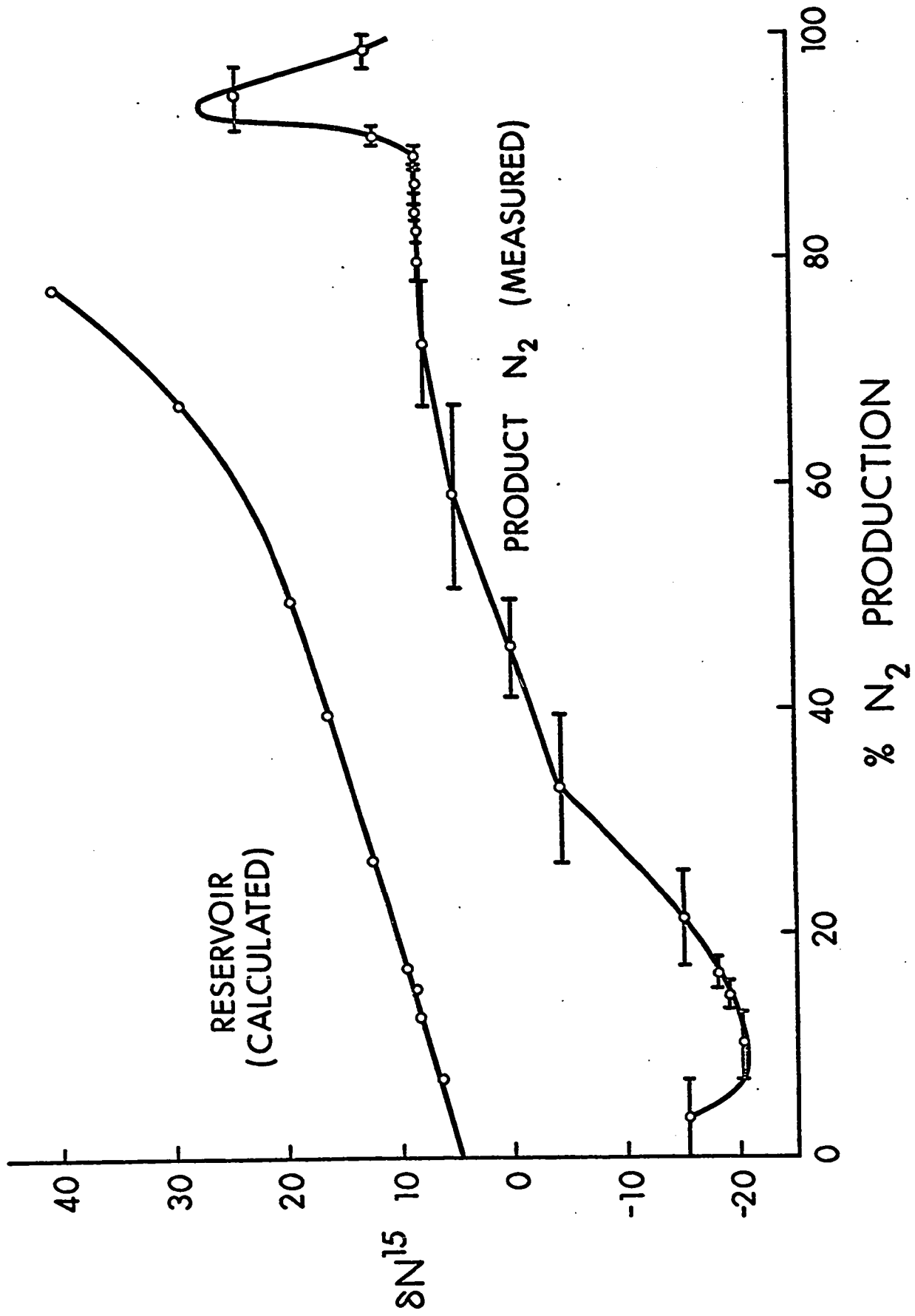
628

Figure 6.29 Logarithmic plot of percent N_2 production; microbiological
nitrate reduction "D"



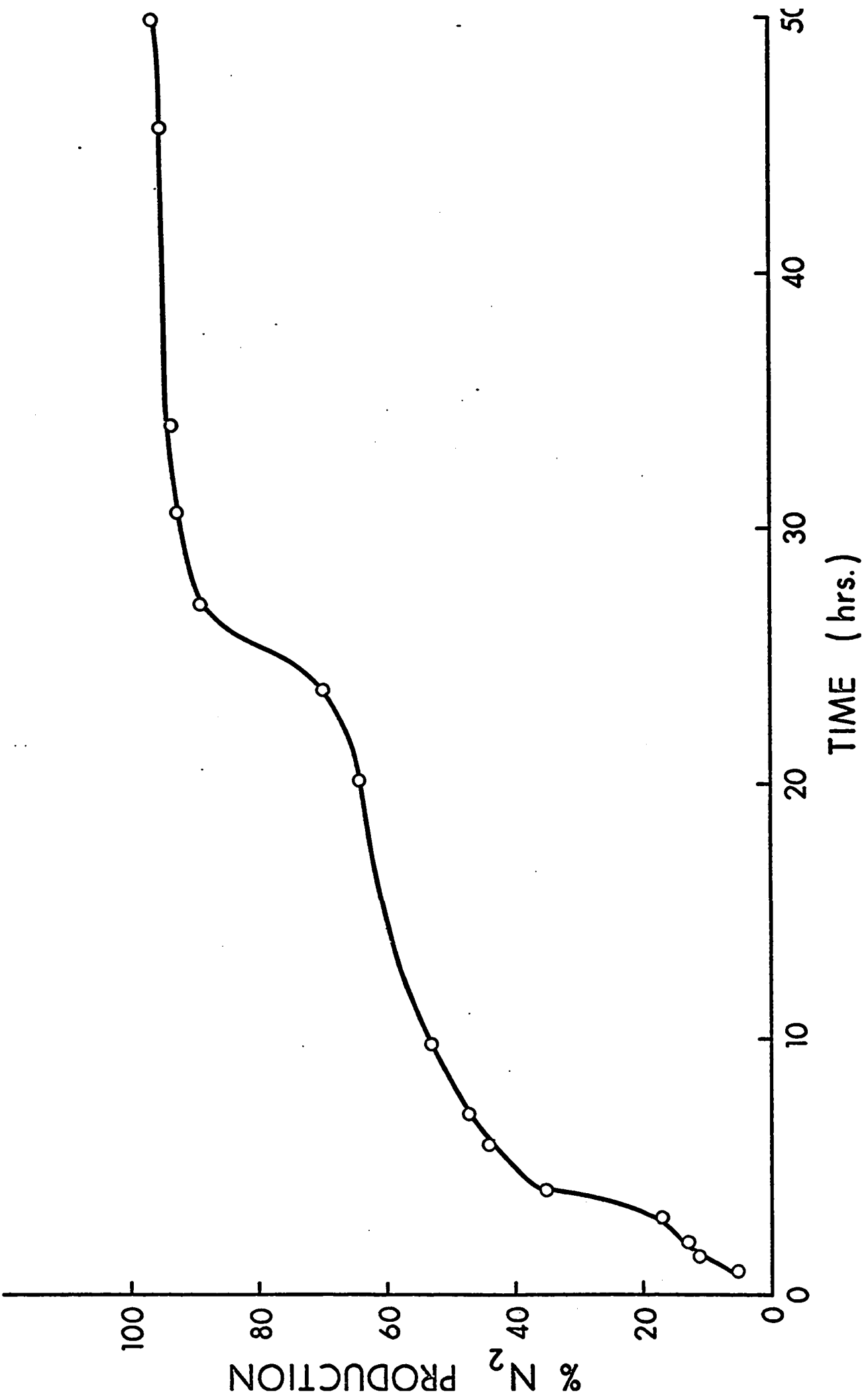
629

Figure 6.30 Isotopic fractionation in microbiological nitrate
reduction "D"



620

Figure 6.31 Percent N₂ production vs time; nitrate reduction "G"



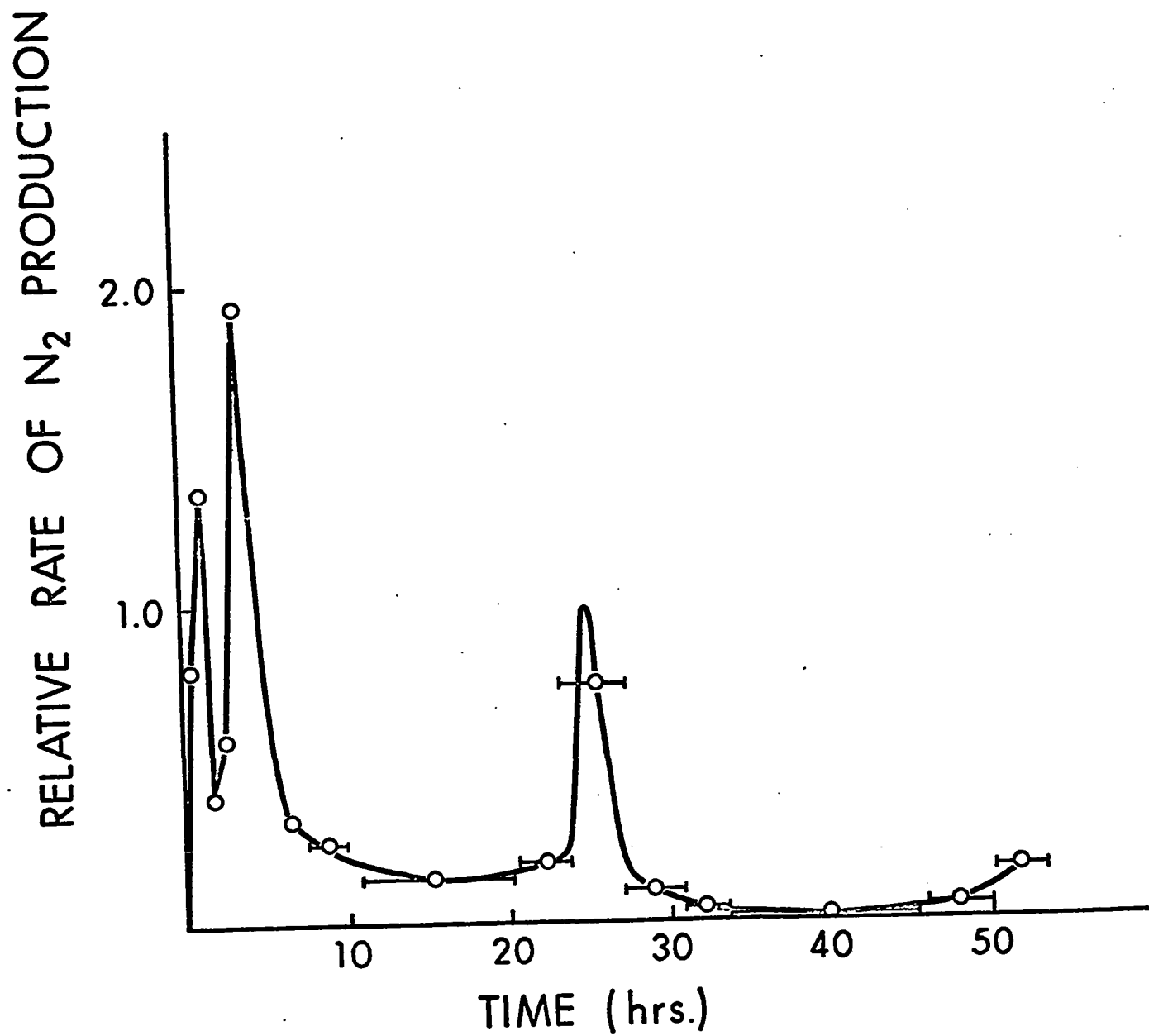


Figure 6.32 Relative rate of N₂ production vs time; nitrate reduction "G"

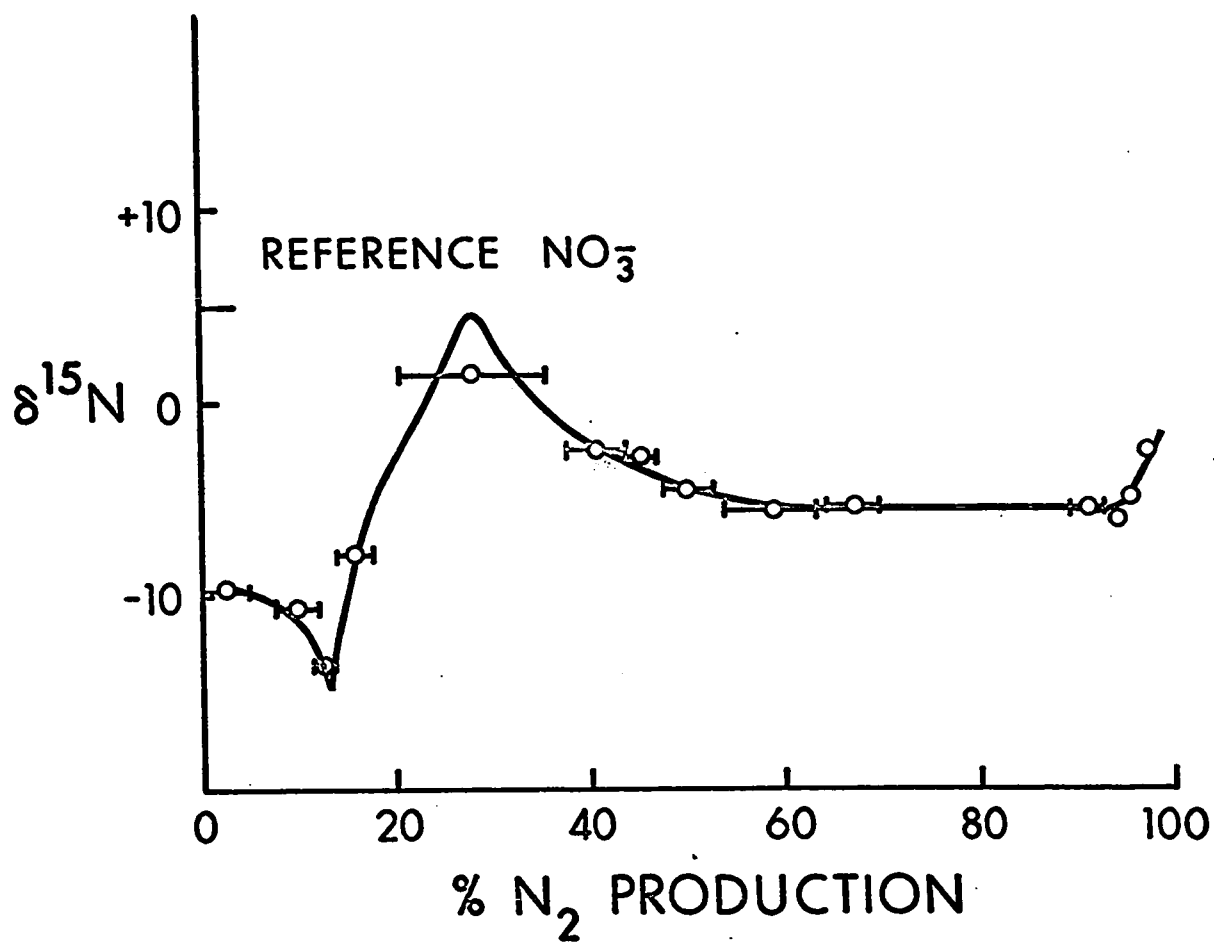


Figure 6.33 Isotopic fractionation in microbiological nitrate reduction "G"

633

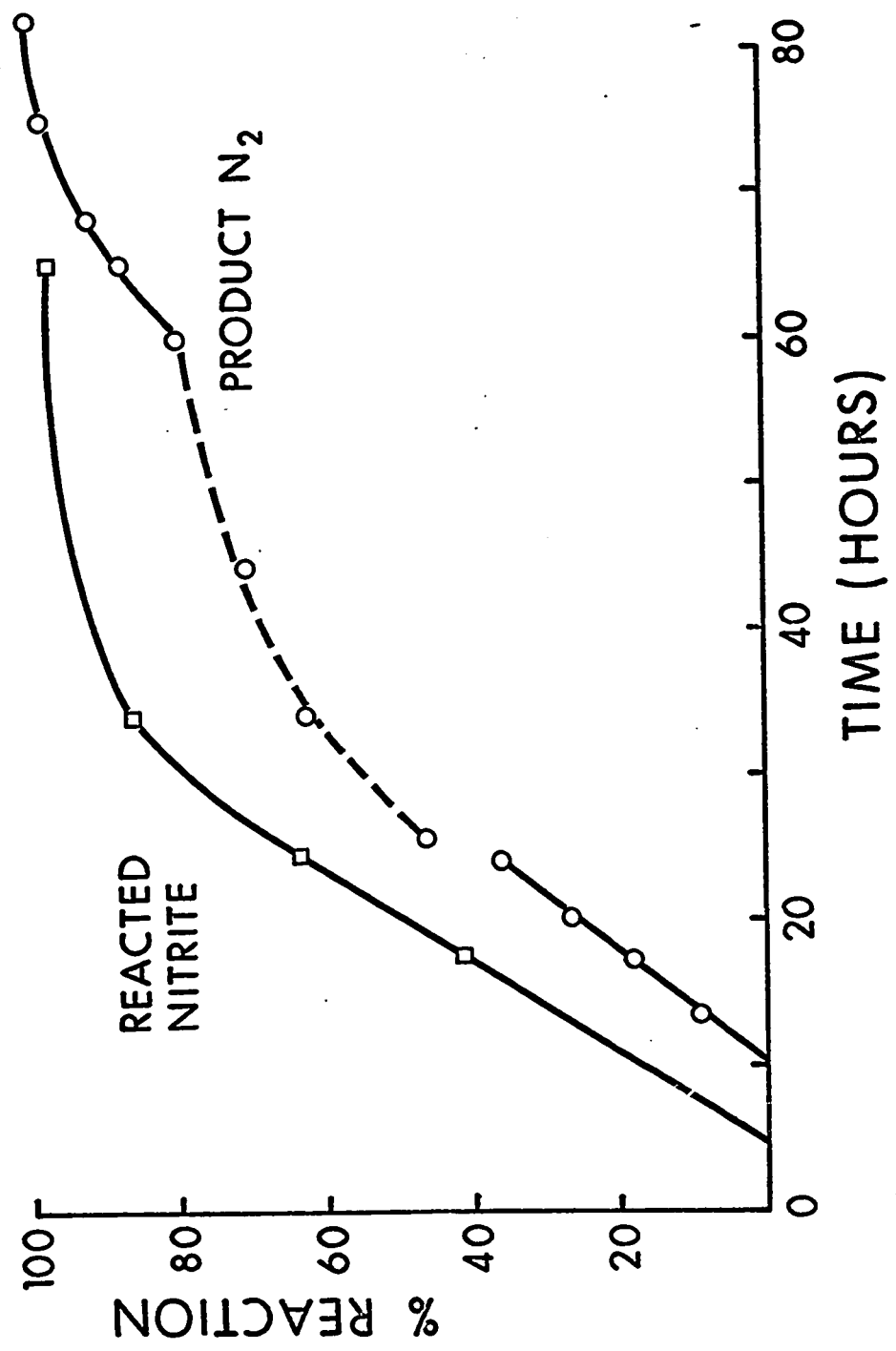


Figure 6.34 Percent N₂ production vs time in microbiological nitrite reduction "E"

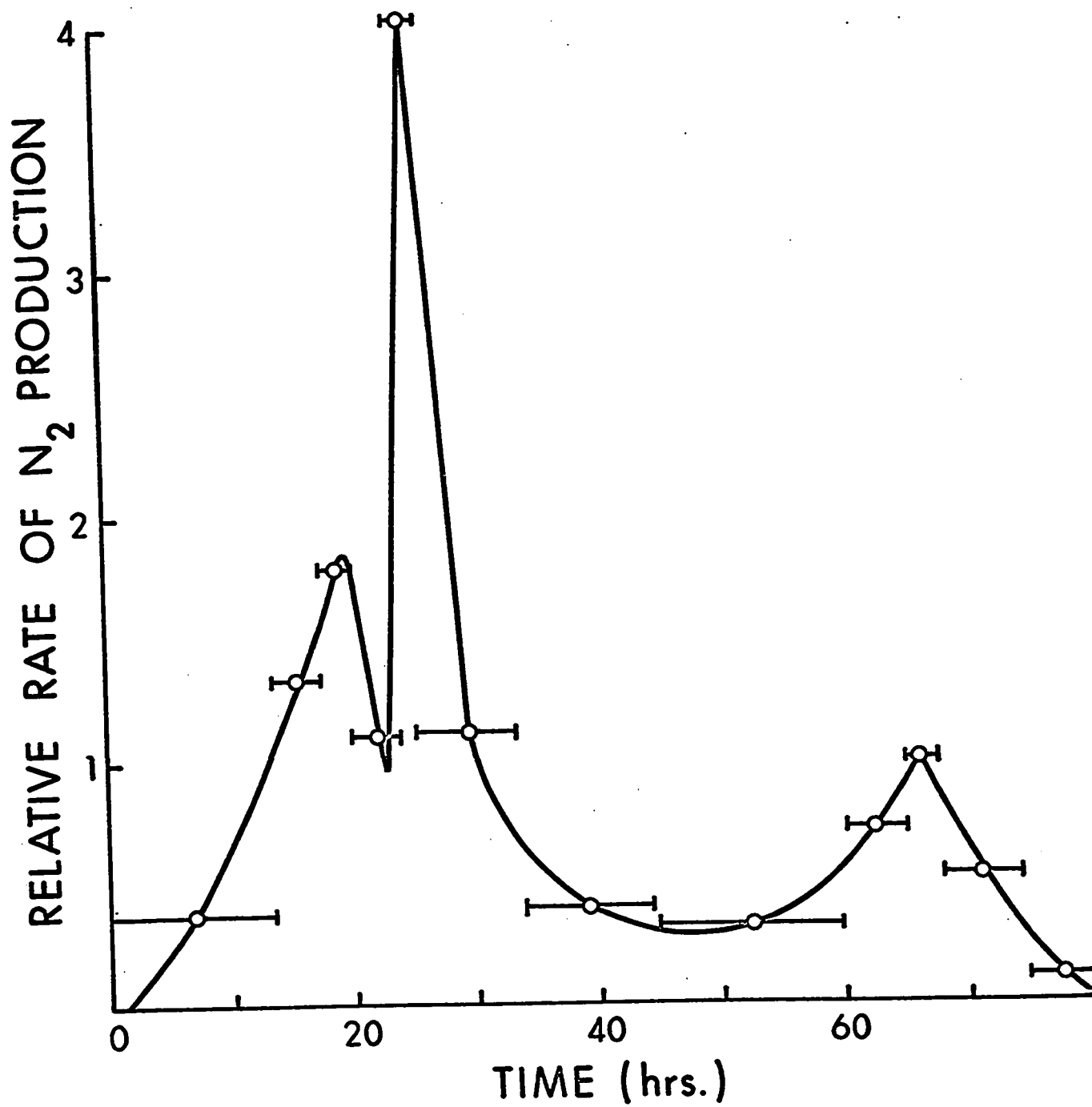
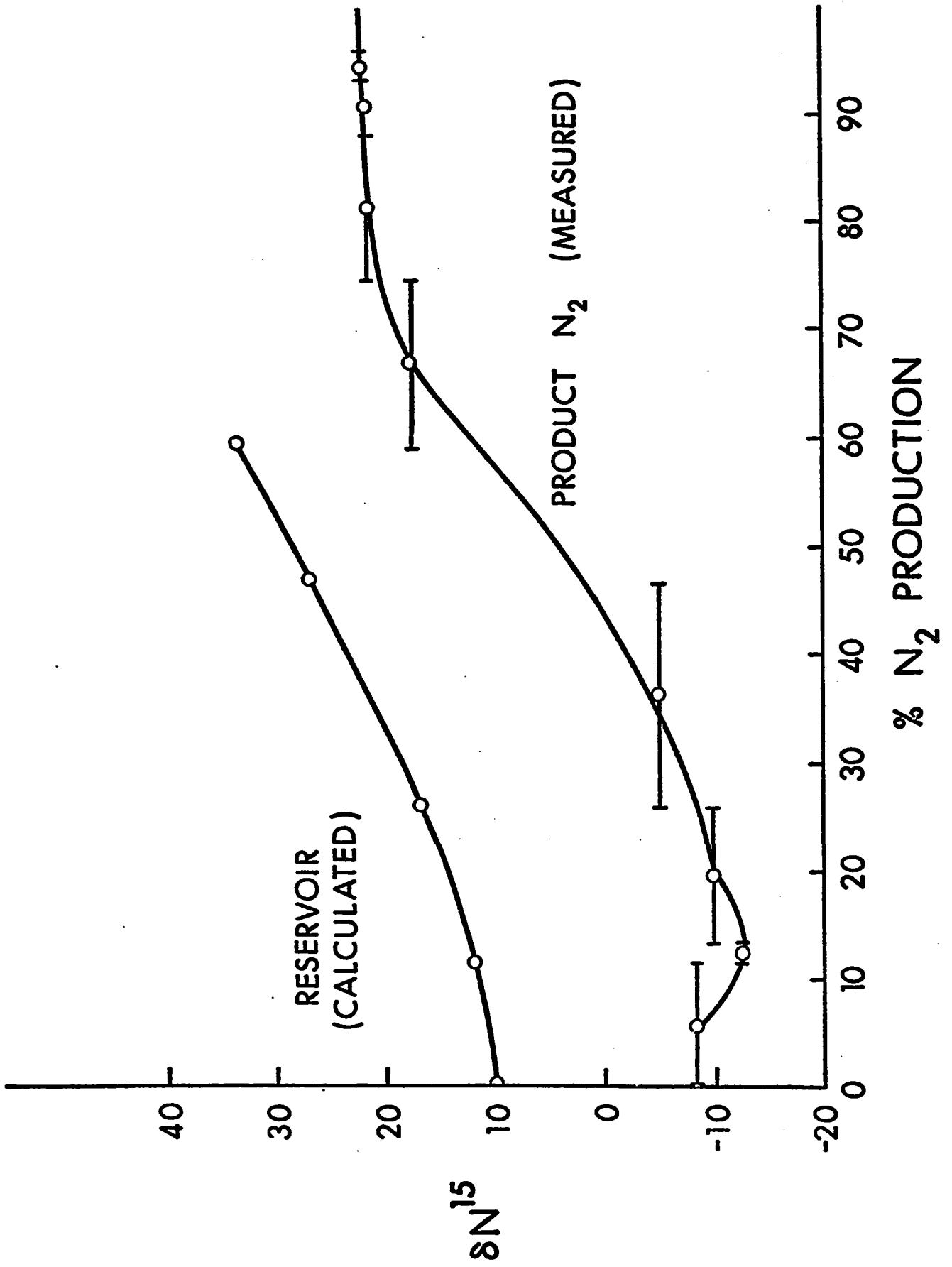


Figure 6.35

Relative rate of N_2 production vs time; microbiological nitrite reduction "E"



636

Figure 6.36 Isotopic fractionation in microbiological nitrite
reduction "E"

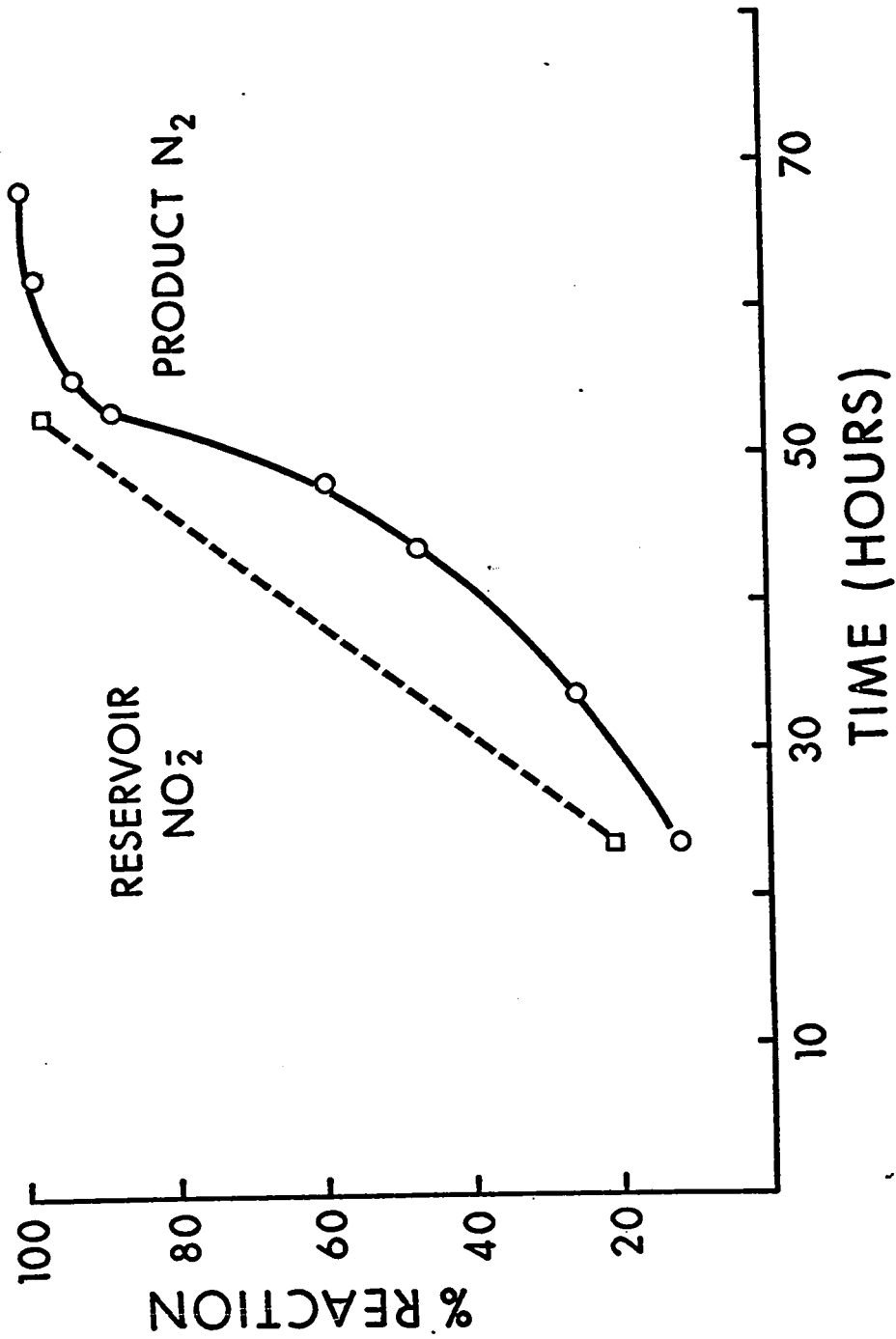


Figure 6.37 Percent reaction vs time; microbiological nitrite reduction "F"

637

Figure 6.38 Relative rate of N_2 production vs time in microbiological nitrite reduction "F"

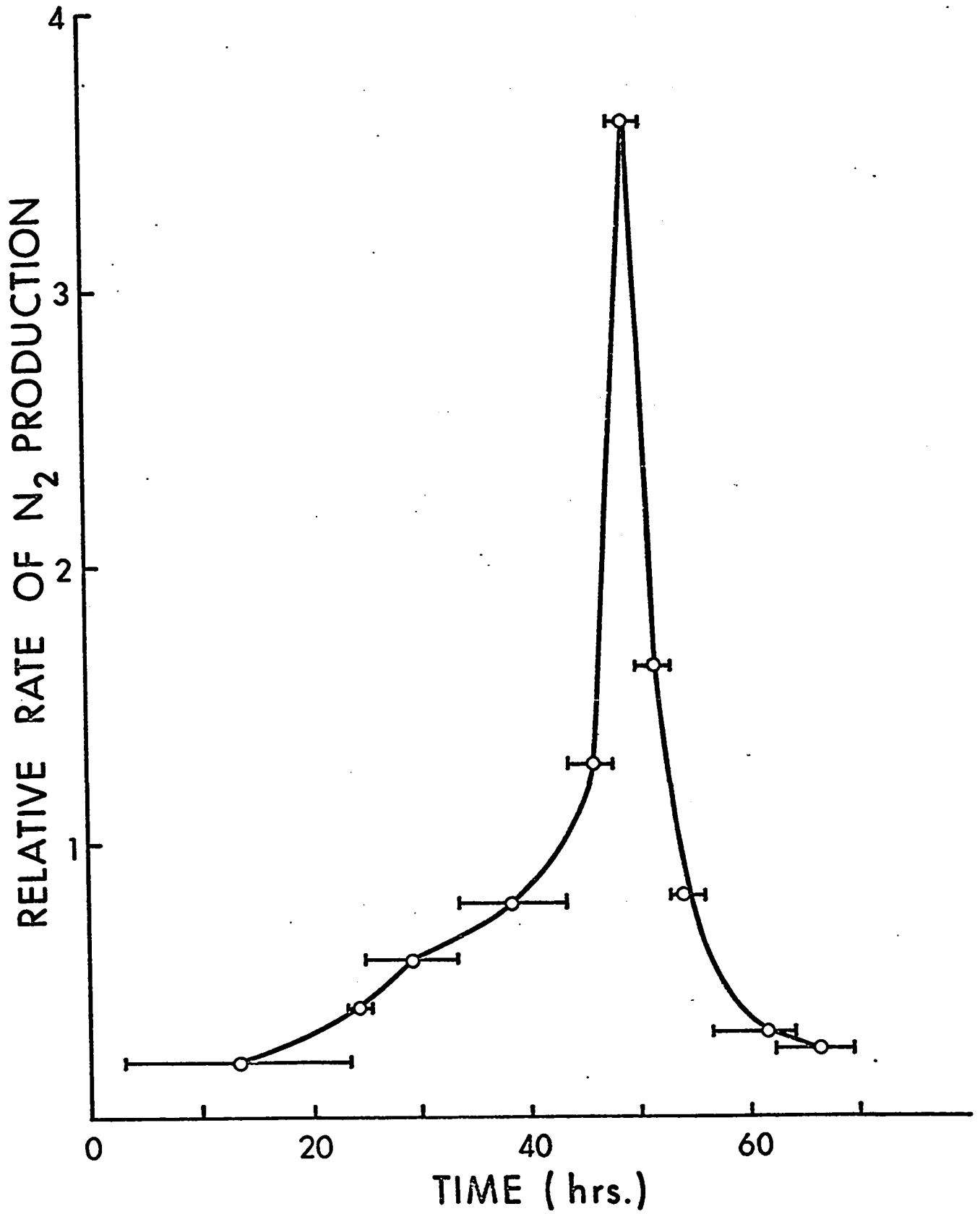
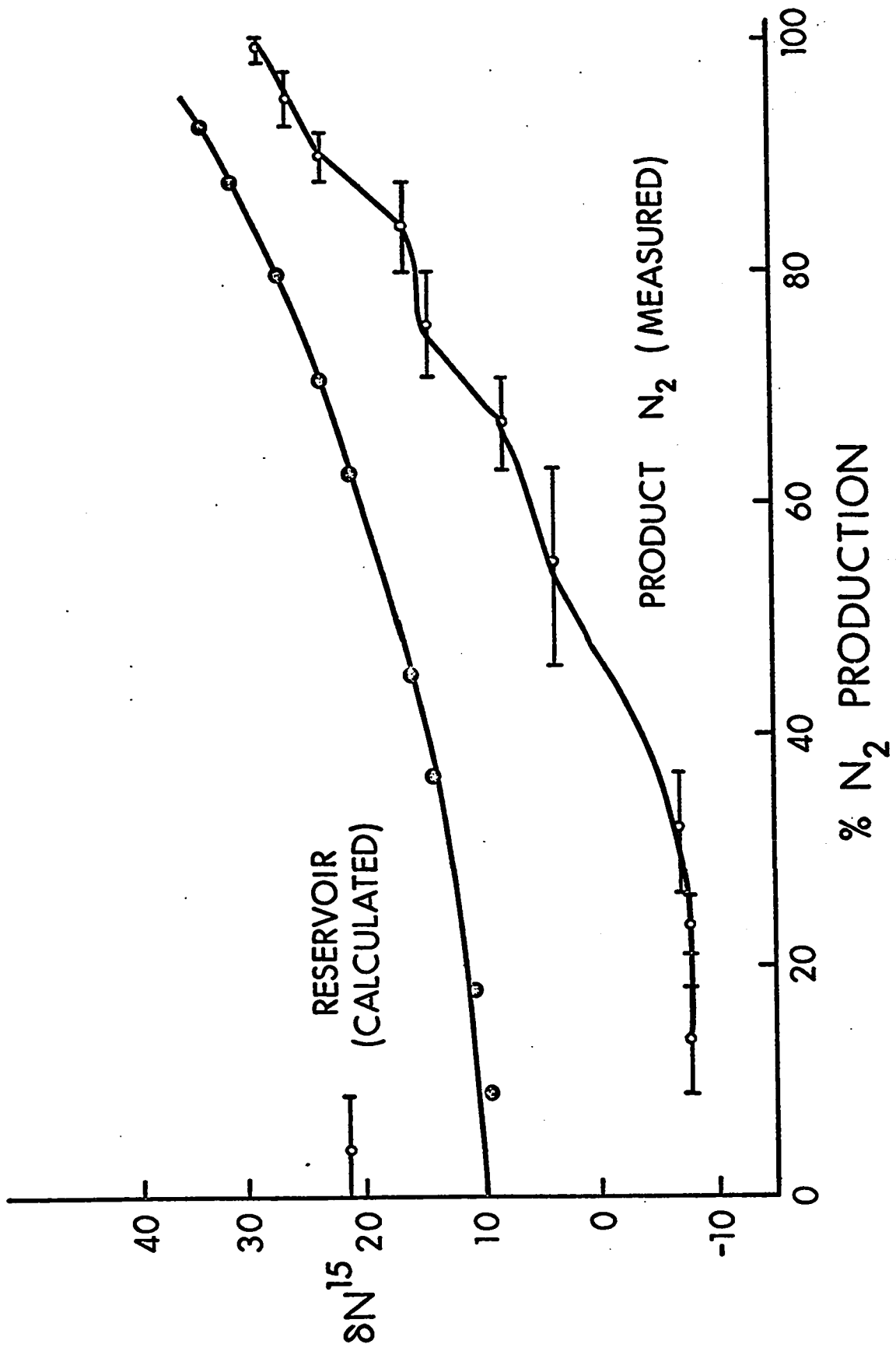


Figure 6.39 Isotopic fractionation in microbiological nitrite
reduction "F"



CHAPTER 7

DISCUSSION AND CONCLUSIONS

7.1 Experimental Procedures

In a number of cases, our results have suggested modifications in technique, and in certain cases new experiments.

Hypobromite oxidation: The hypobromite conversion is easily performed and our modification seems satisfactory. The reaction is very rapid, taking place in less than ten seconds at room temperature. The hypobromite solution had a higher density than the ammonia solution so it was necessary to shake the conversion tube. If the tube is simply tipped, the hypobromite settles to the bottom and a complete conversion may not result.

The largest source of error is oxygen contamination. The rate of production of oxygen varies with both age, and the sample preparation technique. The use of warm sodium hydroxide, or the too rapid addition of bromine to the hydroxide which produces considerable heat seems to give a final solution more prone to oxygen production. Replicate conversions on identical samples yielded variable results unless oxygen was removed from the sample by heating with copper. In one particular nitrate reduction which is not reported here, all isotopic measurements were greater than +5, the reference value. The general features of this run were similar to other nitrate reductions. This resulted presumably because the same amounts of ammonia and hypobromite were used for all samples and the percent

oxygen contamination was about the same. In this experiment, the presence of oxygen caused a systematic error of about 40 % on all samples. The error is of the same order as the kinetic isotope effect being measured and no attempts were made at salvaging the data. In measurements of nitrate references prior to improving our technique with the copper treatment, only two samples gave the accepted value of +5 % . Four other samples during this period gave values as high as +19 % . These values were due to the use of different quantities of different hypobromite solutions of varying age. It is interesting to note that HOERING (1955) used a mixture of CuO-Cu at 700°C to purify his samples. He required this rigorous treatment since he dealt with natural materials. The CuO would serve to oxidize hydrocarbons (and perhaps CO) to CO₂ so that this contaminant could be frozen out of the N₂ sample. Our products did not require so severe a treatment since we dealt with relatively clean chemical reactions.

A second source of error would be isotopic selectivity associated with the hypobromite oxidation. In several cases, incomplete conversions were done deliberately on replicate samples to see if this altered the results. Incomplete conversion results in a loss of N¹⁵ but the effect is small. The largest effect observed was about 3 % . The oxidation is so rapid that we were unable to devise a method of following it isotopically. A sequential experiment proved inconclusive in that no definite trend could be established, although results were variable.

7.2 Nitrate Reduction Experiments

Stoichiometry: BROWN and DRURY (1967) report the reduction reaction to be quantitative. In our experiments, lack of stoichiometric agreement showed that this was not always true. Initially we thought that there was error due to the use of phenolphthalein as an indicator. This indicator changes colour at a pH of about 10. Ammonium chloride on the other hand is acidic due to hydrolysis. Methyl red is the optimum indicator. Since we were interested in obtaining approximately equal sequential samples for isotopic analysis the choice of indicator is not relevant if there is no ammonia loss. The kinetic data verifies that any losses of ammonia were negligible. Since purge rate was constant during a run, ammonia loss would show up during the later part of the experiment when sampling times were relatively long.

In a carefully controlled experiment, product ammonia was collected in a trap using sulfuric acid. A second trap containing indicator was installed to check the efficiency of the first. The reduction was performed in the usual manner. After an hour and a half, an excess of sodium hydroxide was added to the reactor. This did not result in any appreciable product ammonia. 25 ml of ferrous sulfate along with 10 mls of saturated silver sulfate were then added. Within a short period, ammonia was produced. A titration indicated this to be about 5% of the total product. Since the ferrous sulfate was initially added in almost a 2:1 excess, it is probable that the silver catalyst plays an important role in determining the completeness of the reaction. In the reduction experiments we were primarily interested in the kinetics and the isotope fractionation so all reagents were rapidly

mixed at the start of the experiment.

Conversion of Nitrate Samples: In the light of the above experiment, we have modified the procedure for sample conversions. The following modifications have been introduced. (1) The silver sulfate is mixed with the ferrous sulfate and this is added slowly over a 30 minute period. (2) We feel that sulfuric acid is to be preferred in collecting the product ammonia since there is the possibility of the purging gas flushing HCl from the solution. (3) 30 ml of NaOH solution represents a good compromise between reagent quantity and time, and higher concentrations lead to bumping of the solution. (4) A sintered tip was initially tried on the purge but this was dissolved by the sodium hydroxide within a short time. The capillary also has a tendency to dissolve or weaken and should be replaced after every run.

Kinetic Data: In these experiments kinetics are pseudo-first order. In one of the preliminary experiments (not reported here) we obtained kinetics of fractional order but this was found to be entirely due to inadequate purging. The percent reaction was calculated on the basis of total product ammonia. Attempts to evaluate the data in terms of initial concentration, i.e. nitrate present yielded curves rather than the line on semi-log plots. This has serious implications in terms of the fractionation and will be discussed later. The initial rate seems to be slower than the "steady state" rate. This may be due to several things. (1) The addition of the ferrous sulfate cools the solution so the initial temperature is low. (2) The reaction has its greatest rate initially and the purge may be insufficient during this time. (3) The kinetics may be complicated and a

certain time may be required to achieve "steady state" conditions.

Isotopic Data: BROWN and DRURY (1967) report a value of 1.057 ± 0.004 for k_{14}/k_{15} for nitrate reduction at 120°C. This corresponds to an instantaneous fractionation of -57 % . In two of our reductions, we obtain very similar results. (Tables 6.2 and 6.7; figure 6.4 and 6.6). Their experiments were carried out over the range of 6 to 30% reaction. Our values are valid to about 50% reaction at which point errors in the reservoir calculation tend to make the instantaneous fractionation uncertain. This limit depends on the accuracy of the isotopic measurements and also on the stoichiometry, which affects the percent reaction.

In one particular experiment (Table 6.5 ; figure 6.5) the instantaneous fractionation starts high, but diminishes to a constant limit of about 15- % . Since neither kinetic nor isotopic data show abnormalities, it is difficult to explain the low fractionation. The only non-reproducible experimental condition involves the purge rate. A low purge rate may allow a "pool" of ammonia or intermediate to build up in the solution. The rate of the reaction governs the amount flowing into this pool, while the hydroxide concentration and the purge rate govern the amount flowing out. This "pool" tends to integrate out the isotopic fractionation. Initially, the rate of reaction is high and the pool is growing. During these stages, the flushed ammonia will represent the instantaneous isotope fractionation. As time goes by, this pool represents a mixture of product from previous times and therefore the measured isotope effect will be smaller than the true one.

A second serious question concerns the extent to which phase partitioning of the product ammonia affects the observed fractionation. In this particular

set of experiments, the solution is heated under reflux conditions so that water is always present in the condenser in contact with the product ammonia gas. Ammonium ion in the water is returned to the boiler where the ammonia is released. If isotopic equilibrium were achieved, the gas phase would be lighter than the solution by about -23 % (THODE et al, 1945). The reservoir would become progressively enriched in N^{15} . If the $NH_3 \leftrightarrow NH_4$ equilibration time is sufficiently short, then part of the observed fractionation may be due to ammonia exchange. The observed fractionation is, however, in excellent agreement with the predictions of statistical mechanics. BROWN and DRURY (1967) have calculated the kinetic isotope effect to be 1.049 - 1.058 depending upon the theoretical model chosen.

7.3 Nitrite Reduction Experiments

In these experiments, both the stoichiometry and the isotopic balance were good. The reduction rate is much faster than in the case of the nitrate.

The runs at 2.66, 1.33 and 0.67M NaOH appear to yield first order kinetics on the basis of the kinetic data. (Figure 6.8 - 6.10) In earlier runs which are not reported here, the plots of the kinetic data indicated an order of less than unity. Repetition of these experiments with a higher nitrogen purge rate gave the present plots. Therefore, the lower purge rates were not sweeping the system effectively of product ammonia.

Kinetic Isotope Effects: BROWN and DRURY (1967) reported a k_{14}/k_{15} value of 1.029 ± 0.003 for the kinetic isotope effect in NO_2^- reduction. The maximum effect which was observed (1.027) is within the experimental

error of Brown and Drury. However, this value was obtained only near the beginning of the reductions and lower values were obtained during later stages.

To discuss these results, it is necessary to describe the differences in the approaches of the two laboratories toward these studies with reference to figure 7.1. This figure depicts how various parameters of a system alter isotopically with percentage reaction in the case of a simple one-step kinetic isotope effect. The remaining reactant becomes increasingly enriched in the heavy isotope as the lighter isotope is preferentially removed. At any point of the reaction, the instantaneous product differs from the reactant by the kinetic isotope factor. The integrated product in the limit of 100 percent reaction has the same isotopic composition as the initial reactant. BROWN and DRURY (1967) examined the integrated product with the extent of the reaction ranging from near zero up to 50 percent reaction in the case of nitrite reduction. In contrast, our laboratory analyzes successive increments of the product and in effect constructs the instantaneous product curve of figure 7.1. Despite some of the disadvantages discussed in section 6.1, it is felt that the latter approach provides more information about the reaction and the experimental conditions.

It is seen that our nitrite isotopic data is not consistent with the simple first order single process as would be predicted by our kinetic curves. This is not surprising since in fact, BROWN and DRURY (1967) list the following reaction sequence for the reduction of nitrate.

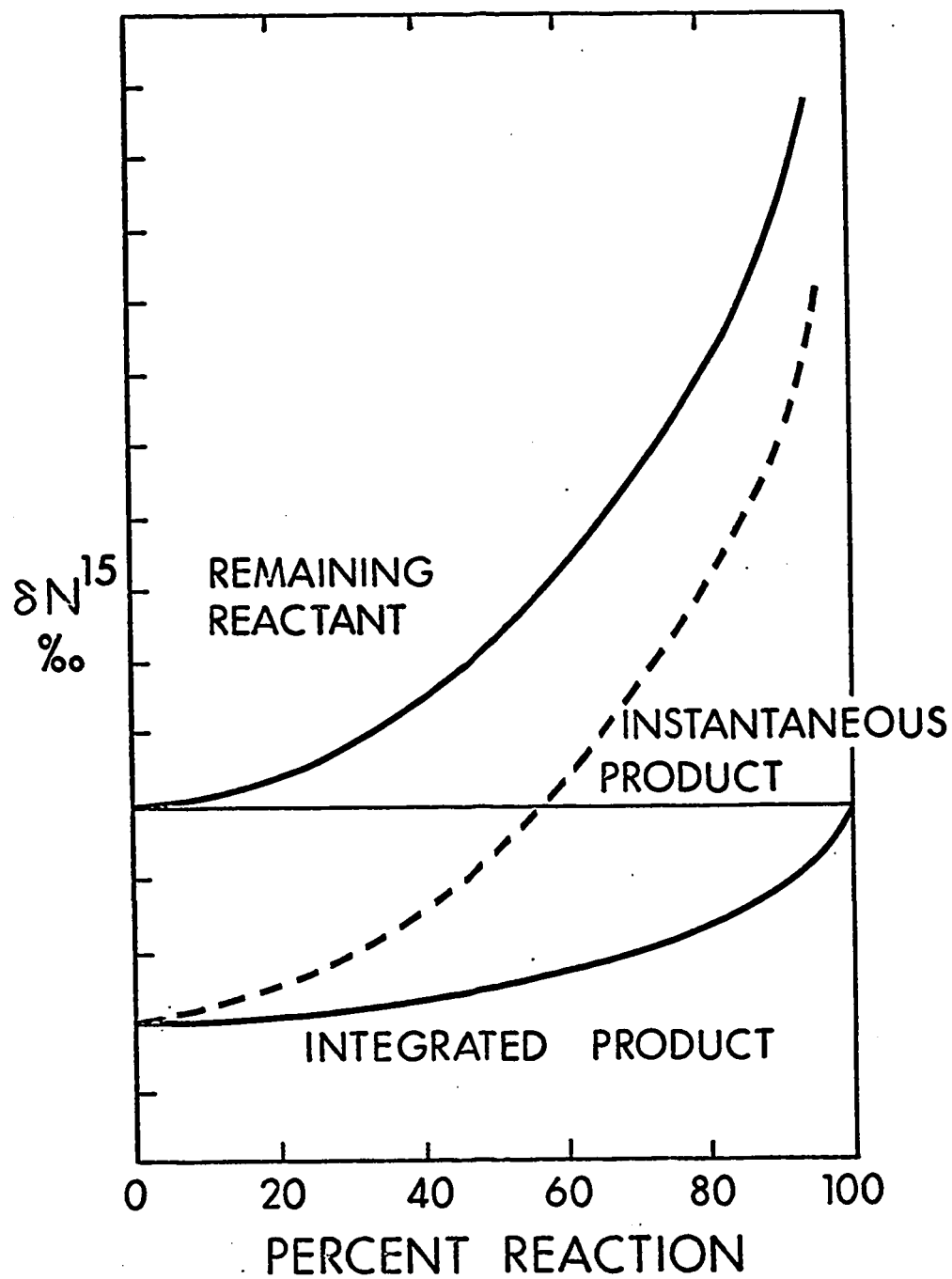
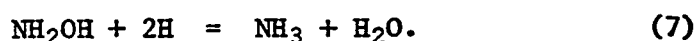
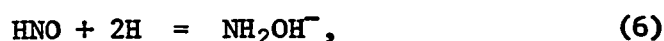
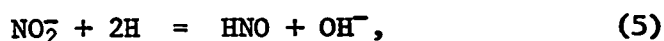
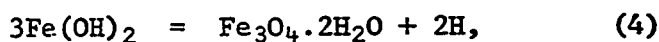
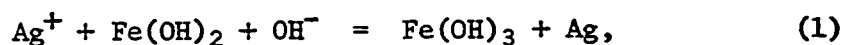


Figure 7.1 Isotopic fractionation in an ideal single step system



To correct their data to zero reaction and hence evaluate k_{14}/k_{15} , Brown and Drury used the equation

$$k_{14}/k_{15} = \log(1-f)/\log(1-\sigma f) \quad (8)$$

where f is the fraction of reacted

$$\text{NO}_3^- \text{ or } \text{NO}_2^- \text{ and } r = \frac{(\text{N}^{15}/\text{N}^{14})_{\text{sample}}}{(\text{N}^{15}/\text{N}^{14})_{\text{reference}}}$$

This equation was derived in section 2.5 of this thesis and is only valid for first order single stage isotopically competing reactions. In a number of reported studies, this equation has been applied since conventional assessment of kinetics seemed to suggest that it was valid. It is, in fact, a very good approximation if there is one step in the process which is overwhelmingly rate-controlling and the isotope fractionation occurs in this step. This appears to be true for the nitrate reduction since equation (2) above is by far the slowest step. Hence our data on nitrate reduction was

in good agreement with BROWN and DRURY (1967) to well beyond the extent to which they carried out their reaction. Our results on nitrite reduction are in fair agreement if we likewise consider only the integrated product curve for lower percentage of conversions. However, our instantaneous product curves show marked departure from the ideal case of figure 7.1. Further, the instantaneous isotopic fractionation in all of our runs had higher values in the initial stages of the reduction but these became progressively lower and then appeared to reach a steady value as the reaction proceeded. This behavior is quite in line with the overall findings of Brown and Drury that the experimentally measured kinetic isotope effect (~ 1.029) was considerably lower than the theoretical one expected in initial N-O bond rupture of NO_2^- (1.048). This means that a step in the process with little or no isotopic selectivity competes with the N-O bond rupture for rate control. The rates of these steps must have been comparable since the measured value is roughly half way between 1.000 (no isotopic selectivity) and 1.048 (isotopic step fully rate controlling). The decrease in the isotope fractionation with percent reaction in our experiments may simply represent the build up of an intermediate pool. If the step which depletes this pool is not isotopically selective while at the same time is competitive in controlling the rate, then we expect the overall isotopic fractionation to decrease as the pool is formed. This results because the pool represents a sort of isotopic average of material obtained over all reaction time.

It should be emphasized that both laboratories examined the behavior of the product. This procedure is the easiest to perform and is, in fact,

of more practical significance. If there are large amounts of intermediates, however, then the appearance of product NH_3 is not directly related to the disappearance of reactant NO_2^- . Equation (8) really applies to the disappearance of reactant. Therefore, the concentration of NO_2^- and its isotopic composition should be measured directly and not ascertained by measurements on the product. The fact that kinetic data based on the product NH_3 , appeared to be straightforward, suggests that intermediates can be low in concentration but yet promote a complex isotopic behavior for the overall process.

7.4 Urea Hydrolysis

The kinetics are pseudofirst order on the basis of product ammonia. i.e. the OH^- concentration is much greater than that of urea. A few preliminary experiments showed the reaction to be relatively independent of the hydroxide concentration over the range studied.

The isotopic fractionation is quite unusual. The results of hydrolysis under two different hydroxide concentrations are shown in figures 6.16 to 6.18.

Low to medium hydroxide concentration yields an isotopic fractionation curve not unlike those encountered in the nitrate and nitrite reduction experiments (figure 6.5). The isotopic fractionation is initially greater than 1.015, but approaches a limit of about 1.010. It would, therefore, seem that the buildup of an intermediate pool as discussed above may also exist here.

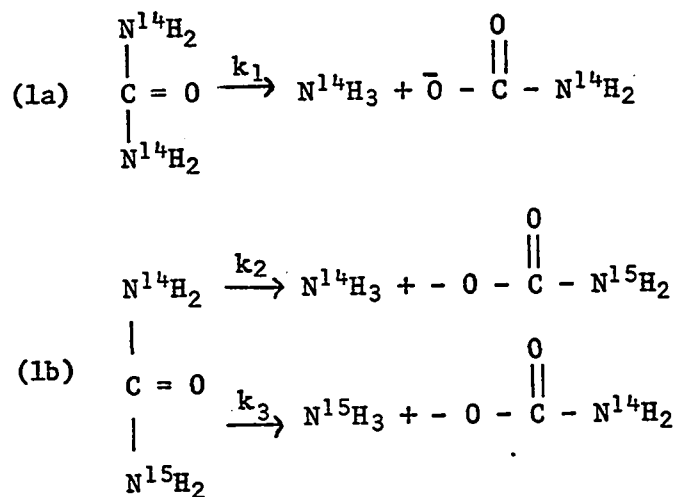
If, as the work of LYNN (1965) and WARNER (1942) suggests, the principle intermediate is cyanate, then the hydrolysis of the cyanate would probably be the slow non-fractionating step and the intermediate pool would be composed of this material. The decomposition of the carbamate would appear rapid.

At high OH^- concentrations, an unusual isotopic behavior appears in that the curve for the isotopic composition of the instantaneous product displays a double inflection. The possible mechanisms for this reaction were discussed in chapter 3. It is a multistep process with NH_3 being produced in two steps so that the double inflection may very well be indicative of these two sources, urea and cyanate or carbamate. If the increased OH concentration altered the rate of hydrolysis of the cyanate significantly, then rate control could be shifted at least partially to the decomposition of the carbamate. If the initial step in the hydrolysis of the urea was still much faster than the second step, than both steps would be fractionating. Under such conditions, the isotopic composition of product NH_3 would become increasingly enriched in N^{15} as the urea became exhausted (reservoir effect). However, when the carbamate commences to react, it will contribute N^{14} enriched ammonia to the product and hence this causes the first inflection. As time goes by, the intermediate (cyanate or carbamate) is increasingly enriched in N^{15} (reservoir effect) so that the curve undergoes another inflection as enriched ammonia is formed at the end of the reaction.

It would seem that the inflections should have the following limits.

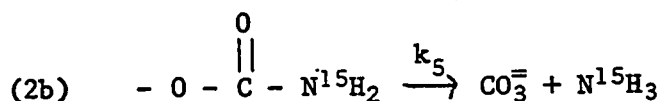
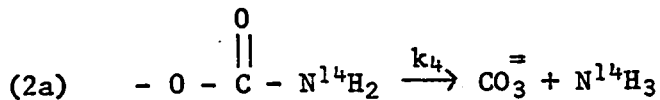
If all of the urea reacted before any carbamate reacted, then there would be an infinitely large inflection at the 50 percent NH_3 production point. (Figure 7.2A) The other limit would be when the carbamate produces ammonia immediately upon formation in which case no inflection would be evident. (Figure 7.2B) It should follow that intermediate cases will produce inflections between zero and 50 percent NH_3 production. Inflections closer to the 50 percent production point should be more pronounced than those closer to zero reaction. Comparison of figures 6.17 and 6.18 suggest that this is the case.

The isotope effect is complicated by the existence of both inter- and intra-molecular isotopic fractionation during reaction (1). This is illustrated as follows:



Process (1a) proceeds at a faster rate than (1b) and this competition is termed an inter-molecular isotope effect. In process (1b), the reaction designated by k_2 is faster than that designated by k_3 . This is called an intra-molecular isotope effect.

The second step (carbamate hydrolysis) involves inter-molecular fractionation alone with (2a) being faster than (2b)



Having considered the inter- and intra-molecular possibilities, some further comments can be made regarding the limiting cases presented in figure 7.2. In case (A), isotopic rate constants k_1 , k_2 and k_3 are involved up to 50% reaction in the NH_3 production while k_4 and k_5 are involved during the latter 50 percent of the NH_3 production. In limit (B), since the carbamate formed hydrolyses immediately, there is no intra-molecular effect. The instantaneous product curve is only determined by competition between $(N^{14})_2$ and (N^{14}, N^{15}) containing molecules.

Theoretical estimates of the fractionation can be obtained by considering a simple model of diatomic C-N bond breakage. SCHMITT *et al* (1952) give the fundamental frequency for the $C^{12}-N^{14}$ bond as $2.715 \times 10^{13} \text{ sec}^{-1}$. If the force constants are assumed to be the same for CN^{14} and CN^{15} , then the isotopic frequencies are related to the reduced masses, then

$$\frac{\nu_{C^{12}N^{14}}}{\nu_{C^{12}N^{15}}} = \left(\frac{\frac{12 \times 15}{12 + 15}}{\frac{12 \times 14}{12 + 14}} \right)^{\frac{1}{2}} = 1.0157$$

$$\Delta\nu = .042 \times 10^{13} \text{ sec}^{-1}$$

$$u = \frac{h\nu}{kT} = 3.312 \text{ at } 120^\circ\text{C}$$

$$\Delta u = \frac{h}{kT} \Delta v = .05124$$

Using the table of BIGELEISEN and MAYER (1947) and neglecting the activated complex term, we find:

$$\frac{k_{14}}{k_{15}} = 1.0157(1 + G(u)\Delta u) =$$

$$(1.0157)[1 + (.23526)(.05124)] = 1.0280$$

Therefore, in the rupture of a simple CN diatomic bond, the fractionation can be expected to fall within the limits of 1.0157 and 1.028. The first limit corresponds to the activated complex resembling the reactant and the fractionation is then temperature independent. The latter limit corresponds to $G(u)\Delta u$ for the activated complex being zero.

Near the beginning of the urea hydrolyses, it is seen that the instantaneous isotope fractionation factor corresponds to the range theoretically estimated. Since the initial carbamate concentration is zero, the NH_3 must come mainly from urea decomposition. This strongly suggests that we are observing the isotope effect in C-N bond rupture and that this step is strongly competitive for rate control during the urea decomposition. After the initial stages, there are five possible isotopic rates involved in ammonia production and it is difficult to further elucidate the process. In run #3, reservoir calculations were possible because the behavior of the instantaneous product approached the limit considered in figure 7.2(B). However, the actual kinetic isotope effects cannot be

calculated because the reservoir consists of both carbamate and urea in unknown relative concentrations.

This hydrolysis of urea experiment emphasizes a number of the points previously considered under nitrate and nitrite reduction. It is an obvious example of where the kinetic data suggests a straightforward first order reaction, yet the isotopic data are not compatible with this suggestion. It is also seen that if isotopic data are obtained along the instantaneous product curve, that much more information can be obtained on a system than by the use of conventional kinetics or the isotopic measurement of integrated product.

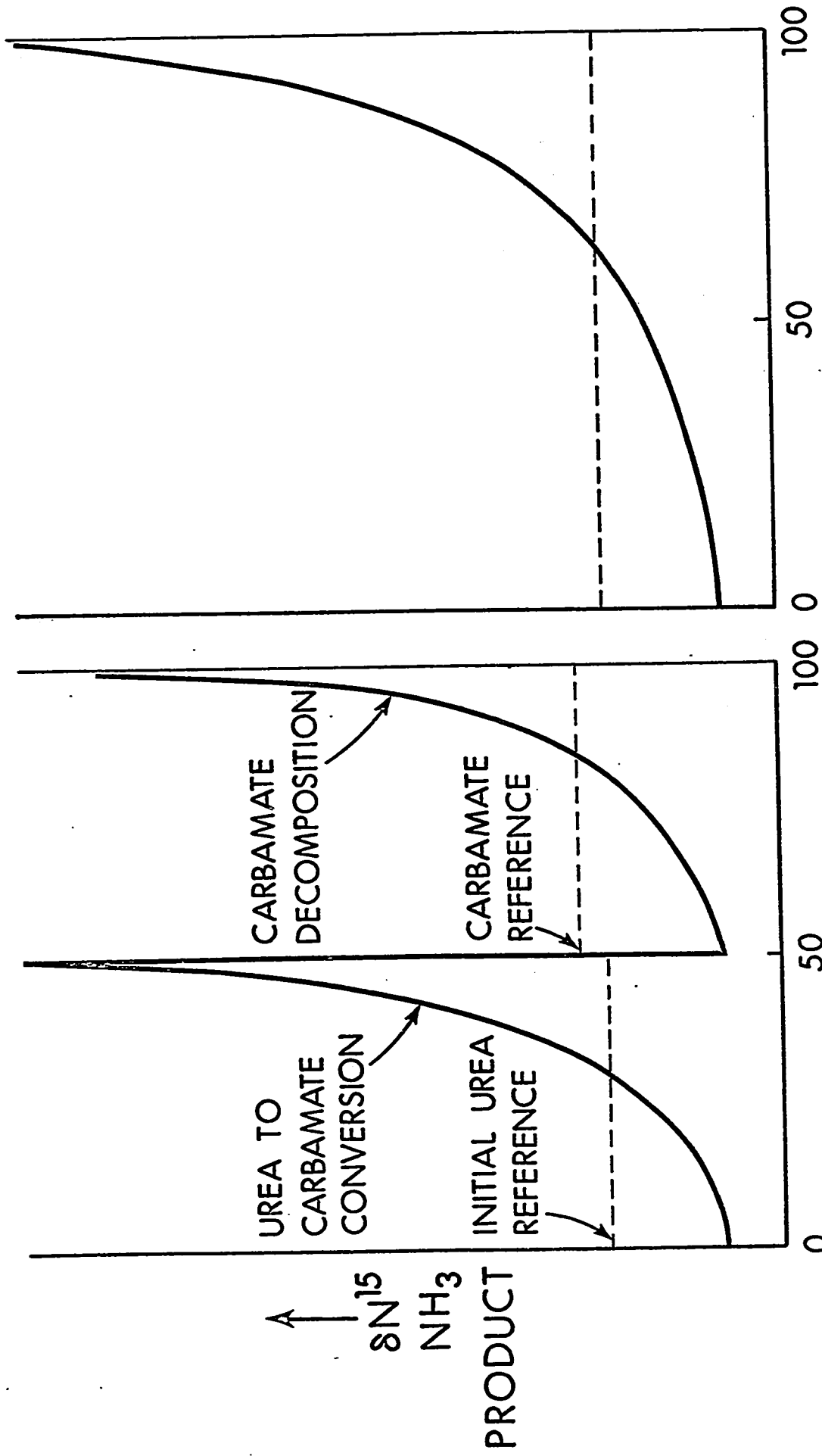
In the case of urea, the data of this thesis strongly supports a multi-step mechanism.

7.5 Microbiological Reduction of NO_3^- and NO_2^- to N_2

In these experiments, nitrate is reduced microbiologically to nitrogen.

Figure 7.2 Limits in isotopic behavior of instantaneous ammonia product during urea hydrolysis.

- (a) All urea reacts before carbamate decomposes.
- (b) As soon as urea reacts, the carbamate decomposes immediately.



(B)

(A)

which can be directly analysed on the mass spectrometer. The problems of sample conversion are not encountered. CO_2 and perhaps N_2O are produced but the use of liquid nitrogen traps during collection and at the mass-spectrometer sample line effectively removes these.

Runs A and B:

Reductions A and B (P. stutzeri, NO_3^-) gave good isotopic balance so this reduction appears quantitative. The media used in (A) was slightly more concentrated than that used in (B). The instantaneous fractionation is somewhat different which demonstrates that growth conditions effect the reduction sequence in some way. More information is contained in (B) in that the reservoir was sampled for residual nitrate several times.

The results of this study of nitrate reduction by Pseudomonas Stutzeri (NCIB 9040) are depicted in figures 6.22 and 6.23. Figure 6.22 shows that the N_2 production rate maximized very sharply at about 26 hours after inoculation and then declined rapidly. The small plateau in the production rate between samples number 7 and 8 is meaningful as discussed below. In figure 6.23, the isotope fractionation and % reaction of nitrate are plotted as a function of % N_2 production. The behavior of the instantaneous fractionation is very complex. In all denitrifying systems studied, the isotope fractionation has been smaller during the initial states of the reduction (samples 1 and 2 of the present study). This phenomenon apparently depends upon cell population variation. Analogous behaviors have been reported during microbiological $\text{SO}_4^{=}$ and $\text{SO}_3^{=}$ reductions to H_2S ; namely that during population growth, the isotope fractionation is lower than during the

stationary phase.

In this experiment, the instantaneous isotope fractionation factor remains relatively constant at 1.02 from sample #3 to #7 (figure 6.23). It then decreases in the vicinity of the small plateau in the N_2 production rate previously mentioned. This plateau appears near the point of total depletion of NO_3^- . The N_2 subsequently produced, must have originated from an intermediate. The disappearance of NO_3^- also corresponds to a marked discontinuity in the isotope composition of product N_2 (and instantaneous isotope fractionation factor). This further attests that a different metabolic process became dominant at 60% N_2 production.

Reduction C (Bacillus sp. 6251, NO_3^-) - The isotopic balance was very poor. It is evident that considerable amounts of material enriched in N^{15} are accumulating in the system, or are given off as N_2O . One of the latter samples was enriched by over 100 % in N^{15} , but samples taken later showed no such enrichment. This phenomena has been encountered elsewhere in the microbiological reductions. (Figures 6.30

Reduction D (Bacillus sp. I₂, NO_3^-) - The isotopic balance is also poor indicating that not all of the NO_3^- was converted to N_2 . One of the latter samples also showed great N^{15} enrichment as discussed in run C. The rate of N_2 production is complex. Two processes are evident from the semi-log plot (figure 6.29). The reservoir was sampled but analysis of the unreacted NO_3^- was not possible because of a large NO_2^- concentration which interfered in the procedure employed. (Standard phenoldisulfonic acid method). It is interesting that this NO_2^- appeared as free ion in solutions and its identification revealed one reason why isotopic balance was

lacking.

Reduction G (P. stutzeri, NO_3^- , resting cell) - This experiment did not proceed at the expected rate. A possible explanation is that the medium may have been too hot at the time of inoculation and this killed many of the cells. The rate curves (figure 6.32) are extremely complicated. The isotopic curve shows poor balance indicating that N^{15} was accumulated somewhere in the system. This is an interesting contrast in that the growing cell experiments with P. stutzeri were almost quantitative. In view of these observations, the possibility exists that our species of interest may not have been dominant. Nevertheless, it is noteworthy that nitrogen isotope fractionation is very much in evidence.

Reduction E (Bacillus sp. 625, NO_2^-)

Since four NO_2^- determinations were carried out during the course of this reduction, one is able to arrive at an assessment of the time factors in the reduction (figure 6.34). For example, the time between 20 percent reduction of nitrite and 20 percent production of nitrogen is about 6 hours. Another appraisal of the reaction kinetics can be obtained by considering relative concentrations at a given time. For example, at 20 hours, 50 percent of the NO_2^- has disappeared but only 26 percent of the N_2 has been produced. These phenomena can result from at least two possibilities. The initial N-O rupture is not rate-controlling and/or there is another product such as N_2O produced.

With the exception of the first sample, the isotopic fractionation is reasonably well behaved. (Figure 6.36) There is an indication of small

inflection in the region of sample number 8 which may be related to the inflection in the N_2 product curve (figure 6.35).

Reduction F (Alcaligenes faecalis 4456, NO_2^-)

The relative production rate of N_2 in this experiment exemplifies almost ideal behavior - namely exponential production of N_2 in the early exponential growth stages and then exponential decay as the energy supply is exhausted and the viable population decreases. This is in marked contrast to many of the runs described above.

The isotopic composition of the product N_2 in this experiment is well behaved for samples up to 70 percent of the total collected. The kinetic isotope effect is quite comparable to that found in the inorganic reductions. It must be remembered, however, in making this comparison that the end products are different (N_2 , microbiologically; NH_3 , chemically). The last inflection (figure 6.39) corresponds to nitrite depletion.

7.6 Summary and Implications

The more significant contributions of this thesis and associated implications are now summarized in point form.

1. Our experience with the hypobromite oxidation procedure suggest that traces of O_2 can cause severe difficulties. Therefore, steps must be taken to provide for its removal such as heating the N_2 sample with a metal.
2. Our chemical reduction studies show that NO_3^- and NO_2^- can be converted to N_2 successfully if precautions are taken. In parti-

cular, the reduction may not go to completion if the catalyst becomes poisoned.

3. Our chemical reduction studies on NO_3^- and NO_2^- gave results in general agreement with other workers. However, a number of points were evident.
 - (a) Low purging rates may influence the isotopic composition of the product. This results because another step which can compete for rate control is inserted in the reaction.
 - (b) It is not correct to assume that if kinetic data reveal a simple first order reaction, that the isotope effects will exhibit a similar behavior.
 - (c) In line with (a) and (b), the procedure of examining increments of the product isotopically throughout the reaction is preferable to sampling integrated product.
4. The hydrolysis of urea demonstrated a case where two isotopic selection processes can occur simultaneously and yield the same product. These experiments also demonstrate the validity of points 3(b) and (c). In particular, the kinetic data based on the product NH_3 suggest a simple first order behavior whereas the isotopic data shows that the overall reaction is more complex.
5. The microbiological reduction studies reveal the following points:
 - (a) Micro-organisms can significantly alter the $\text{N}^{14}/\text{N}^{15}$ abundance during NO_2^- and NO_3^- reduction. This finding is important to the interpretation of terrestrial $\text{N}^{14}/\text{N}^{15}$ abundance variations. HOERING (1960) interpreted his results on

nitrogen fixation by Azotobacter as indicating no fractionation of nitrogen isotopes during transfer from the atmosphere to the biosphere. This generalization should be checked using other fixation systems. Our data signify that transfer from the terrestrial inorganic pool (nitrates, nitrites) through the biosphere to the atmosphere results in the return of N_2 to the atmosphere which is significantly depleted in N^{15} in comparison to the inorganic pool.

- (b) Microbiological alteration of the N^{14}/N^{15} abundance in any given reaction has a complex dependence upon the specific organism and conditions in the medium.
- (c) In growing cell experiments, the N^{14}/N^{15} isotope effects in the initial stages of the reduction are invariably lower than when the cell population is going through its stationary phase. Analogous effects in S^{32}/S^{34} fractionation have been reported many times in microbiological $SO_4^{=}$ and $SO_3^{=}$ reductions. (JONES and STARKEY, 1957; HARRISON and THODE, 1957; KAPLAN and RITTENBERG, 1964; KROUSE et al, 1968; KROUSE and SASAKI, 1968). Therefore, this may be a general phenomenon in microbiological isotopic studies involving reduction.

6. This thesis demonstrates that reasonably detailed isotopic studies of a system (chemical or microbiological) reveal more information than comparable kinetic studies.

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