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Environmental Protection Technology Series

# AN INDEX OF REFRACTORY ORGANICS

Robert S. Kerr Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Ada, Oklahoma 74820

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# AN INDEX OF REFRACTORY ORGANICS

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## FOREWORD

The Environmental Protection Agency was established to coordinate administration of the major Federal programs designed to protect the quality of our environment.

An important part of the agency's effort involves the search for information about environmental problems, management techniques and new technologies through which optimum use of the nation's land and water resources can be assured and the threat pollution poses to the welfare of the American people can be minimized.

EPA's Office of Research and Development conducts this search through a nationwide network of research facilities.

As one of these facilities, the Robert S. Kerr Environmental Research Laboratory is responsible for the management of programs to: (a) investigate the nature, transport, fate and management of pollutants in groundwater; (b) develop and demonstrate methods for treating wastewaters with soil and other natural systems; (c) develop and demonstrate pollution control technologies for irrigation return flows; (d) develop and demonstrate pollution control technologies for animal production wastes; (e) develop and demonstrate technologies to prevent, control or abate pollution from the petroleum refining and petrochemical industries, and (f) develop and demonstrate technologies to manage pollution resulting from combinations of industrial wastewaters or industrial/municipal wastewaters.

This report contributes to the knowledge essential if the EPA is to meet the requirements of environmental laws that it establish and enforce pollution control standards which are reasonable, cost effective and provide adequate protection for the American public.

W. C. Galegar
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## ABSTRACT

Refractory waterborne organics resist biodegradation, accumulate in the environment and can inhibit life forms. This research develops laboratory techniques for, and interpretations of, a Refractory Index (R.I.) to assess quantitatively the persistency of refractory organics and uses the R.I. determination to evaluate some 38 industrial, natural and combined organics. R.I. values close to 1.0 characterize readily biodegradable organics; intermediate R.I. values, approximately 0.3 to 0.7, indicate partial degradation; R.I. values approaching zero indicate refractory organics; negative R.I. values indicate inhibitors. The coefficient of variation for R.I. values is 13%. Since negative R.I. values are of qualitative significance only, a Biological Inhibition Value (B.I.V.) is developed and used to assess quantitatively those organics found to interfere with the long-term ultimate Warburg Respirometer determinations, which were the biochemical tests used. In a few cases, confirmation of the R.I. interpretation was performed by specific analysis using a model activated sludge unit. Correlations between oxygen demand tests ($BOD_5$, $BOD_u$, TOD) and organic parameters (TOC, TN) are presented.

Suggestions for required pretreatments of industrial wastewaters before allowing discharge into municipal sewage treatment plants are included as an application of the Refractory Index criterion. The bibliography contains 49 references.



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CONTENTS


| | |
|---|---|
| Foreword | iii |
| Abstract | iv |
| Figures | vi |
| Tables | vii |
| Abbreviations and Symbols | viii |
| Acknowledgment | ix |
| 1. Introduction | 1 |
| 2. Summary | 3 |
| 3. Conclusions and Recommendations | 6 |
| 4. Main Text | 8 |
| Objective | 8 |
| Background | 8 |
| Derivation and Laboratory Methods | 10 |
| Explanation of Terms | 11 |
| 5. Refractory Index Data and Interpretations | 14 |
| General Explanations for Persistence | 14 |
| Presentation and Interpretation of R.I. Data for Naturally Occurring Organic Compounds | 15 |
| Presentation and Interpretation of R.I. Data for Synthetic and Industrial Organic Compounds | 20 |
| Presentation and Interpretation of R.I. Data for Combinations of Organic Materials | 26 |
| Test Procedures: Interferences, Limitations, Correlations and Confirmations | 30 |
| 6. Closing Statement | 39 |
| References | 40 |
| Appendix | 45 |
| A. Derivation of UOD Measurement | 46 |
| B. Laboratory Methods and Materials | 52 |
| C. Warburg Respirometer | 66 |
| D. Suggested Pretreatment Requirements for Industrial Waste | 123 |

FIGURES

Page

Figure 1. Laboratory scheme for determining a refractory index. . . . . 9

Figure 2. Metal interference with $BOD_5$ data. . . . 31

TABLES


| | | Page |
|---|---|---|
| Table 1. | List of Refractory Indices for Organic Materials Tested | 5 |
| Table 2. | Refractory Index Data for Naturally Occurring Organics | 16 |
| Table 3. | Refractory Index Data for Synthetic and Industrial Compounds | 21 |
| Table 4. | Chemicals Which Inhibit Respirometer Response | 24 |
| Table 5. | Refractory Index Data for Combinations of Organic Materials | 27 |
| Table 6. | Color Analysis of Textile Dye | 28 |
| Table 7. | Color Analysis of Industrial Dye Waste | 29 |
| Table 8. | Models for Quick-Time Oxygen Demand Measurements of Raw Sewage | 34 |
| Table 9. | TOD Response for D-Glucose/Sewage Effluent | 36 |
| Table 10. | TOD Response for L-Lysine/Sewage Effluent | 37 |
| Table 11. | TOD Response for p-Chlorophenol/Sewage Effluent | 38 |
| Table A. | Suggested Limits and Ranges for Industrial Wastewater Pollutant Inputs to Municipal Sewage Treatment Plants | 126 |
| Table B. | Interpretation of Refractory Index Values | 127 |

## LIST OF ABBREVIATIONS AND SYMBOLS

B.I.V. - Biological Inhibition Value, Relative Decrease in Ultimate Biochemical Oxygen Demand of Sewage due to presence of an inhibitory substance, expressed as a fraction

$BOD_u$ - Ultimate Biochemical Oxygen Demand, mg/L as $O_2$

$BOD_u'$ - Ultimate Biochemical Oxygen Demand Measurement Without Nitrate Corrections, mg/L as $O_2$

$\Delta NO_3^-$ - Nitrate Corrections Terms, mg/L as $O_2$ Demand

$|NO_2^-|$ - Concentration of Nitrites, mg/L as $O_2$

$|NO_3^-|$ - Concentration of Nitrates, mg/L as $O_2$

R.I. - Refractory Index, Normalized Value Indicating Degree of Degradation, $\frac{BOD_u}{UOD}$

TN - Total Nitrogen, Expressed as mg/L as $O_2$

TOD - Total Oxygen Demand, mg/L as $O_2$

TOxN - Total Oxidizable Nitrogen, mg/L as $O_2$

UOD - Ultimate Oxygen Demand, mg/L as $O_2$

s - Standard Deviation of a Population

$\bar{x}$ - Average of a Population

$(s/\bar{x})$ - Coefficient of Variation

< - Less Than

> - Greater Than

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## SECTION 1

## INTRODUCTION

The purpose of this research has been to develop and accumulate data on a Refractory Index as a quantitative measure of persistence of organic wastes especially those of industrial origin. The discharge of non-biodegradable (refractory) organic wastes into receiving waters poses a threat to the aquatic environment which is even greater than that of the oxygen depletion realized when readily biodegradable materials are released into the environment. Some refractory organics originate from industrial waste either directly or via municipal wastewaters which receive waste from industry. There are also, however, naturally occurring refractory organics; some of these can be converted to harmful materials during such processes as the chlorination of water supplies.

The subject of refractory organic materials as water pollutants is a relatively new area of concern for environmental engineers, scientists and administrators. Previously emphasis had been placed on immediate effects of contamination such as oxygen depletion, toxicity responses and general aesthetic properties. In contrast, the more recent concern is with the long-range, perhaps more subtle, effects of bio-persistent organic contamination. These materials which originate from point sources such as industrial waste discharges and non-point sources such as agricultural runoffs have been detected in waterways and potable water supplies after extensive exposure to both the natural environment and controlled degradation through wastewater treatment facilities. This detection of bio-persistence must be regarded as a warning to begin a control strategy to prevent such organic material accumulations in water systems.

Because the classic five-day Biochemical Oxygen Demand ($BOD_5$) has been a standard of wastewater quality, industries with high BOD values for wastewater have tried to lower the BOD value of their discharge. A valid approach to lowering BOD level is the use of effective treatment processes; a non-appropriate approach to lowering the BOD level is to switch from readily biodegradable organics to refractory organics which do not create an immediate high biochemical oxygen demand because they resist degradation. These substitutes, whose use is sometimes encouraged when regulatory agencies rely solely on a five-day BOD criterion, can in fact be far more polluting to the environment due to persistence, toxicity, effects on flora and fauna and long-term depletion of oxygen levels in receiving waters. An example of this is the switch by textile mills from starch to non-biodegradable sizers.

Certain industrial chemicals should be restricted from entering the aquatic environment because of their persistence, the high energy expenditures needed to decompose them by conventional methods (such as the activated sludge bio-reactor), their accumulative degradation off products and/or their inhibition to aquatic flora and fauna. The first step in organizing an effective strategy is to develop a means of classifying materials with respect to biopersistency in the aquatic environment. The index of

refractory organics discussed and developed in this report is such a quantitative parameter. The Refractory Index used here is essentially the ratio of long-term aerobic biochemical oxygen demand under standard conditions to the complete theoretical oxygen demand. This fraction expresses the amount of the organic material that persists in receiving waters.

Ample evidence is presented here and in the literature to demonstrate the existence of refractory organics (1) (3) (6) (13) (17) (18) (19) (22) (24) (25) (27) (28) (33) (37) (43) (45) (48) (49). For those organics that show a zero biodegradability plus an inhibiton to the bacterial systems, an additional analysis to measure refractoriness is needed; this is a bioassay measure that quantifies the degree of interference with normal biochemical oxygen demand reactions.

Even some relatively harmless organics, if they persist in the aquatic environment, can be converted to harmful substances (e.g., humic acid to chlorinated organics by means of disinfection practices). It is necessary to lower the risk caused by the presence of harmful persistent organics from industry and natural sources.

## SECTION 2

## SUMMARY

### AN INDEX OF REFRACTORY ORGANICS

The objective of this research has been to develop a Refractory Index as a quantitative measure of persistence of organic residuals, industrial and natural, that are frequently found in wastewaters and in aquatic environments. The Refractory Index is formulated based on the ratio of long-term ( > 20 days) ultimate (Total) Biochemical Oxygen Demand to real-time measures of Theoretical (Total) Oxygen Demand and Total Oxidizable Nitrogen.

A method of evaluating the total quantity of oxygen needed for complete oxidation to ultimate and products $CO_2$, $H_2O$, and $NO_3^-$ and termed Ultimate Oxygen Demand (UOD) is derived. In addition, the Warburg Respirometer Biochemical Oxygen Demand test, selected to predict optimum biodegradability properties, and termed the Ultimate Biochemical Oxygen Demand ($BOD_u$) is used. The ratio of these measurements ($BOD_u$ to UOD) is called the Refractory Index (R. I.) and is offered as a parameter for estimating biodegradability and treatability of organic materials found in and entering into the aquatic environment.

$$(R.I.) = \frac{BOD_u}{UOD} \qquad [1]$$

The R.I. data presented in this report have a statistically determined coefficient of variation ($s/\bar{x}$) of 0.13.

Lists of Refractory Index values for common industrial organics and natural materials are presented along with a discussion on the significance of the Refractory Index and its interpretation. Some materials, for example glutamic acid and glucose, are readily degraded and have R.I. values approaching 1.0; other materials, for example DDT, show negative R.I. values interpreted as bacterial inhibition response. The most difficult R.I. values to interpret are those between extremes, for example lysine, which has an R.I. value of about 0.5; explanations for these intermediate values are offered.

Organic materials, especially those originating from industry and characterized as refractory (i.e., having R.I. values close to zero) are considered persistent for stipulated environmental conditions. This persistency is extrapolated to the bio-reactors of wastewater treatment and the receiving aquatic environment. Certain materials, inhibitors, produce a R.I. number of less than zero; this can be used as a bioassay parameter for inhibitory organics and heavy metals of industrial wastes. A new term, the Biological Interference Value (B.I.V.) is used to quantify inhibitor responses; the greater the B.I.V., the greater the toxicity inhibition response. Table 1 is a list of compounds tested with either the R.I. value or, in the case of inhibitors, the B.I.V. value.

Correlation models for different oxygen demand tests (TOD, BOD) and tests for organics (TOC, TN and others) are presented with special attention to real-time parameters (quickly determined measurements); however, great caution should be exercised in using any such correlation for industrial and variable wastes.

From the collected experimental evidence basic theoretical and empirical reasons are offered for the cause of persistence in certain organics.

TABLE 1.

LIST OF REFRACTORY INDICES FOR ORGANIC MATERIALS TESTED

| Organics | R.I. | B.I.V. |
|---|---|---|
| Acetic Acid | 0.6 | |
| Acetone | 0.8 | |
| Adenine | 0.1 | |
| Aniline | 0.6 | |
| Antifreeze | 1.0 | |
| Arginine | 0.7 | |
| L-Aspartic Acid | 0.8 | |
| Benzene | 0.2 | |
| Biphenyl | 1.0 | |
| Bipyridine | <0 | 0.6 |
| Butyric Acid (Na Form) | 0.8 | |
| Carboxymethyl Cellulose (CMS) | 0.0 | |
| Chloroform | <0 | 0.7 |
| p-Chlorophenol | 0.0 | |
| Cyanuric Acid | <0 | 0.3 |
| DDT | <0 | 0.4 |
| DDT with Carrier | <0 | 0.3 |
| Dichlorobiphenyl | 0.2 | |
| Dichlorophenol | <0 | 0.6 |
| Ethylene Glycol | 0.8 | |
| Ethylene Glycol with Inhibitor | 0.0 | |
| Gasoline | 0.2 | |
| D-Glucose | 0.9 | |
| D-Glutamic Acid | 1.0 | |
| L-Histidine | 0.5 | |
| Humic Acid | 0.0 | |
| Hydroquinone | 0.5 | |
| L-Lysine | 0.5 | |
| Phenol | 0.9 | |
| Propionic Acid (Na Form) | 0.8 | |
| Propylene Glycol | 0.8 | |
| Sevin (Carbaryl) with carrier | 1.0 | |
| Sewage (Domestic) | 0.7 | |
| Starch | 0.6 | |
| Textile Dye | 0.6 | |
| Textile Dye - Industrial Waste | 0.1 | |
| L-Valine | 0.9 | |
| Vinyl Chloride | 0.0 | |

## SECTION 3

## CONCLUSIONS AND RECOMMENDATIONS

Since there are non-biodegradable organics of industrial and natural origin, new approaches to measuring, treating and otherwise managing these refractory materials are necessary to avoid the hazards due to these persistent contaminants in the aquatic environment. Quantitative determination of persistent contaminants is an essential step towards understanding and dealing effectively with these materials.

This study develops and tests a method for determining the total amount of theoretical oxidizable matter in wastewaters and expresses this amount as Ultimate Oxygen Demand (UOD); for evaluating the total amount of aerobic biodegradable matter present, an Ultimate Biochemical Oxygen Demand ($BOD_u$) is used. Ultimate Oxygen Demand (UOD) is determined by combining the Total Oxygen Demand (TOD) and Total Nitrogen (TN) measurements with each expressed as mg/L $O_2$. UOD defines the amount of oxygen needed for a complete oxidation of organic matter to ambient aquatic environmental end products - $CO_2$, $H_2O$ and $NO_3^-$.

The Ultimate Biochemical Oxygen Demand ($BOD_u$) is determined by measuring the total oxygen uptake of microorganisms originating from domestic sewage over an extended period of time ( > 20 days), while exposed to the specific organic material being examined in a Warburg Respirometer apparatus. Conditions for the test include: $20^oC$, buffered neutral pH test conditions, absence of sunlight, sufficient nutrients, heterogenous bacterial populations and an aerobic environment. A nitrification correction term is used to account for unequal nitrate production between a blank (raw sewage) and test solution (raw sewage and test organic material); however, in most cases this correction is small.

The ratio of $BOD_u$ to UOD for a material indicates the fraction degraded. This fractional ratio is expressed as a Refractory Index (R.I.) in a normalized range of 1.0 to 0.0; however, negative values of R.I. which have been noted (e.g., DDT) suggest that this test procedure could be useful as a bioassay test to screen for inhibitors. For certain waste materials, either organic or metallic, that inhibit biochemical degradation a quantitative term, the Biological Interference Value (B.I.V.) is used to express the interference in the long-term BOD test. Refractory Index values of zero indicate no measurable degradation while Refractory Index values of one imply complete degradation to the final end products. Organic materials which either decompose to end products slowly or not at all are known as bio-refractory.

Correlation coefficient models for different oxygen demand parameters ($BOD_5$, TOD) and between other measures of organics (TOC, TN, UV, Turbidity) are offered since there is a need for relating real-time parameters to classic standards tests; however, great caution should be exercised in using any such correlation for variable waste.

The R.I. parameter is designed to assist engineering judgment in determining acceptability of a waste for discharge. It is useful in evaluating degradability of specific industrial waste compounds, pesticides and natural compounds. This information can be used to classify waste materials in order to make decisions about the acceptability of discharge to the environment or to biological reactors, or to indicate when other treatment techniques (such as activated carbon) are necessary.

There are water-borne organic constitutents, both natural and industrial, that resist degradation. The R.I. parameter provides a means to quantify the persistency of such refractory organics. It is recommended that the procedures developed here be used to evaluate the nature of organic wastes by testing more materials for persistency, especially those of industrial origin that waste energy by passing through bio-reactors unaltered and that may end up as accumulated materials in the aquatic environment.

## SECTION 4

## MAIN TEXT

### A. OBJECTIVE

The primary objective of this study is to develop laboratory techniques for a general parameter defining persistent organics. The parameter presented in this report is termed Refractory Index (R.I.) and is defined as the ratio of biochemical oxidation to complete oxidation in the aquatic environment. The principal uses for this parameter include:

1. Classification of specific compounds such as industrial by-products, pesticides and natural compounds in terms of their ability to degrade.

2. Classification of industrial wastes for Sewer-Use-Ordinances. Wastes found to resist oxidation should be restricted from discharge to biological treatment systems including the environment. Alternatives include providing pre-treatment or changing the industrial processes to eliminate the refractory materials.

3. Classification of waste discharges in terms of appropriate or non-appropriate discharge to the environment. Materials which have been found to resist biodegradation and, therefore, to accumulate in the environment can pose health and other dangers.

4. Preliminary evaluation of the degradability of a wastewater, waste constituent or specific compound in a biological treatment system. After receiving R.I. data, a complete treatable study can be planned.

### B. BACKGROUND

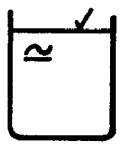
#### 1. Meaning of Refractory

Terms such as refractory, bio-refractory, stable, residual, recalcitrant and persistent have been used to describe degradability characteristics of organic materials found in wastewaters. Ludzack and Ettinger use the term resistance for material that is "more difficult to destroy by biological methods than domestic sewage..."(24). A. A. Rosen also applies the term refractory to those substances resistant to biodegradation (38). Rosen extends the meaning to include "organics which are not truly resistant to oxidation but which persist sufficiently long in a receiving water to exert a characteristic pollutional effect". G. C. McCallum also uses refractory to describe materials "resistant to conventional treatment" (30).

In this study refractory refers to a material and/or its breakdown products which fail to degrade significantly in an aerobic environment with organisms found in domestic sewage during a sufficiently long period of time.

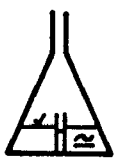
Fig. 1 LABORATORY SCHEME FOR DETERMINING A REFRACTORY INDEX

QUICK-TIME MEASUREMENTS

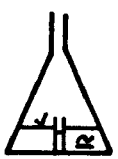


UOD = TOD + TN

WARBURG RESPIROMETER



TEST =
ORGANIC + SEWAGE + NUTRIENTS



BLANK =
SEWAGE + NUTRIENTS

$$BOD'_U = BOD'_{U_{TEST}} - BOD'_{U_{BLANK}}$$

NITRATE PROBE

$$\Delta NO_3^- = NO_{3_{BLANK}}^- - NO_{3_{TEST}}^-$$

$$RI = \frac{BOD'_U + \Delta NO_3^-}{UOD} = \frac{BOD_U}{UOD}$$

The key words in this definition are breakdown products, aerobic environment, degrade significantly, sewage organisms and long period of time.

### 2. Recent Evidence of Refractory Materials

Since refractory materials persist in the biologically active environment (such as biological treatment process* or receiving waters) they exert pollutional effects longer than easily degraded materials (38). Examples of such materials include chlorinated phenols and biphenols, pesticides, mercuric organic complexes and materials found in runoff waters such as humic acids and motor oils (9) (23) (45). Although the impact of refractory substances is not completely understood, such properties as color, taste and odor have been found characteristic of waters containing bio-resistant materials. Refractory organics that exhibit color, taste and odor properties at high concentrations can also be responsible for public health dangers and environmental deterioration at concentrations that do not affect permissible drinking water standards for sensory properties. In addition, many residual organics that are not readily observable may be concentrated in living tissue with adverse effects. Refractory organic materials in a water system, regardless of their concentration, should be considered potentially harmful. Possible effects include biological magnification, toxicity, carcinogenicity and mutagenicity effects on man as well as on various aquatic animals and plants. Other difficulties with refractory materials include bio-treatment overloads from accumulations (29), fouling of ion exchange resins and chelating trace metals (38) and, of course dangers of overloading the ecological system and exposing people, plants and other animals to excessive dosages of refractory materials.

Recent investigations have described accumulations of refractory materials (48). The Mississippi River is reported to contain at least 34 different chemicals originating from industrial waste. Drinking water supplies to Evansville, Indiana are reported to contain industrial contaminants from a discharge 150 miles upstream. Humic acids have been reported to be converted to harmful materials by the standard water treatment practice of chlorination. It is, therefore, becoming evident that materials previously unrecognized, undetected or assessed as non-pollutants (because of low BOD) can in fact present dangers.

## C. DERIVATION AND LABORATORY METHODS

### 1. Derivation and Laboratory Procedure for UOD

The Ultimate Oxygen Demand Measurement (UOD) is designed to quantify the total amount of oxygen needed to complete oxidation of a waterborn organic material to the end products of $CO_2$, $H_2O$ and $NO_3^-$. Since no single analyzer is capable of yielding a direct measure of this value, the UOD

---

*Biological treatment implies biodegradation and, therefore, excludes the flocculation, precipitation and sorption mechanisms found in activated sludge and trickling filter treatment; however refractory material is found in the sludges of such wastewater treatments.

parameter is derived from a combination of Total Oxygen Demand (TOD) and Total Nitrogen (TN) measurements. An examination of the TN and TOD measurement capabilities along with a systematic derivation of the UOD parameter with particular regards given to the assumptions and limitations characteristic of this parameter is presented in Item A of the Appendix.

2. Laboratory Manual for UOD and $BOD_u$ Evaluations

A working knowledge of the laboratory methods used to generate Refractory Index (R.I.) data is necessary in order to interpret R.I. evaluations and to apply them correctly. Item B in the Appendix presents detailed step-by-step laboratory procedures for R.I. evaluations. Explanations of the reasons for each procedure are included. This Appendix is designed to assist laboratory personnel to organize the analytical steps and to enable the environmental engineer and scientist to develop an understanding of R.I. data. Figure 1 illustrates schematically the procedures for determining R.I. values.

D. EXPLANATION OF TERMS

The following terms or expressions are used in this work:

1. Gross Parameter - A measurement that is a general evaluation and therefore describes a number of entities collectively. Most environmental engineering measurements (suspended solids, BOD, TOC, etc.) can be classified as gross parameters. A specific measurement, such as the concentration of oxalic acid, is not a gross parameter. Such specific analyses of wastewaters cannot always be used because of their complex mixture of transient forms of numerous organics at low concentrations. In addition, knowledge of specific organic residuals does not always help in treatment design.

2. mg/L as $O_2$ - Concentration (weight per unit volume) of a material expressed as molecule oxygen equivalence. This value is obtained by multiplying the material's concentration (mg/L) by the molecular weight of $O_2$, 32 gm/g-mole, and dividing this product by the molecular weight of the original material. For example:

$$100 \text{ mg/L TOC as C} = 100 \times 32/12 = 250 \text{ mg/L TOC as } O_2 \qquad [2]$$

3. mg/L as $O_2$ Demand Equivalence - Concentration of a material (weight per unit volume) expressed as the amount of oxygen needed for biochemical oxidation; i.e., one mole of ammonia ($NH_3$) requires 2.0 moles of oxygen ($O_2$) for complete conversion to $H_2$) and $HNO_3$.

$$NH_3 + 2.0\ O_2 \longrightarrow HNO_3 + H_2O \qquad [3]$$

For example: 20 mg/L $NH_3$ = 20 x 32/17 x 2.0 = 75 mg/L $NH_3$ as $O_2$ demand equivalence.

4. Refractory, Biorefractory, Recalcitrant, Persistent - These terms are used to define the biodegradability of organic materials in the aquatic

environment. A material described as refractory is characterized by a resistance to degradation to $CO_2$, $H_2O$ and $NO_3$ (end products of the ambient aquatic oxidation).

5. TOD, Total Oxygen Demand - Determined on the Ionics Model 225 TOD analyzer (mg/L as $O_2$). This is a high temperature (900°C) catalyzed aerobic oxidation.

6. TN, Total Nitrogen - Determined on the Dohrmann MCTS-10 nitrogen analyzer (mg/L as N). In this study TN is converted to mg/L as $O_2$.

7. UOD, Ultimate Oxygen Demand - Calculated from the TOD and TN measurements. UOD defines the total quantity of oxygen needed to oxidize a material to $CO_2$, $H_2O$ and $NO_3^-$. This gross parameter is used to quantify the total amount of material present.

8. TOxN, Total Oxidizable Nitrogen - The quantity of nitrogen present that is theoretically capable of oxidization to $NO_3^-$. This is a term used in the UOD derivation, not a measured quantity, and is expressed as oxygen demand equivalence.

$$TOxN = 3/2\ (\text{Organic N} + NH_3) + 1/2\ NO_2^- \qquad [4]$$

all expressed in mg/L as $O_2$ demand equivalence.

9. $BOD_u$, Ultimate Biochemical Oxygen Demand - Determined on the Warburg Respirometer with a nitrification correction term. $BOD_u$ defines the amount of oxygen required to degrade organics under the specific test conditions. This gross parameter is used to quantify the degradable organic material present.

10. $NO_3^-$, Nitrification Correction - This term is used to correct for unequal degrees of nitrification (nitrate production) in the Warburg test, and is expressed in mg/L as $O_2$ demand equivalence.

11. R. I., Refractory Index - A ratio of $BOD_u$/UOD, used to indicate the bio-degradability property of an organic material. A Refractory Index of 1.0 indicates 100% degradation, while a Refractory Index of 0.0 indicates no degradation. Intermediate values indicate partial degradation.

12. Warburg Respirometer Test Solutions - The $BOD_u$ test procedure is designed to determine the biochemical oxygen uptake response for known chemical solutions of mixtures of unknown chemicals (e.g.; industrial wastes). In the method adopted, the chemical is added to a solution of raw sewage plus additional nitrogen and phosphorous nutrients if necessary. This mixture (raw sewage, ammonia, phosphate buffer and test chemical) is referred to as the Test Solution.

13. Warburg Blank Solution - A $BOD_u$' value is determined by subtracting the $BOD_u$' value of raw sewage, ammonia chloride and phosphate buffer from the $BOD_u$' of the test solution. The raw sewage, ammonia chloride and phosphate buffer solution is referred to as a Blank Solution.

14. Biological Interference Value - Some of the compounds tested exhibited an inhibition or interference response to the $BOD_u$ process; that is, there is more uptake for the sewage alone than for the sewage plus the tested compound. In calculating the R.I. for such cases, the numerator of the ratio $BOD_u/UOD$* is a negative number, thus also making the R.I. negative; this is difficult to analyze quantitatively. For this reason the B.I.V. (Biological Interference Value) was developed. The B.I.V. represents the reduction in BOD of the sewage due to the presence of the compounds

$$B.I.V. = (BOD_{sewage} - BOD_{sewage + compound})/BOD_{sewage} \qquad [6]$$

The range of B.I.V. is zero to one. Zero indicates complete or 100 percent reduction of the sewage BOD, while 1.0 indicates no interference at all with the BOD process.

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$$*R.I. = (BOD_{sewage + compound} - BOD_{sewage})/UOD_{compound} \qquad [5]$$

## SECTION 5

## REFRACTORY INDEX DATA AND INTERPRETATIONS

The Refractory Index data that follow are grouped into the categories natural, synthetic, industrial and combinations because these categories lend themselves to discussion; however, the distinction between categories is to a large extent arbitrary. Along with the data presentation are offered explanations and rationales for the biodegradability or refractoriness of individual compounds and groups of compounds.

### A. GENERAL EXPLANATION FOR PERSISTENCE

Specific reasons for the persistence of individual organic compounds are presented with the data on refractory organics which follow. There are also some general explanations of the phenomenon.

1. Insufficient time - The ultimate BOD test normally allows a 30 day degradation period. This test time is extended if a measurable oxygen uptake surge is noted. The time limit is used because the ultimate BOD test is designed to have implications for actual treatment situations in which detention time is limited. This is not to be interpreted as stating that the 30 day plus time period can be extrapolated to indicate the ultimate fate (in terms of aerobic degradation) of a material. Thus, it can be suggested that the time limit on the R.I. classification determines persistency. On the other hand, 30 days is far in excess of treatment times in wastewater treatment plants.

2. Non-optimal environment - If a naturally occurring material is found to resist degradation under the conditions provided in an experimental system, the most obvious explanation for this persistency is that the environmental conditions of the test are inappropriate. In this case, the environmental conditions include fixed temperature, nutrients, constant pH (buffered), heterogenous bacterial seed, absence of light and sufficient substrate. The interaction between time and this environment in the presence of microorganisms creates the biochemical environment, an enzymatic system which whould degrade the compound of interest. These tests thus take place under specific environmental conditions which may be non-optional for the degradation of specific materials.

Although it is possible to conduct further study to determine what conditions or series of conditions would accelerate the degradation process, the information gained would not be directly applicable to the existing biological wastewater treatment systems. It should be noted that the main intent of this study is to determine persistence under conditions which could be directly applied to existing systems. Therefore, extensive acclimation procedures are not used since acclimated bacterial populations are difficult to maintain in wastewater treatment plants. There is a reasonable degree of acclimation in the 30 day test period used.

The environment of the $BOD_u$ test was chosen to coincide with the standard BOD technique; this approximates the optimum condition of a biological treatment process and is judged to be a generally optimum condition for aerobic degradation.

3. Missing Link in Path of Degradation - Degradation is promoted by enzymes produced by microorganisms. The microorganisms involved coexist in a trophic dynamic balance with each other and within an entire ecosystem. If the balance normally found in the natural aquatic environment of a lake or stream is critical to the degradation pathway for the material being tested and is not present in the ultimate BOD test, the material would be classified as refractory. It should be noted, however, that in the $BOD_u$ test sewage seed was used; the bacterial population found in sewage is more heterogenous than that of most natural environments.

## B. PRESENTATION AND INTERPRETATION OF R.I. DATA FOR NATURALLY OCCURRING ORGANIC COMPOUNDS

Naturally occurring compounds are defined as those compounds commonly found in metabolic pathways. This semi-arbitrary classification distinguishes these organic materials from the manufactured or process compounds of industry. It is, of course, not possible nor correct to have an exact distinction between natural and synthetic materials.

Table 2 lists compounds considered natural in this report; these include simple substrates (e.g., glucose), building blocks of complex life forms (e.g., amino acids), enzyme and co-enzyme intermediaries (e.g., adenine) and plant organic extracts (e.g., hydroquinone). Because these compounds are naturally occurring, one might intuitively expect close to complete degradation in the long-term BOD test as well as other aerobic biological reactors.* R.I. values for readily degradable organics might be expected to range above the 0.8 or 0.9 levels. Note that, since the coefficient of variation ($s/\bar{x}$) is 0.13 for the R.I. determination, for a totally degraded material, ideal values of 0.87 to 1.13 would correspond to a spread of one standard deviation.

Compounds such as glucose and glutamic acid do fall in this range. However, many other natural materials fall short of this degree of degradation, most notably the basic amino acids lysine and histidine, the coenzyme intermediary adenine and also acetic acid which is a product of partial degradation.

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*After all, it can be reasoned that we would be knee-deep in organic debris if naturally occurring organics resist degradation. But there are, in fact, persistent organic materials in the environment: life forms, petrochemicals, humic materials. This brings into question the too often accepted statement that natural organics are readily biodegradable. Thermodynamically they are unstable but the pathway for degradation may not be available; inorganic nutrients can be limiting and inhibition can occur. Further, can degradation of organic material occur without synthesis of the complex organic material of the microorganisms involved with the decomposition?

TABLE 2

REFRACTORY INDEX DATA FOR NATURALLY OCCURRING ORGANICS

| Material | R.I. | $BOD_u$ | TOD | TN | $BOD_5$ | TOC | $NO_3^-$ |
|---|---|---|---|---|---|---|---|
| | | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as C / 100 mg | mg as $O_2$ demand / 100 mg |
| Acetic Acid | 0.61 | 66 | 125 | 0 | 71 | 47 | none |
| Adenine | 0.12 | 0 | 207 | 120 | 12 | 44 | none |
| L-Aspartic Acid | 0.81 | 98 | 97 | 24 | 69 | 36 | none |
| L-Arginine | 0.65 | 166 | 196 | 60 | 90 | 45 | -71 |
| Butyrate (Na) | 0.84 | 148 | 175 | 0 | 135 | 55 | none |
| D-Glucose | 0.93 | 98 | 107 | 0 | 77 | 40 | -39 |
| D-Glutamic Acid | 0.98 | 139 | 121 | 22 | 86 | 41 | none |
| L-Histidine | 0.52 | 127 | 181 | 62 | 69 | 47 | none |
| Humic Acids | 0.0 | 0 | 240 | 2 | 0 | 64 | 91 |
| Hydroquinone | 0.41 | 78 | 190 | 0 | 2 | 66 | -19 |
| L-Lysine | 0.47 | 120 | 208 | 44 | 82 | 49 | 2 |
| Propionate (Na) | 0.80 | 115 | 143 | 0 | 108 | 49 | none |
| Starch | 0.63 | 61 | 127 | 0 | 50 | 44 | 11 |
| L-Valine | 0.93 | 210 | 198 | 27 | 185 | 51 | none |

Table 2 presents the accumulated data for the naturally occurring organic group. This includes the R.I. values and normalized (per 100 mg of tested organic) values for the ultimate Biochemical Oxygen Demand ($BOD_u$), the Total Oxygen Demand (TOD) and the Total Nitrogen (TN) content as well as the nitrate correction value and related classic organic measurements: 5-day Biochemical Oxygen Demand ($BOD_5$) and Total Organic Carbon (TOC). The graphical presentation of these data can be found in Part C of the Appendix grouped alphabetically.

Certain individual compounds are described in the following paragraphs with possible explanations for their R.I. values.

1. Glucose and Glutamic Acid were found to degrade almost completely (R.I. values of 0.93 and 0.98, respectively). Such a response is expected since both compounds readily enter energy producing metabolic pathways, are converted to $CO_2$ and $H_2O$ and do not usually form stable intermediaries. If the energy produced in the degradation is not immediately released, it is stored in other compounds (e.g., ATP) rather than as the compound itself or a partially oxidized intermediate. The purpose for presenting R.I. values for these readily degradable organics is to demonstrate the reliability of the measurements.

L-Valine, $(CH_4)_2CHCH(NH_2)COOH$, an acidic amino acid, was tested and found to be readily degradable with an indicated R.I. of 0.93. Compounds like glutamic acid and valine are known to be transformed to $\alpha$-ketoglutarate and ammonia by dehydrogenase enzymes during conditions that favor amino acid oxidative degradation. The $\alpha$-ketoglutarate molecule is an intermediate in the tricarboxylic acid cycle and consequently should degrade almost completely to $CO_2$, $H_2O$ and $NH_4^+$.

2. The relatively low R.I. value for the basic amino acids, lysine, histidine and arginine concurs with previous research by Helfgott et al (19) which identifies organic residuals from biological process effluents as: carbonaceous, nitrogen bearing, cationic at high pH (electropositive at pH ≈ 9), dispersed, non-biodegradable and non-sorbable onto activated carbon. Possible explanations for intermediate R.I. values include the following which are not necessarily mutually exclusive.

a. A stable intermediary is produced that is more persistent than the parent constituent. For lysine, for example, this might be the decarboxylated compound cadaverine associated with decaying flesh and toxic materials.

b. Some fraction of the material is converted to a refractory form of the parent compound; e.g., lysine distributes itself between parent L-lysine to a mixture of biodegradable L-lysine and refractory D-lysine; hence the R.I. value of about one half.

c. The high solubility of certain organic constituents especially small and charged species like lysine, causes a very slowly changing distribution between the aquatic phase and the bacterial mass where sorption and degradation occur. The cationic nature of the basic amino acid may limit the attachment to the bacterial cell mass. The portion of soluble organic form in the water phase appears as persistent.

d. The eventual ecosystem developed in the bio-reactor used, the Warburg Respirometer in this case, is an aquatic environment not adapted to use all the lysine available as an energy source. The system is satisfied in this specific substrate requirement at some low concentration.

e. The basic amino acids as building blocks of protoplasm and cell walls could be cycled in and out of the life forms without being degraded to ultimate end products. Bacterial and algal cell wall structure, for example, are known to persist in aerobic bio-reactors (11)(19)(49), and the residual basic amino acids could reside there.

f. The basic amino acids are converted to polymers (perhaps the end chain amino acid of cell wall structure of microorganisms). These polymer forms of the amino acids appear as refractory.

3. Adenine, another basic nitrogen bearing organic (6 aminopurine), is extremely refractory, R.I. = 0.12. Some of the same reasons for partial stability of the basic amino acids apply here, but a strong case can be made for conservation of the co-enzyme constituent by the bacteria that cycle this chemical to support metabolisms of readily degradable substrates like glucose and polymerize structural building materials like lysine. Adenine is conserved in the microorganisms and not degraded as a substrate energy source.

4. Certain other amino acids, valine, asparatic acid and glutamic acid degraded almost completely (R.I.=0.93, 0.81, 0.98, respectively) in a pattern anticipated for natural organics. These acidic amino acids can serve either as energy substrate or as building material. In water of neutral pH these amino acids are present as anions and can be readily sorbed by microorganisms and metabolized.

5. Two organic acids, butyric and propionic, tested in sodium salt forms showed ready degradation (0.84 and 0.87, respectively) as anticipated for natural organic degradation intermediaries, but acetic acid shows only partial degradation with an R.I. value of 0.61. It is thought that the larger organic acids degrade to acetic acid (by means of a Beta oxidation pathway), but acetic acid can either be further oxidized or enter into enzymatic pathways that build to larger organic materials of life. Like lysine and adenine acetic acid is a relatively stable intermediary that is conserved by cycling in and out of bacterial structures. (Synthesis of cellular material is a necessary half of the process of oxidative degradation.)

Acetic acid as a small highly soluble organic also leaves a large portion in solution resisting adsorption into the biomass where degradation and synthesis occurs. It is probably the protonated (un-ionized) form that

absorbs and diffuses through bacterial cells by a hydrogen bonding mechanism. It is noted that effluents of sewage treatment plants contain several mg/L of volatile organic acids especially acetic acid.

6. Starch is not a simple homogenous material but a polymer that degrades first by hydrolysis to yield glucose, maltose and a high molecular weight amylopectin which has a number of nonacetyl "ends"; thus, there are branching and cross-linkages in natural starch in addition to linear glucose polymers. That portion of the starch that yields simple sugars of hydrolytic degradation undoubtedly degrades almost completely. The unhydrolyzed and branched portion of starch appears relatively refractory. This would account for the R.I. value of 0.63 showing partial degradation. Starch is not hydrolyzed completely because of some branched linkage that offers obstruction to enzyme attachment.

As a general rule for refractoriness, branching is an accepted empirical factor. The infamous case of "hard" (biorefractory) branched alkylbenzenesulfonate vs. "soft" (biodegradable) linear alkys sulfonates of the detergent industry illustrates this point of branching limiting degradation.

7. Hydroquinone ($HO-C_6H_4-OH$) is a natural material of plants that also showed surprisingly high resistance to degradation (R.I. = 0.4). This is difficult to explain but it is an empirical fact that symmetric compounds (e.g., benzene) seem to show higher resistance to biodegradation than unsymmetrical compounds (e.g., phenol).

8. Humic acids were found to resist degradation almost totally, R.I. = 0. Such a result is consistent with known properties of this residual found in natural waters. Humic acids, a non-chemically specific group of large molecular weight that is soluble at low pH, are known for imparting yellow color and for long-term persistence. In the ambient environment, some humic acids probably disappear by adsorption, assimilation and degradation.

It is interesting to speculate as to what causes the formation of humic acid in the environment in order to obtain hints as to the persistent nature of the material. The organic debris of plants and animals enters into an aquatic environment that is generally nitrogen deficient. Bacteria and algae utilize the organic nitrogen or mineralized nitrogen for growth. The carbonaceous residual skeleton of decaying plants is thus a localized environment that is nutrient unbalanced so that it no longer constitutes a suitable substrate for microorganisms. Thus, it persists for long periods of time. Some of the humic acids are the stable intermediaries of biodegradation of resistant cellulose and natural starch forms that were previously mentioned. They are high molecular weight, condensed, aromatic compounds low in nitrogen but high in partially oxidized intermediary groups (e.g., carboxyl groups). The size, branching and resistance to hydrolysis fragmentation create a natural refractory residual.

## C. PRESENTATION AND INTERPRETATION OF R.I. DATA FOR SYNTHETIC AND INDUSTRIAL ORGANIC COMPOUNDS

The distinction between synthetic or industrial organic compounds and natural compounds is to some extent arbitrary and is used here only to make a convenient category for discussion. This category includes a wide variety of compounds: common, simple organics of industry (e.g., phenols and benzene); widely used materials that find their way into municipal treatment plants (e.g., glycols and gasoline); the chemicals of agriculture (e.g., pesticides); and some representative chlorinated organics (e.g., chloroform) that may be produced during chlorination practices of water and wastewater treatment.

Materials found to fall into the refractory classification (low R.I. values of <0.6; i.e., $1.0 - 3(s/\bar{x})$) are not expected to degrade in an aerobic wastewater treatment system such as an activated sludge process are expected to persist in natural water systems such as aquifers, rivers and lakes. The practice of discharging such persistent industrial materials into the environment should be discouraged.

Table 3 lists the compounds under this category for R.I. value, nitrate correction and normalized parameters of oxygen demand and organic content. Item C of the Appendix includes the graphic presentation of this information.

Many of the reasons for the occurrence of refractory natural organics also apply to synthetic organic materials; in addition, much research and development has been done by industry to create materials which persist; i.e., do not degrade on the shelf, hold up under severe environmental conditions and generally are not readily altered by bacterial actions. For example, dyes are often selected that resist degradation by sweat, light, microorganisms, severe cleaning conditions and are not resolubilized.

1. Acetone (dimethyl ketone) falls into the category of readily degradable industrial organic materials. Acetone is a product of the anerobic fermentation of material carbohydrate materials such as potato starch, maize, etc. Acetone bodies are also found in sewage wastewaters as a by-product of urine and the biodegradation of fats. The average R.I. value of 0.8 (separate determinations of 0.93 and 0.71) for acetone demonstrates its degradation to ultimate end products in the aerobic and nutrient rich environment provided by the test.

There may have been volatile losses of acetone during the test procedure but this common solvent of industry also has an easy entrance mechanism through bacterial cell walls where oxidative degradation occurs.

2. Benzene and Phenol show disparate R.I. values that reflect findings reported elsewhere (21) (39). Phenol undergoes biochemical oxidation in the respirometer environment (R.I. = 0.87), while benzene resists degradation (R.I.= 0.23). This presents an interesting example of how apparently similar materials demonstrate very dissimilar degradation characteristics. Resonance structure and symmetry are the factors imparting persistence to benzene; the

TABLE 3

REFRACTORY INDEX DATA FOR SYNTHETIC AND INDUSTRIAL COMPOUNDS

| Material | R.I. | $BOD_u$ | TOD | TN | $BOD_5$ | TOC | $NO_3^-$ |
|---|---|---|---|---|---|---|---|
| | | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as C / 100 mg | mg as $O_2$ demand / 100 mg |
| Acetone | 0.93 | 220 | 220 | 0 | 124 | 62 | 0 |
| Aniline | 0.58 | 208 | 283 | 34 | 150 | 77 | -18 |
| Benzene | 0.23 | 69 | 308 | 0 | -86 | 92 | -12 |
| Biphenyl | 1.14 | 198 | 174 | 0 | 64 | 78 | 13 |
| Bipyridine | < 0 | - | 273 | 41 | - | 70 | 0 |
| Chloroform | < 0 | - | 34 | 0 | - | 10 | 0 |
| p-Chlorophenol | 0 | -1 | 169 | 0 | -16 | 64 | -57 |
| CMC | 0 | 0 | 115 | 0 | 0 | 43 | -6 |
| Cyanuric Acid | < 0 | - | 93 | 74 | - | 28 | 0 |
| DDT | < 0 | - | 141 | 0 | - | 53 | 6 |
| Dichlorophenol | < 0 | - | 136 | 0 | - | 47 | 0 |
| Ethylene Glycol | 0.76 | 112 | 129 | 0 | 85 | 38 | 0 |
| Gasoline | 0.21 | 70 | 334 | 0 | 54 | 96 | 93 |
| Phenol | 0.87 | 206 | 239 | 0 | 172 | 77 | -44 |
| Propylene Glycol | 0.78 | 134 | 168 | 0 | 108 | 47 | 0 |
| Sevin | 1.0 | 95 | 90 | 5 | 9 | 20 | 0 |
| Vinyl Chloride | 0 | 0 | 140 | 0 | 0 | 30 | 0 |

ability for the un-ionized (pK = 10) phenol to diffuse by a hydrogen bond mechanism through microorganisms is thought to be the reason for the ready degradation of phenol.

3. Aniline, in contrast to both refractory benzene and biodegradable phenol, showed an intermediate refractory index of 0.58. Aniline is known to be very sensitive to oxidation but is converted into a host of partial oxidation products including azobenzene, azoxybenzene, nitrobenzene, quinone, dyestuff Aniline Black and several other intermediate end products resulting from secondary products of condensation. The test method developed for R.I. evaluation does not indicate intermediate hydrolysis products or stable carbonaceous structures such as ring configurations. Thus, those degradation products of aniline analogous to benzene appear refractory and those analogous to phenol appear biodegradable. The heterogenous nature of the oxidation products of aniline results in an intermediate indication of biodegradability.

4. Ethylene Glycol and Propylene Glycol with R.I. values of 0.76 and 0.78 fall into a category of compounds with high but not complete degradation. These are materials of commerce which are used in such large quantities for antifreeze and road de-icing that persistence in the environment would be a problem. These materials are generally considered to degrade well but not completely. This is probably a function of the high solubility of these low molecular weight, double alcohol group materials. It is not expected that these organics would be a serious problem except for the oxygen demand.

5. Sevin (Carbaryl Sevin) is used as a pesticide, often replacing DDT because sevin is known not to persist but rather to degrade almost totally. The R.I. determined as 1.0 confirms this complete degradation. It is interesting to compare this unchlorinated pesticide ($C_{12}H_{11}O_2N$) with the more recalcitrant chlorinated pesticides.

6. Chlorinated organics have been found to fall into the category of persistent organics by many current investigations (12)(15) (35) (38). Of special current concern is the creation of persistent organics by chlorination for disinfection of water supplies. The zero values of R.I. for p-chlorophenol and vinyl chloride, indicating no degradation, as well as the negative calculated values of R.I. for DDT, chloroform and dichlorophenol, indicating an inhibition of the bacterial system in the respirometer, are of particular concern.

Materials in the chlorinated hydrocarbon category responded differently to the ultimate BOD test than did the more readily degradable materials. These chlorinated organics are found either to resist degradation or to inhibit the bacterial populations in the sewage of the test solutions. The ultimate BOD measurement is therefore found to have an application in quantifying the degree of inhibition. For instance, the $BOD_u$ for dichlorophenol plus sewage was evaluated at 260 mg/L $O_2$, while the sewage alone had

a $BOD_u$ of 630 mg/L $O_2$. This response not only indicates a resistance to degradation by dichlorophenol but also an inhibition of sewage degradation in the respirometer environment of roughly 60%. The R.I. calculation from such data results in a negative number which, although indicative, is difficult to interpret quantitatively; therefore, the B.I.V. is used to quantify the response of the microorganisms in the respirometer environment to these inhibitory organic materials.

Another interesting set of R.I. responses from chlorinated organics is the negative oxygen uptake values ($BOD_u$ of test lower than $BOD_u$ of blank) from DDT samples. Since, as noted, a negative R.I. value has qualitative but not quantitative usefulness, a different way to express the degree of problem of these organics is suggested. The relative loss of ultimate BOD of the test organic material spiked into sewage of a determined $BOD_u$ seems to be a more meaningful way of suggesting quantitative inhibition. This calculation is termed the Biological Interference Value (B.I.V.).

$$B.I.V. = (BOD_{u2} - BOD_{u1})/BOD_{u2} \qquad [6]$$

where $BOD_{u1}$ = $BOD_u$ of sewage plus test organic

and $BOD_{u2}$ = $BOD_u$ of sewage alone.

Table 4 gives the B.I.V. numbers for the limited number of inhibitory organics tested.

7. Biphenyl and Bipyridine illustrate some interesting contrasts to the chlorinated organics. Chlorinated biphenyls have been reported as inhibitory to enzymatic systems; some are reported to be carcinogens as well as refractory residuals. In these tests, the unchlorinated biphenyls showed complete degradation with R.I. of 1.14 (considering the $s/\bar{x}$ of 0.13, this value is an indication of complete degradation). On the other hand, biphenyls have been found in the natural environment (polar ice cap cores). It should be stressed that the respirometer environment provides a more suitable ecosystem for biological degradation than most natural ecosystems. Natural environments, such as polar ice caps, tend to be nutrient deficient whereas the biochemical oxygen demand test conditions of the respirometer provide nutrient excesses.

In bipyridine, one C on each phenolic ring is replaced with N. Bipyridine is a refractory chemical that inhibits biochemical degradation of sewage. The R.I. is negative since the ultimate BOD of the test chemical bipyridine, is less than the ultimate BOD of the sewage into which it is spiked. The B.I.V. number for bipyridine was found to be 0.56 as listed in in Table 4.

To verify the results of high degradability for biphenyl, gas chromatography analysis were conducted on the raw and bio-oxidized biphenyl solutions. The gas chromatography data were collected to determine the presence of biphenyl before and after biochemical oxidation under test conditions. The biphenyl, which was assayed at 99% pure could not be detected at the end of the test period.

TABLE 4

CHEMICALS WHICH INHIBIT RESPIROMETER RESPONSE

| Chemical | B.I.V. | calculated R.I. |
|---|---|---|
| Bipyridine | 0.56 | -0.47 |
| Chloroform | 0.71 | -0.27 |
| Cyanuric Acid | 0.26 | -0.22 |
| DDT | 0.42 | -0.21 |
| DDT with carrier | 0.34 | -0.25 |
| Dichlorophenol | 0.59 | -0.77 |

8. CMC (Carboxymethyl cellulose) is replacing starch as a sizer in the cloth fabrication industry. In contrast to starch, which has the relatively high R.I. value of 0.7 to 0.8, CMC has an R.I. value of 0, indicating a refractory nature.

Users of sizers had been told to stop using starch because its biodegradability exerts a large biochemical oxygen demand on treatment plants and the receiving aquatic environment. The industrial concerns, by switching to CMC, avoided the oxygen demand problem but placed into the environment the refractory CMC, which passes unaltered through treatment plants. This is a clear-cut case of perversion of purpose which occurs when only one measure of pollution (BOD) is used. A single measure of pollution can be manipulated to show no apparent pollution, while the actual consequence to the environment is worse since the substitute persists, accumulates in the environment, and eventually does harm. If, as advocated here, the ultimate BOD is compared to the total oxygen demand, the pollutional effects of materials like CMC become evident. Then alternative treatment processes can be selected to remove if not reclaim this material.

9. Cyanuric acid, $N = \overset{OH}{\overset{|}{C}} - N = \overset{OH}{\overset{|}{C}} - N = \overset{OH}{\overset{|}{C}}$, is one of the few organic materials that appears to be more degradable in an anaerobic environment than in an aerobic environment. It is discussed here because of its uniqueness (40).

Cyanuric acid is a well-oxidized organic with a symmetrical ring structure which implies high resistance to degradation. It can be degraded more quickly by hydrolysis, a process which is accomplished both in aerobic and anerobic environments. Since this material does not serve well as an energy source, it has little oxidative potential. Further, cyanuric acid is inhibitory to bacterial growth with a B.I.V. number of 0.26.

In attempting to develop general rules for which organic materials are degradable or persistent, there is the danger of overgeneralizing. Cyanuric acid is an exception to the general rule that anaerobic degradation is quicker and more complete in reaching ultimate end products.

## D. PRESENTATION AND INTERPRETATION OF R.I. VALUES FOR COMBINATIONS OF ORGANIC MATERIALS

This section of the report includes a discussion of data on organic materials that are composites from municipal and industrial sources. The absolute concentration of materials in composite wastewaters and mixtures of organics is seldom known; therefore, the R.I. value as a normalized ratio of ultimate biochemical oxygen demand to ultimate total oxygen demand is particularly useful. The test technique can be applied to composite wastes for which individual component assays are very difficult. Table 5 lists the data for combinations of materials.

1. Domestic sewage is a heterogenous mixture of numerous organics in transient form under bacterial modification. The organics are of natural, synthetic and industrial origin. This dynamic mixture of thousands of different organics in dilute solution can contribute refractory organics to the environment via the effluents and/or sludges of wastewater treatment plants. Some refractory organics can even be created during the treatment of wastewaters; for example, endogenous respiration during activated sludge treatment contributes refractory bacterial cell walls (11) (19) and chlorination produces refractory chlorinated hydrocarbons.

The R.I. values shown in Table 5 are averages of 11 different determinations in sets of at least three. These data illustrate the relatively high average Refractory Index (R.I. = 0.7). The range is also shown as an indication of sewage variability. Although raw sewage is biodegradable, the R.I. value of 0.7 indicates the presence of some refractory material.

2. Gasoline showed a low R.I. value of 0.21; it is known to be a relatively persistent organic. The low solubility of gasoline is thought to make it relatively unavailable for bacterial degradation in most situations; however, a special procedure was used to disperse the gasoline in the respirometer for the $BOD_u$ determination. The R.I. value is for the soluble and micro-colloidal gasoline constituents.

When a commercial unleaded gasoline was tested, the $BOD_u$ for the sewage plus gasoline was slightly less than for sewage. The presence of gasoline, however, completely inhibited the nitrification of the nitrogen compounds in the sewage. When the $\Delta NO_3^-$ correction was added to the $BOD_u'$, an R.I. of 0.21 is calculated. Published data indicate that gasoline degrades 22% in a $BOD_5$ test. The nitrification problem is displayed in the Appendix; the typical nitrification hump exhibited for sewage Warburg Respirometer response did not occur for gasoline plus raw sewage.

The gasoline was prepared for this test as follows: 50 ml gasoline was added to 450 ml distilled water. This was stirred for several days, then put in a separator funnel for approximately 30 days. The water was carefully drawn off at the bottom in order not to include the lighter gasoline fraction visible at the top. Tests of this mixture showed a TOC

TABLE 5

REFRACTORY INDEX DATA FOR COMBINATIONS OF ORGANIC MATERIALS

| Material | R.I. | $BOD_u$ | TOD | TN | $BOD_5$ | TOC | $NO_3^-$ |
|---|---|---|---|---|---|---|---|
| | | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as $O_2$ / 100 mg | mg as C / 100 mg | mg as $O_2$ demand / 100 mg |
| Antifreeze | 1.12 | 208 | 180 | 0 | 171 | 39 | 0 |
| DDT (with carrier) | 0 | 0 | 93 | 0 | 0 | - | 16 |
| Ethylene Glycol (with inhibitor) | 0 | 0 | 129 | nil | 38 | 38 | - |
| Gasoline | 0.21 | 70 | 334 | nil | 54 | 96 | 93 |
| Humic Acid | 0.0 | 0 | 240 | 2 | 0 | 64 | 30 |
| | | mg/L $O_2$ | mg/L $O_2$ | mg/L $O_2$ | mg/L $O_2$ | mg/L C | mg/L $O_2$ |
| Textile Dye - Industrial Waste | 0.05 | 3.2 | 527 | 71 | 0 | - | 95 |
| Dye Material | 0.58 | 314 | 468 | 97 | | - | 92 |
| Raw Sewage | 0.7 | 380 | 470 | 105 | 220 | - | - |
| Raw Sewage-Range | 0.5-0.9 | 160-650 | 180-930 | 75-190 | 130-400 | | |

of 76 mg/L C and a TOD of 270 mg/L $O_2$. This is estimated to be 80 mg/L as octane which yields a theoretical oxygen demand of 277 mg/L $O_2$ and theoretical carbon of 67 mg/L C. In the test solution, 100 ml of raw sewage were placed with 250 ml gasoline, and mixture aliquots of this were placed in the Warburg flask.

It is particularly interesting to note that the presence of gasoline seems completely to inhibit the nitrification of sewage. Gasoline is, of course, the product of distillation of crude oil, a naturally occurring refractory organic mixture.

3. Dyes represent typical refractory materials in that they resist by design alteration in use. A dye was tested and found to have an R.I. value of 0.56, indicating partial resistance to degradation in the test used. The dye was most likely a combination or organics rather than a single pure material. In the manufacture and application of dyes, inhibitors are commonly used to impart greater stability.

From the same manufacturer that produced the dye discussed above, some industrial wastewater was obtained and tested under the same procedures described in this report. The R.I. value of this waste was found to be 0.05, indicating almost total refractoriness. This wastewater normally passes through a rather elaborate treatment plant, primary sedimentation and activated sludge, then into local rivers. It is interesting to note that the dyes can be seen passing through these waste treatment steps and into the receiving environment, clearly indicating the inappropriateness of these treatment steps for this product.

Many of the organic constituents of this particular industry resist biochemical degradation. Obviously, treatments other than bio-reactors should be used for such wastes; in this case, activated carbon might be more effective in protecting the receiving environment.

Color measurements were conducted on the initial and final test solutions containing the individual dye in order to quantify the degree of color removal due to long-term ($\gtrsim$ 30 days) biodegradation procedures of the Warburg Respirometer. Table 6 shows predominant wavelength, percent luminance and percent colorimetric purity for the dye waste before and after degradation in the Warburg Respirometer.

TABLE 6

COLOR ANALYSIS OF TEXTILE DYE

| | Before Oxidation | After Oxidation |
|---|---|---|
| Predominant Wavelength (nm) | 576.0 | 560.0 |
| Luminance (%) | 60.2 | 84.0 |
| Colorimetric Purity (%) | 7.5 | 3.0 |

This table shows that there was a 40% increase in total luminance and a 60% decrease in color at the predominant wavelength. This response indicates a reduction in color influence during biological oxidation and corroborates the R.I. evaluation.

The industrial waste discharge of the manufacturer was also found to resist biological degradation in the Warburg Respirometer (R.I. = 0.05). Spectrophotometric color analysis of the filtered effluent, however, shows a 200% increase of total luminance and a 70% decrease of color purity, displayed in Table 7. These data indicate removal of color during the biological degradation process and, therefore, disagree with the R.I. evaluation. Such an apparent contradiction of results between the R.I. value and the color loss can be explained by observing another mechanism of pollution removal during the biological treatment (i.e., other than biodegradation). At the end of the test, it was observed that the biomass in the Warburg Respirometer contained color while the filtrate was

TABLE 7

COLOR ANALYSIS OF INDUSTRIAL DYE WASTE

| | Before Oxidation | After Oxidation |
|---|---|---|
| Predominant Wavelength (nm) | 508 | 500 |
| Luminance (%) | 31.4 | 92 |
| Colorimetric Purity (%) | 40 | 12 |

relatively clear. The conclusion, therefore, is that dye constituents are concentrated by biochemical or physical sorption. The implication of this mechanism is that dye becomes a refractory constituent of sludge: i.e., the problem material is only transferred to another area, not degraded. In fact, the writers have observed that dried activated sludge from a municipal sewage treatment plant receiving industrial dye waste had a dye color. Since this particular sludge is disposed of at a land fill, leachate may contain pollutants from the dye.

4. Commercial grade antifreeze, largely ethylene glycol, was tested and found to have an R.I. of essentially 1.0. (The actual value was 1.12 but there is 13% relative error). Although the antifreeze did not appear to be pure ethylene glycol, the UOD was the same as ethylene glycol. Undoubtedly, there were additives (or diluents) in the commercial antifreeze. Since the exact chemical composition of commercial products and industrial waste is rarely known, the UOD measurements and comparisons are particularly useful.

6. Laboratory ethylene glycol listed as having an unspecified inhibitor additive had a negative R.I. value. This compares with a pure ethylene glycol R.I. of 0.8 and an antifreeze R.I. of about 1.0. The concentration of the ethylene glycol and other additives in antifreeze were not known. The presence of inhibitors in commercial composite materials is a factor to be taken into account in wastewater treatment.

7. DDT in a carrier is reported on as a composite material. The test also indicates an inhibitory response for the composite (B.I.V. = 0.34) which is close to that of DDT alone (B.I.V. = 0.42).

8. Humic acid data are repeated in Table 5 since this is not a single material but a classification grouping developed by soil scientists for refractory organics that are insoluble at low pH.

## E. TEST PROCEDURES: INTERFERENCES, LIMITATIONS, CORRELATIONS AND CONFIRMATIONS

### 1. Interferences

When industrial wastes include high concentrations of heavy metals or sulfur, the R.I. technique must be interpreted carefully in evaluating the persistence of organics. Heavy metals and sulfur can exert an apparent high TOD and interfere with the long-term biochemical oxygen demand evaluation. For example: $Cd^{++} \longrightarrow CdO$ and Organic S $\longrightarrow SO_2$. The sewage used for the tests reported here had low metal content (e.g., Cd < 20ug/L) and low sulfur (organic sulfur < 1.1 mg/L; sulfides < 2.0 mg/L as a maximum); these values are well below the oxygen demand of the tested solutions and are too low to have caused significant interferences.

In order to quantify the effect of metals on the test procedures, three heavy metals, cadmium, mercury and lead, were tested. Figure 2 shows the interference with $BOD_5$ for increments of metals added. The amount by which $BOD_5$ is diminished by the metal is expressed as a fraction:

$$f = BOD_5 \text{ with metal}/BOD_5 \text{ of sewage alone} \qquad [7]$$

In order to normalize the metal concentration it is expressed as mg/L $CaCO_3$ eq. (divide by 50 for meq/L). The $BOD_5$ determinations were made by the classic Standard Methods bottle BOD technique.

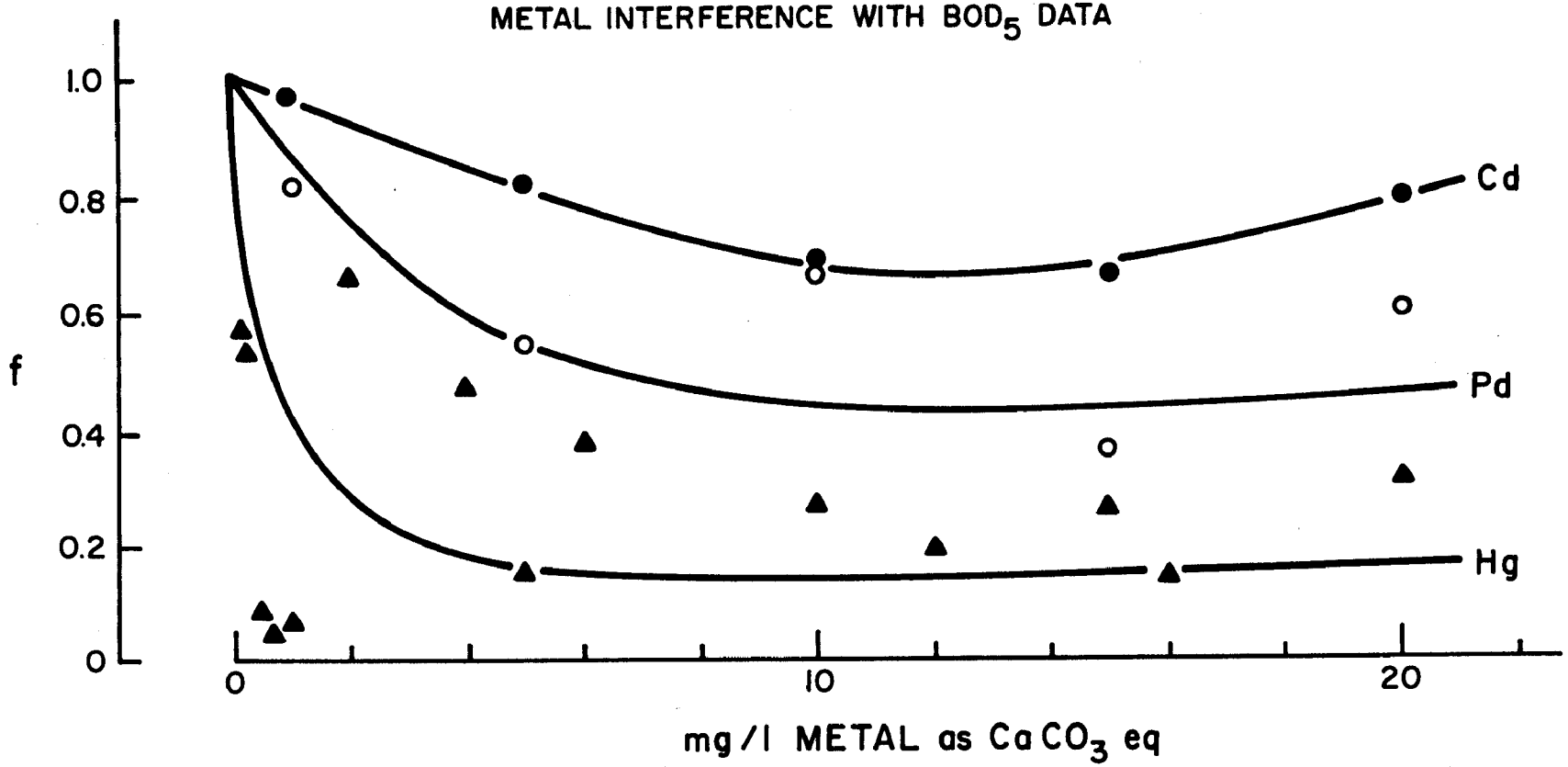
a. Cadmium - The interference from Cadmium is marginal although present and seems to reverse itself at higher concentrations.

b. Lead - The interference from lead is pronounced and has high scatter.

c. Mercury - The interference from mercury is the most pronounced of the three metals, also with scatter.

FIGURE 2

METAL INTERFERENCE WITH $BOD_5$ DATA



For inhibitory industrial wastes containing metals or toxic organics, the calculated R.I. values will be negative, indicating a problem. Quantitatively, this can be expressed as a B.I.V. number. Specific analysis would then be necessary to determine the interfering agent. The test procedure detects the presence of inhibitory materials.

The Appendix includes a set of curves for the Warburg Respirometer BOD determinations for these metals.

2. Limitations

The limitations of the R.I. evaluation are discussed here in terms of its purpose and applicability. The purpose of the R.I. is to determine if an organic compound or a waste is biodegradable. The organic compound or waste is subjected to bio-oxidation by sewage microorganisms. The total amount of oxygen used in aerobic biodegradation ($BOD_u$) is measured by means of a Warburg Respirometer and is compared to the total amount of oxygen that would have been used had all of the compound been oxidized to the natural end products of complete aerobic biodegradation (UOD). The UOD can be measured by using TOD and TN or it can be calculated from the compound formula and quantity added to the sewage (if known). The R.I. is the ratio of $BOD_u$ to UOD.

a. UOD - The UOD determines the "strength" of the waste. For a pure organic compound, the UOD can be calculated. For industrial wastes, the "strength" depends on what compounds are present and in what concentrations. Since this specific information is not always known, it is determined by use of the TOD and TN measurements. For these evaluations to be accurate, the waste must have a relatively high TOD value; TOD loses its sensitivity below 10 mg/L $O_2$. All of the work done in this research was performed in the 200 to 300 mg/L $O_2$ range, the range of highest precision.

The TOD parameter cannot be used on wastes containing high levels of certain oxidizable inorganic substances. Ammonia, the exception, is measured as part of the TN determination. At the high temperatures involved, certain inorganics exhibit oxygen demand or release oxygen, casting doubt on the TOD evaluation. In this category are included compounds that contain sulfur, nitrate, nitrite and wastes containing metals in high concentrations. In some cases, corrections can be made if detailed analyses of the wastewater are done. In the tests conducted in this campaign, these interferences were minimal.

b. $BOD_u$ - The $BOD_u$ is determined by use of a Warburg Respirometer. This instrument uses a manometer to measure partial pressure changes due to the removal of oxygen from the atmosphere of the flask. It is assumed that the only gas exchanged and measured is $O_2$ and that any $CO_2$ evolved from the biological reaction is converted to the carbonate-bicarbonate system either in the test solution or the alkali solution in the center well which contains KOH. Since the pressure is measured,

wastes exhibiting significant volatilization, like chloroform, cannot be evaluated for R.I. by use of the manometric technique. A bottle BOD series is more appropriate for these.

The Warburg $BOD_u$ is assumed to represent as complete a biodegradation response as is possible for the specific compound being tested. Optimum conditions are provided for nutrients, pH, temperature and oxygen tension. It should be noted, however, that not all possible conditions are tested for. Some compounds, like cyanuric acid, are more susceptible to anaerobic biodegradation. Microorganisms not found in sewage that exist in nature may be able to degrade the compound. Perhaps an acclimated population would be best as in the case of CMC. For the purposes of waste treatment, however, "special" conditions and "special" bacterial populations are hard to maintain as they are easily imbalanced by variations of industrial wastes in treatment plants.

One major disadvantage of the Warburg $BOD_u$ test is time. Because of the nature of the biodegradation process, and to assure as complete a reaction as possible, the $BOD_u$ test is extended to 30 days, more when the curve is not on a plateau. When results from this test should be verified by retesting, the time element becomes an increasing limitation. Correlations are provided in the next section between quick-time measurements, TOD, TN and TOC and the naturally slow biochemical oxygen demand tests.

It was mentioned earlier that wastes tested must be of high UOD classification for the purposes of TOD analysis. This also holds for $BOD_u$. The combined response of the compound plus sewage must be significantly above that of sewage alone. The $BOD_u$ has an error associated with it of as much as 11.5%. One way to insure the desired response is to put more waste with the sewage, but then the size of the Warburg reaction flask becomes a limiting factor. The total volume of liquid in the flask should not exceed about 30 ml for 125 ml flasks.

These limitations, however, do not reduce the usefulness of the R.I. to evaluate the biodegradability of organic wastes and compounds, provided the limitations are known and understood. Inasmuch as these limitations would exclude organic wastes exhibiting low oxygen demand (UOD) and compounds exhibiting interference and high volatilization, all other waterborne organic wastes, the vast majority, can be tested for R.I. with a high degree of confidence.

### 3. Correlations

Correlations between bioassay measurement techniques, such as the classic slow time BOD tests, and the real-time (quick determination) of oxygen demand, TOD, or measure of organics, TOC and TOrganicN, as well as oxidizible nitrogen, TOxN, for ammonia and organic nitrogen can supply useful information for operational management of wastewaters, but must be carefully considered.

The technical paper "Quick-Time Instrumental Measurements of Organic Characteristics" provides the derivation of correlation equations along with the rationale and test procedures to arrive at the model equations shown in Table 8. (18).

The correlation coefficients (r) demonstrate the reliance on the TN determination in this work for estimating the organic content and oxygen demand of wastewater along with the TOD value. TOC, while an effective measure of carbonaceous organics, is not complete in determining an organic response to oxidation.

It must be cautioned and emphasized, however, that all correlations, like crude analogies, are fraught with danger of hidden "lurking" variables (e.g., the presence of refractory organics and/or inhibitors) and should be used with great care in evaluating variable and industrial wastes. In many cases, it is more appropriate to use the complete (total and gross) measurements such as TOD, TN and TOC rather than the indirect, incomplete biochemical response measurement such as $BOD_5$.

TABLE 8

MODELS FOR QUICK-TIME OXYGEN DEMAND MEASUREMENTS

OF RAW SEWAGE

| Model | Relationship Equation | r |
|---|---|---|
| I | $BOD_u = 0.90\ (TOD + TN) - 32$ | 0.98 |
| II | $BOD_5 = 0.51\ (TOD - TN/2) + 67$ | 0.97 |
| III | $BOD_{uc} = 0.48\ (TOD - TN/2) + 151$ | 0.57 |
| IV | $BOD_5 = 0.58\ (TOC) + 106$ | 0.74 |

4. Correspondence of R.I. Value to Activated Sludge Treatment for Selected Compounds

In order to evaluate the appropriateness of the R.I. value to sewage treatment, three compounds were tested in a model activated sludge unit.

a. Experimental Procedure

The treatment system used in this experiment has the following design characteristics (42):

| | |
|---|---|
| Aeration Detention Time | 6 hours |
| Sludge Recycle Rate | 45% |

| | |
|---|---|
| Primary Settling Detention | 90 minutes |
| Secondary Settling Detention | 105 minutes |
| Secondary Settling Overflow Rate | 21,000 liters/day/$m^2$ |

Wastewater was pumped to this system at a constant rate (76/liters/day) from a 60-liter holding tank. A previous study of this laboratory scale treatment system showed TOD reduction in the range of 60 to 88% with an average TOD reduction of 75%. An outline of the experimental procedure is given below:

(1) The system is fed with domestic sewage for at least two hours. Normal operation of this plant is to feed domestic sewage during the day and rest the plant during evenings. The procedure of feeding raw sewage for at least two hours prior to the experiment is to obtain sufficient background data.

(2) A mixture of domestic sewage and chemical solution (50/50 percent by volume) is fed to the system at a total strength (TOD) approximately equal to the raw sewage. This precaution is followed to prevent an organic overload on the biological processes. It should also be noted that this mixture is similar to the solution used for the Warburg BOD analysis.

(3) TOD analyses are conducted on the secondary effluent throughout the test to see if the TOD reduction is changed. An increase of TOD in the effluent is interpreted as a negative process response to the biological processes. No significant change in the TOD effluent is interpreted as a positive degradation response. Gas chromatography analyses of the effluent were performed to determine if the added compound passes through the biological process.

It should be noted that a thorough evaluation of the treatment response phenomena for these chemicals would involve a number of runs and statistical analyses of the results to verify that the biological treatment variables, such as sludge age, settling characteristic of floc, and variation in removal capabilities, are independent of the final conclusion. Such a procedure was not followed in this case because the data collected are only intended to give a general concurrence with the R.I. values.

b. Glucose

Table 9 lists the secondary effluent TOD concentrations from time zero (when glucose and raw sewage mixtures are first introduced) until the holding tank contents are exhausted. The raw glucose/sewage mixture has a TOD of 189 mg/L. The data show no pronounced change in TOD reduction for the glucose/sewage mixture as compared to the sewage alone and, therefore, indicate a removal of glucose in the biological process. Because glucose is classified in the very high R.I. range, the activated sludge treatment response and the R.I. evaluation are in concurrence.

TABLE 9

TOD RESPONSE FOR D-GLUCOSE/SEWAGE EFFLUENT

| Time, Hours | Secondary Effluent TOD (mg/L as $O_2$) | % TOD Removal |
|---|---|---|
| 0 | 73 | 61 |
| 2 | 87 | 54 |
| 5 | 96 | 49 |
| 6 | 67 | 65 |
| 7 | 91 | 52 |
| 8 | 76 | 68 |
| 9 | 70 | 63 |
| 11 | 58 | 69 |
| 12 | 70 | 63 |
| 14 | 67 | 65 |

c. Lysine

Table 10 lists the secondary effluent TOD concentration from the time of the lysine/sewage mixture injection to completion of the run. The raw lysine/sewage solution has a TOD of 208 mg/L. The data show a gradual decrease in the percent TOD reduction. Such a treatment response concurs with the low R.I. characteristic of L-Lysine. The average R.I. value of lysine is 0.54. This index value indicates a lower degradability potential than domestic raw sewage (R.I. = 0.7). The increase of TOD after biological treatment, therefore, is judged to be due to a decrease in biodegradation for the lysine/sewage mixture as compared with the raw sewage alone.

TABLE 10

TOD RESPONSE FOR L-LYSINE/SEWAGE EFFLUENT

| Time, Hours | Secondary Effluent TOD (mg/L as $O_2$) | % TOD Removal |
|---|---|---|
| 0 | 105 | 50 |
| 2.5 | 106 | 50 |
| 4.5 | 99 | 52 |
| 6.5 | 111 | 47 |
| 7.5 | 99 | 52 |
| 9.5 | 128 | 38 |
| 10.5 | 157 | 25 |
| 11.5 | 144 | 31 |
| 12.5 | 161 | 23 |

d. p-Chlorophenol

Table 11 lists the secondary effluent TOD concentration from time zero to completion of the run for a mixture of sewage and p-chlorophenol. The raw p-chlorophenol/sewage mixture has a TOD of 228 mg/L. Table 11 shows a reduction in TOD removal and, therefore, concurs with the negligible R.I. evaluation of p-chlorophenol. Gas chromatography analysis of the secondary effluent shows a gradual increase of p-chlorophenol from 0.0% at time zero to 57% at seven hours and 98% at twelve hours. Because p-chlorophenol is identified as refractory, the expected treatment response of this material is to pass through the process. An increase of secondary effluent TOD is judged to indicate this type of response, i.e., the presence of a refractory organic if an inhibitor of the activated sludge process.

TABLE 11

TOD RESPONSE FOR p-CHLOROPHENOL/SEWAGE EFFLUENT

| Time, Hours | Secondary Effluent TOD (mg/L as $O_2$) | % TOD Removal |
|---|---|---|
| 0 | 117 | 48 |
| 2 | 117 | 48 |
| 4 | 125 | 45 |
| 6 | 174 | 23 |
| 7 | 165 | 27 |
| 8 | 171 | 25 |
| 12 | 156 | 31 |

## SECTION 6

## CLOSING STATEMENT

This report presents quantitative evaluation techniques for determining if specific organic constituents, either singly or in combination, are resistant to biodegradation or are inhibitory to the bacterial systems used in wastewater treatment plants to process organic materials before they enter the aquatic environment.

In addition to the measurement techniques, this report presents the test results of a Refractory Index (R.I.) evaluation for over 38 waste constituents. A Biological Inhibition Value (B.I.V.) was developed and is used to assess six of the tested organic materials which were found to interfere with the biochemical tests used (long-term Warburg Respirometer BOD determinations). In addition, confirmation of Refractory Index values has been made for three specific organic materials in a model (activated sludge) bio-reactor. Correlations between the oxygen demand tests used ($BOD_5$, $BOD_u$, TOD) and other measures of organics (TOC, TN) are also presented.

Industries should be encouraged to reclaim those materials of value from their effluent including organic constituents and the water itself (36). If, however, the wastewaters are sent into municipal treatment plants, there should be assurances that the specific wastes are treatable; i.e., degradable in the bio-reactors of the treatment plant and non-interfering (non-inhibitory) with the available processes of the treatment plant. A set of suggestions for pretreatment requirements for industrial waste is included in the Appendix to the report as Item D.

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49. Fate of Organic Pesticides in the Aquatic Environment. Advances in Chemistry Series III, American Chemical Society, Washington, D. C., 1972.

# APPENDIX


Item A Derivation of UOD Measurement

B Laboratory Methods and Materials

C Warburg Respirometer Curves for Ultimate Biochemical Oxygen Demand and Refractory Index Determination

D Suggested Pretreatment Requirements for Industrial Waste Prerequisite to Discharge into Municipal Sewage Treatment Plants

ITEM A

## Derivation of UOD Measurement

### 1. TOD MEASUREMENT RESPONSE

The Total Oxygen Demand Analyzer* reports (8) the amount of oxygen needed to complete the following oxidation reactions for elements commonly found in organic materials:

$$C_{organic} + O_2 \longrightarrow CO_2 \qquad [8]$$

$$2H^+ + 1/2\ O_2 \longrightarrow H_2O \qquad [9]$$

$$N_{organic} + 1/2\ O_2 \longrightarrow NO \qquad [10]$$

$$O_{organic} \longrightarrow 1/2\ O_2 \qquad [11]$$

$$S_{organic} + 1/2\ O_2 \longrightarrow SO_2 \qquad [12]$$

Oxyanions commonly found in wastewaters undergo the following reduction reactions and therefore yield a negative TOD response (2) (8) (41).

$$NO_3^- \longrightarrow NO + O_{2(g)} \qquad [13]$$

$$NO_2^- \longrightarrow NO + 1/2\ O_2 \qquad [14]$$

$$SO_3^- \longrightarrow SO_2 + 1/2\ O_2 \qquad [15]$$

The TOD analyzer, therefore, yields a general measure directly related to the organic materials present in water, but as with all gross measurements found in environmental engineering laboratories, responses from other impurities can yield an output not related to the organic concentration. This characteristic is considered during the UOD derivation and also must be noted during recommended measurement application (14).

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* Ionics Model 225 Total Oxygen Demand Analyzer

2. TN MEASUREMENT RESPONSE

The Total Nitrogen Analyzer* reports all bounded nitrogen present in water (20) but not dinitrogen, $N_2$. This includes ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$) and organically bounded nitrogen. Because this measurement does not separate nitrogen concentrations according to the oxidation state (e.g., $NH_3$ vs. $NO_3^-$), adjustments must be made when applying the TN measure for quantifying an oxygen demand parameter. This adjustment is accomplished by deriving a Total Oxidizable Nitrogen (TOxN) expression (16) (34).

3. DERIVATION OF UOD

UOD is defined as the amount of oxygen needed for complete oxidation (end products of $CO_2$, $H_2O$ and $NO_3^-$). The following assumptions are made to generate this value:

a. Inorganic materials, such as heavy metals capable of TOD oxidation, are negligible. This is especially true for non-industrial wastewaters.

b. The TOD Measurement is a response from 100% oxidation of organic carbon (to $CO_2$), 100% oxidation of organic hydrogen (to $H_2O$) and 33.3% oxidation of organic nitrogen (to NO) (14).

c. Oxidation from organic sulfur is negligible. Average organic sulfur found in raw sewage at the University of Connecticut is 1.1 mg/L as S (31). There is little if any industrial input here.

d. Total Nitrogen (TN) data report 100% of all nitrogen materials present.

e. The expression Total Oxidizable Nitrogen (TOxN) used in this derivation defines the quantity of oxygen required to oxidize all nitrogen (organic and inorganic) to $NO_3^-$.

The general formula for Ultimate Oxygen Demand (all expressed in mg/L as $O_2$) is:

$$[UOD] = [TOD] + [1.12\ NO_3^-] + [1/2\ NO_2^-] + 2/3\ [TOxN] \qquad [16]$$

1.12 $NO_3^-$ is added to this expression because it is reduced to $NO_{0.75}$ during high temperature TOD combustion, yielding additional 1.12 $O_2$. This was experimentally determined. The $NO_2^-$ also reduces to NO yielding 1/2 $O_2$. These quantities, therefore, are added to the TOD value to arrive at a true expression of oxidizable materials present. The resulting (TOD + 1.12 $NO_3^-$ + 1/2 $NO_2^-$) expression represents carbonaceous oxygen demand plus 1/3 nitrogen oxygen demand. Because the UOD value also includes nitrogen oxygen demand, two-thirds of the remaining oxidizable nitrogen is added (2/3 TOxN).

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* Infratronics Model Dohrmann MCTS-10 Nitrogen Analyzer

TOxN can be expressed as:

$$TOxN = 3/2\ (\text{Organic N} + NH_3) + 1/2\ NO_2^- \qquad [17]$$

The 3/2 (N organic + $NH_3$) + 1/2 $NO_2^-$ expression respresents the quantity of oxygen required to completely oxidize all nitrogen forms to $NO_3^-$. Noting that total nitrogen equals organic nitrogen plus ammonia plus nitrate plus nitrite (TN = N organic + $NH_3$ + ($NO_3^-$ + $NO_2^-$ from total nitrogen (TN)). The total oxidizable nitrogen value, therefore, is derived as:

$$TOxN = 3/2\ (TN - NO_3^- - NO_2^-) + 1/2\ NO_2^- \qquad [18]$$

which simplifies to:

$$TOxN = 3/2\ TN - 3/2\ NO_3^- - NO_2^- \qquad [19]$$

Referring back to expression [10], UOD is now expressed as:

$$UOD = TOD + 1.12\ NO_3^- + 1/2\ NO_2^- + 2/3\ [3/2\ TN - 3/2\ NO_3^- - NO_2^-] \qquad [20]$$

which simplifies to:

$$UOD = TOD + TN + 0.12\ NO_3^- - 1/6\ NO_2^- \qquad [21]$$

Average nitrate values for raw sewage at the University of Connecticut were found to be 2.4 mg/L as N, while nitrite values were undetected (31). The raw sewage entering the University of Connecticut Sewage Treatment Plant is usually aerobic and little opportunity of denitrification ($NO_3^- \rightarrow N_2$) occurs. Therefore, assuming the quantities 0.12 $NO_3^-$ and 1/6 $NO_2^-$ are negligible, a final expression for Ultimate Oxygen Demand is given as:

$$UOD = TOD + TN \text{ as mg/L as } O_2 \qquad [22]$$

## 4. LABORATORY PROCEDURE FOR UOD

A complete step-by-step procedure for determining the UOD of a water sample is presented in the laboratory manual section of this report. A general outline of the steps needed to determine UOD is presented below:

a. To evaluate the TOD concentration:

1. Calibrate TOD Analyzer with standard solutions of potassium biphthalate.

2. Prepare sample (dilute for proper range, homogenize sample).

3. Determine at least three to ten TOD values and use average for TOD concentration expressed in mg/L as $O_2$.

b. To evaluate the TN concentration:

1. Calibrate TN Analyzer with standard solutions of ammonia chloride, potassium nitrate or urea.

2. Prepare sample (homogenize).

3. Determine at least three to ten TN values and use average for TN concentration expressed in mg/L as N.

4. Change TN expression from mg/L as N to mg/L as $O_2$ by the factor:

$$\text{TN mg/L as } O_2 = \text{TN mg/L as N} \times \frac{\frac{32\text{g}}{\text{g-mole } O_2}}{\frac{14\text{g}}{\text{g-mole}}\ \text{N}}$$

The UOD value is found by adding TOD and TN

$$\text{UOD} = \text{TOD}_x + \text{TN (all expressed as } O_2) \qquad [23]$$

## 5. LABORATORY PROCEDURE FOR $BOD_u$ MEASUREMENT

Ultimate Biochemical Oxygen Demand ($BOD_u$) determines the amount of oxygen required by a mixed and heterogeneous community of bacteria as found in domestic sewage under the following conditions (15):

- Aerobic environment.
- 20°C.
- Neutral pH (approximately 7).

- $NH_3$ nutrient concentration at an approximate level where $BOD_5/N$ equals 100/5. ($BOD_5$ - mg/L as $O_2$; N - mg/L as N).

- $PO_4^{\equiv}$ nutrient concentration at a sewage to buffer ratio as recommended by Standard Methods for the dilution technique (44).

- Trace nutrients supplied from raw sewage.

- Starting microorganism population from domestic sewage.

This test is designed to simulate the degradation process expected to occur if the waste material in question is exposed to a municipal, aerobic biological treatment system or a separate industrial waste biological treatment system using domestic sewage as the seed material. Presented below is a discussion on the standard test conditions.

a. Temperature.

A 20°C temperature is chosen to remain consistent with standard $BOD_5$ test and to yield a conservative evaluation of biodegradability. Higher temperatures (to a limit of about 35 to 40°C) are expected to accelerate the biological process (19) and, therefore, present an ecosystem unrelated to the outside environment. Lower temperatures, on the other hand, slow down the biodegradation process and yield overly conservative results. It is expected that long-term BOD tests conducted at various ambient temperatures above freezing result in the same absolute value provided proper bacterial seed nutrients and time are given.

b. Sewage Organisms.

Raw sewage is chosen as a seed because the large diversity of organisms present provides the greatest possible starting point to develop a microscopic ecosystem for utilizing the material being studied. Also, the biosystem resulting from an exposure of raw sewage organisms and test material during aerobic conditions for an extended period is expected to parallel a municipal biological treatment biosystem. Refractory Index data, therefore, are judged appropriate as indicators of biotreatment ability.

c. Acclimation Time.

Normal biodegradability or treatability studies, as conducted by an environmental engineer, are started by acclimating an activated sludge to the waste material being studied. If successful acclimation is attained, the appropriate treatment coefficients for uptake rates, removal rates, sludge production and sludge characteristics are found to determine if the material is suitable for conventional biological treatment (4)(7)(10)(16)(27)(32)(47).

The purpose of the $BOD_u$ measurement, however, is not to derive treatment coefficient parameters but is to evaluate the feasibility of degrading the material through biological reaction. The period required to degrade this material, is, of course, the primary concern for both industrial and municipal aerobic biological treatment and natural stream and lake purification reactions. The acclimation time, therefore, is included to yield standardized results.

Acclimation studies have been demonstrated in laboratory and even occasionally in treatment plants such as the activated sludge type. However, industrial plants are also subject to bulking, toxic inputs and wide variation in flow and concentrated input, and therefore have difficulty in maintaining a reliable acclimated bacterial population.

d. Nutrients.

To assure that a proper nutrient supply is available for development of the microorganism populations, ammonia chloride, phosphate buffer and domestic sewage solutions are added to the standard BOD test solution.

$BOD_u$ is found by subtracting oxygen uptake values of the blank solution (raw sewage, ammonia chloride, phosphate buffer) from the test solution (raw sewage, ammonia chloride, phosphate buffer and test material). Nitrate readings of these solutions are taken at the completion of respiration. In cases where different degrees of nitrification result, a nitrification correction term is used. The resulting general formula for $BOD_u$, therefore, is:

$$BOD_u = BOD_u' + \Delta NO_3^-$$

where: $BOD_u'$ = difference of oxygen uptake between test solution and blank solution, and $\Delta NO_3^-$ = nitrification correction term.

The nitrification correction term is not applicable to cases where nitrogen is present in the test material and the difference in nitrification between the test solution and blank solution is less than the quantity of possible nitrification from the nitrogen containing test material.

ITEM B

## Laboratory Methods and Materials

### 1. METHODS AND MATERIALS

This section presents detailed instructions for arriving at Refractory Index (R.I.) evaluations. Further details on the specific analyzers can be found in the following references:

- Microcoulometric Titrating System, Instruction Manual, Dohrmann Envirotech, Mountain View, California.

- Total Oxygen Demand Analyzer Operating Manual, Ionics Inc., Watertown, Mass.

- Orion Research, Analytical Methods Guide, Orion Research Inc., Cambridge, Massachusetts.

- Umbreit, W. W., R. H. Burris and J. F. Stouffer, Manometric Techniques, Burgess Publishing Co., Minnesota. 1964, 4th Edition (46).

### 2. PREPARATION OF SOLUTIONS

The procedure for R.I. evaluation requires preparation of TOD, $NO_3^-$, and TN standard solutions; 20% KOH for the Warburg and TOD analyzers; known (quantitative and qualitative) solution of organic compounds for the $BOD_u$ analysis; and test solution for the Warburg analysis.

Methods for preparation of these solutions follow:

a. TOD Standard Solution is made from potassium biphthalate ($KOCOC_6H_4$-2-COOH). An 850.9 mg/L solution of potassium acid phthalate yields a 1000 mg/L TOD as $O_2$ solution.

1. Dry potassium biphthalate powder in a 104° C oven overnight.

2. Cool to room temperature in a desiccator.

3. Weigh chemical on balance to 0.1 mg.

4. Calculate TOD of solution by proportion, e.g., if weighed chemical is 90 mg and this is diluted to 1000 ml, the TOD (900 mg/L potassium biphthalate) is:

$$900 \times \frac{1000}{850.9} = 1050 \text{ mg/L TOD as } O_2$$

5. Dilutions of this standard are made to arrive at a series of TOD standards within the anlayzer operating range; e.g., 200 mg/L, 100 mg/L, 50 mg/L and 20 mg/L as $O_2$ solutions.

6. Cover standards and store at $4^oC$.

b. Nitrate ($NO_3^-$) Standard Solution is made from potassium nitrate ($KNO_3$).

1. Follow the same drying procedure as described above.

2. Prepare a 0.1 Molar solution of $KNO_3$ (10, 100 mg/L).

3. From this standard solution prepare $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$ Molar solutions of $KNO_3$.

4. Adjust pH of standard solution to correspond with the pH of samples to be analyzed (pH = 4).

5. Cover standards and store at $4^oC$.

c. Total Nitrogen (TN) Standard Solutions are made from ammonia chloride ($NH_4Cl$), potassium nitrate ($KNO_3$) and urea [$(NH_2)_2CO$].

1. Follow the same drying procedure as described above.

2. Prepare standard solutions of the compound and express in mg/L of nitrogen. For example, a 100 mg/L solution of urea equals:

$$100 \text{ mg/L Urea} \times \frac{2 \text{ moles N}}{1 \text{ mole Urea}} \times \frac{14\text{g N/Mole N}}{60\text{g Urea/mole Urea}} = 47 \text{ mg/L N as N}$$

3. Cover and store standards at $4^oC$.

d. 20% KOH solution is made by dissolving 236 grams potassium hydroxide (KOH) into one liter of solution.

1. A drying procedure is not required because the solution to be made does not fall into a highly precise range (percent is parts per hundred).

2. Add 236 grams KOH to a liter volumetric flask (Pyrex).

3. Fill the flask with distilled water. Pour cold water on the flask during this step to avoid excess heating.

4. Store KOH in a plastic container. Avoid contact with skin and eyes.

e. Organic compound solutions for $BOD_u$ test fall into four classifications: water soluble, nonwater soluble and compounds that may be chemically or physically affected at 104°C. To make known solutions of these organic materials, the following rules are followed:

1. For compounds that are not affected at a 104°C temperature, follow the same drying procedure as discussed above.

2. For compounds that may be affected at a 104°C temperature (i.e., amino acids), dry in a vacuum desiccator overnight.

3. Water soluble compounds are dissolved in distilled water, covered and stored at 4°C.

4. In some cases, nonwater soluble compounds (e.g., biphenyl) are dissolved in acetone or another suitable solvent, placed in a volumetric flask, covered and stored at 4°C. After a measured volume of this solution is placed in a Warburg flask, the acetone is volatized away by placing the flask in a vacuum desiccator.

In another case the material was added as a dispersion in the best manner possible, but while in this condition, only a fraction entered true solution. Materials like DDT despite limited solubility showed an inhibitory response.

f. Phosphate buffer solution is prepared as follows:

Dissolve 8.5 grams potassium dihydrogen phosphate ($KH_2PO_4$), 21.75 grams dipotassium hydrogen phosphate ($K_2HPO_4$) and 33.4 grams disodium hydrogen phosphate hepahydrate ($Na_2HPO_4 \cdot 7H_2O$) into one liter of solution.

## 3. OPERATION OF TOD/TOC ANALYZER

This section describes routine operating procedure for the Ionics Model 225 Total Oxygen Demand (TOD) and Total Organic Carbon (TOC) Analyzer.

a. Standardization.

1. Prepare standard solutions of potassium acid phthalate as described above.

2. Scrub the standard solution with nitrogen gas to remove dissolved carbon dioxide. This procedure is followed to remove inorganic carbon. The carbon analyzer, therefore, will report organic carbon.

3. Conduct TOD and TOC analyses on the standard solutions. TOC (mg/L as C) values of the standard potassium biphthalate solution are equal to 0.4 times the TOD (mg/L as $O_2$) concentration. Normal calibration procedure is to analyze three or more standards with between three to ten

TOD/TOC values conducted per standard.

4. Compute the TOD and TOC factors (Factor = Actual TOD/Scale reading) and graph the TOD and TOC factor vs. the scale reading.

b. Sample Analysis.

1. Homogenize samples with particulate matter (e.g., raw sewage) in a Waring high speed blender.

2. Acidify and scrub sample as described in the above section.

3. Dilute the sample to fall within the TOD/TOC scale readings.

4. The TOD and TOC values are determined by averaging three to ten outputs.

4. OPERATION OF TN ANALYZER

This section describes routine operating procedure for the Dohrmann MCTS-10 Nitrogen System analyzer.

a. Standardization.

1. Prepare a TN standard solution as described in Section A.

2. Inject standard solutions at the expected unknown sample TN concentraiton range. At least three injections are needed.

3. Calculate the TN recovery factor by the formula:

$$\% \text{ Recovery} = \frac{1.74 \text{ (counts) } (100\%)}{(1\mu L) \text{ (mg/L N) (Range - ohms)}}$$

$$\text{Factor} = \frac{(1.74)}{\% \text{ R (Range-ohms)}} \times \text{TN standard} \qquad [24]$$

b. TN Analysis.

1. The TN (mg/L as N) values for unknown standards are determined by the formula:

$$\text{TN} = \frac{\text{Counts} \times (1.74)}{(\mu L \text{ Sample Volume}) \text{ (Range-ohms)}} \times \text{Factor} \qquad [25]$$

## 5. OPERATION OF NITRATE PROBE

This section describes routine operation of the Orion Nitrate probe.

a. Standardization.

1. Prepare series of $10^{-1}$ to $10^{-5}$ Molar concentration of potassium nitrate as described in Section 2b above.

2. Determine the electrode potential (+mv) direct readout for each standard, and graph electrode potential vs. Molar concentration on a 5 cycle semi-log paper. (Molar concentration on the log scale and electrode potential output on the arithmetic scale.) A straight line is constructed from this data.

b. $NO_3^-$ Analysis.

1. Molar concentrations of nitrate are determined by reading the electrode potential and using the standard graph.

2. mg/L $NO_3^-$ as N values are determined by multiplying the Molar concentration by the molecular weight of nitrogen (14g).

## 6. OPERATION OF WARBURG APPARATUS

This section describes routine operating procedure for the Warburg apparatus. Details on the $BOD_u$ test procedure are given below:

a. Cleaning of Warburg Flasks.

1. Normal cleaning procedure:

- remove grease with paper towel.
- pour concentrated HCl (technical grade) to about 1/3 of flask and swirl.
- transfer HCl to another flask.
- rinse at least five times with tap water and final rinse with distilled water.

2. Special cleaning procedure (to be followed after about three to four runs -- depending on condition of glassware):

- rinse flask with tap water to remove test solutions and KOH.
- soak flask overnight in Chromerage (made from concentrated technical grade $H_2SO_4$ and Chromerage).

- remove flask from Chromerage and rinse at least five times with tap water followed by a final rinsing with distilled water.

b. Cleaning of Warburg manometers.

1. Normal cleaning procedure:

- remove manometer from support.
- remove manometer fluid and fluid reservoir.
- remove grease with paper towel.
- rinse manometer with tap water by hooking rubber pressure line to manometer opening.
- dry in oven (104$^{o}$C).
- install fluid reservoir so fluid rises to about the 5 cm mark with no adjustment.

2. Special cleaning procedure (to be followed after three to four runs -- depending on condition of glassware):

- remove manometer from support.
- remove manometer fluid and fluid reservoir.
- rinse residual manometer fluid.
- soak manometer overnight in Chromerage as above described.
- remove from Chromerage and rinse manometer with tap water.
- final rinse with distilled water.
- dry in oven (104$^{o}$C).
- install manometer fluid reservoir as described above.

c. Preparation of Warburg Flasks for $BOD_u$ Test.

- place absorbent filter paper into center KOH well (paper is shaped as accordian).
- place light coating of grease to outside edge of KOH well (this procedure is followed to prevent KOH from splashing out to test solution).

- add 0.5 ml of 20% KOH to center well.

- seal top of flask with cork.

d. Attaching manometer and flask to Warburg apparatus.

- place anhydrous lanolin on fitted manometer joint and flask side-arm joint.

- attach springs to side-arm.

- add test solution to Warburg flask and attach to manometer joint.

- attach springs to flask and manometer attachment.

- place flask into water bath and allow temperature inside flask to reach water temperature before closing manometer stopcock.

7. $NH_3$ DETERMINATION

$NH_3$ is determined by the Kjeldahl distillation method. Further reference on this method can be found in Standard Methods. A step-by-step procedure is given below:*

a. Add 50 ml sewage, 250 ml distilled water and 25 ml buffer solution to an 800 ml Kjeldahl flask. Also prepare a blank solution by omitting the sewage.

b. Distill this solution into a 100 ml Boric Acid solution until the 300 ml mark is reached.

c. Titrate 0.02 $NH_2SO_4$ into the Boric Acid solution to the original pH.

d. Calculate the ammonia concentration by the formula:

$$NH_3 = \frac{280\ (V_s - V_B)}{50} \quad \text{mg/L as N} \qquad [26]$$

$V_s$ = titration of sample (ml)

$V_B$ = titration of blank (ml)

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*An alternate method for ammonia determination is by means of ion selective probes. These need to be calibrated frequently but are free of interferences and offer quick-time analysis.

## 8. $BOD_u$ TEST PROCEDURE

This section presents step-by-step analytical details used to arrive at the $BOD_u$ value.

a. Preparation of Test Solutions.

Preliminary information needed to prepare these solutions are TOD and TN values of the raw sewage and test compounds or wastes, plus nitrogen concentration of the raw sewage. The procedures followed for these evaluations are discussed earlier.

1. Calculate the estimated $BOD_5$ of raw sewage and the test compound using the formula:

$$BOD_5 = 0.51\ (TOD - TN/2) + 67 \qquad [27]$$

2. Calculate UOD of the test compound or waste solution and the raw sewage using the formula:

$$UOD = TOD + TN \qquad mg/L \text{ as } O_2 \qquad [23]$$

3. Determine the correct volume of test compound solution to be added to the test solution by proportioning according to UOD:
(Xml test compound) (UOD test compound) = (a ml sewage) (UOD sewage)

4. Calculate the combined estimate $BOD_5$ of the test compound plus sewage using the formula:

$$BOD_5\ [\text{combined}] = \frac{(a\ ml\ \text{sewage})\ (BOD_5\ \text{sewage}) + (b\ ml\ \text{compound}\ (BOD_5\ \text{compound})}{a\ ml + b\ ml} \qquad [28]$$

Usually 15 to 20 ml raw sewage is chosen (a = 15 ml).

5. Calculate the required volume of $NH_4Cl$ solution using the proportion:

$$(X ml\ NH_4Cl)\ (-mg/L \text{ as } N) + (\underline{a}\ ml \text{ sewage})\ (-mg/L\ NH_3 \text{ as } N \text{ sewage})$$

$$= (a\ ml + b\ ml)\ (\text{required } NH_3 \text{ concentration}) \qquad [29]$$

The required $NH_3$ concentration is calculated by using the ratio $BOD_5$ combined/N = ratio $\geq$ 100/5.

6. Phosphate buffer solutions are added at the same sewage/buffer ratio as recommended in Standard Methods:

- 1 ml buffer solution/Liter dilution water.

- 3 ml raw sewage/300 ml dilution water.

- Therefore sewage/buffer = 3/0.3x1 = 10/1.

- Because compound + sewage solution has a $BOD_5$ = 2 x $BOD_5$ sewage, use a 5/1 ratio.

- When using 20 ml sewage, add 4 ml buffer solution.

At this point, the following information should be calculated for each test solution:

- ml compound or waste required.

- ml raw sewage.

- ml $NH_4Cl$ solution required.

- ml phosphate buffer solution required.

Because one blank solution is used, all the test solutions are proportioned equally except for the ml compound or waste added. Therefore, the higher calculated values for $NH_4Cl$ and phosphate buffer solutions are used in all the solutions tested.

b. Warburg Test Start Up.

1. Prepare batch solutions (total volume approximately 200 ml) of the Warburg test solutions and Warburg blank solutions using the proportions calculated in Section 1 above.

2. Add aliquot samples of these batch solutions to the Warburg flasks so that the total volume in each flask equals the total volume calculated in Section 1 above. Each test solution is evaluated in triplicate.

3. Acidify the remaining test solution with $H_2SO_4$ to pH = 4.0.

4. Determine the nitrate probe reading for the acidified solutions.

c. Data Collection.

Manometer readings are taken according to the manometer fluid displacement. Normal manometer reading is conducted by setting the right side at 15 cm and reading the left side. During times when the manometer fluid displacement is so large that it is impossible to set the right side at 15, the following procedure is used:

1. Set right side at a point that will allow left side to be on scale.

2. Read left side.

3. Set right side at a point that is twice as far from 15 as from the previous setting (1).

4. Read left side.

5. Calculate where the fluid would be on the left side if the right side could have been set at 15 through proportions.

Other information that is collected during each reading is the time and the barometric pressure. A suggested order of data collection is as follows:

- Set manometers at 15 (right side).
- Record time of reading.
- Read barometric pressure (mm Hg).
- Read left side of manometers.
- Open manometer stopcocks.
- Adjust manometer fluid.
- Close stopcocks.

The $BOD_u$ test is conducted for at least 20 days. If a noticeable oxygen uptake is noted after 20 days, the test is continued to 30 days. After 30 days, the test is continued until oxygen uptake stops. At the completion of oxygen uptake, the Warburg flask contents are transferred to a vial and acidified to pH $\simeq$ 4 with $H_2SO_4$.

Nitrate probe readings are then taken for each sample.

d. Data Analysis.

The BOD value mg/L $O_2$ uptake is determined from the Warburg apparatus by converting the change of pressure (found from manometer readings ) to

loss of $O_2$ within the flask. A derivation of this conversion is arrived at through the following sequence:

- Calculate the ml of $O_2$/ml of air within the flask at STP (gas concentrations at standard pressure and temperature are used in the derivation to eliminate temperature corrections).

- Calculate the ml of $O_2$/ml of fluid space at STP.

- The ml of $O_2$ from time 1 to time 2 is then related to a change in Warburg manometer pressure reading.

- ml $O_2$ is then converted to mg $O_2$ per liter sewage at test conditions. Details of this derivation follow:

e. Derivation of Warburg Equations.

1. ml $O_2$/ml air space @ STP

$$PV/T = P'V'/T'$$

[prime signifies standard conditions]

$P'$ = 1 atm.

$V'$ = gas volume @ STP

$T'$ = $0^{\circ}$ C = $273^{\circ}$ K

$T$ = $20^{\circ}$ C = $293^{\circ}$ K

$V$ = volume of gas @ test condition = $V_g$

$P$ = P-R = partial pressure of gas minus water vapor pressure

$$V' = \frac{V_g \frac{273}{293} (P-R)}{1 \text{ atm.}}$$

2. ml $O_2$/ml fluid

$$\frac{\text{ml } O_2}{\text{ml fluid}} = V_f \; a \; \frac{(P \; R)}{P_o} = V''$$

$V_f$ = volume fluid

a = Bunsen coef. (ml $O_2$/ml water) at 0° C, 1 atm. partial pressure

$\frac{P-R}{P_o}$ = correction factor partial pressure of gas

3. Gas at start = V' + V''

After uptake, the gas partial pressure will decrease by h. h is measured as mm Brodi fluid. To keep consistent units $P_o$ will be expressed as mm Brodi fluid. Proper conversion is as follows:

$$1 \text{ atm} \times \frac{760 \text{ mm Hg}}{1 \text{ atm}} \times \frac{10{,}000 \text{ mm Brodi}}{760 \text{ mm Hg}} = 10{,}000 \text{ mm Brodi fluid}$$

Gas at finish:

$$V_2' = \frac{V_g \frac{273}{293} (P-R-h)}{10{,}000 \text{ mm Brodi}}$$

$$V_2'' = V_f \; a \; \frac{(P-R-h)}{10{,}000}$$

$$V = (V_1' + V_1'') - (V_2' + V_2'')$$

$$= V_g \frac{273}{293} \frac{h}{10{,}000} + V_f \; a \; \frac{h}{10{,}000}$$

$$= (V_g \frac{273}{293} + V_f \; a) \frac{h}{10{,}000}$$

For oxygen, the following conversion is used:

4. Conversion Factor for: ml $O_2$/ml sample to mg $O_2$/L sample

$pV = nRT$

$n = 1$ mole

$V = x$ (volume in liters needed for 1 mole of $O_2$ at the stated conditions)

$R = \frac{0.0821 \text{ atm}}{\text{mole } °K}$

$T = 293$ °K (20 °C)

$p = \frac{p}{760 \text{ atm}}$ (p = actual atmosphere pressure, mm Hg)

$V = nRT/p$

$$V = \text{volume of one mole} = \frac{(1 \text{ mole})\ (0.0821 \frac{\text{atm}}{\text{mole } °K})\ (293 °K)}{p/760 \text{ atm}}$$

$$= \frac{(1)\ (010821)\ (293)\ (760)}{p} \text{ (L/mole)} = \frac{18{,}282 \text{ L}}{\text{mole } p}$$

$$\frac{\text{ml } O_2}{\text{ml sample}} \times \frac{1000 \text{ ml sample}}{\text{L sample}} \times \frac{\text{L } O_2}{1000 \text{ ml } O_2} \times \frac{\text{mole (p)}}{18{,}282 \text{ L}} \times$$

$$\frac{32\text{g O}}{\text{g}\cdot\text{mole } O_2} \times \frac{1000 \text{ mg}}{1 \text{ g}} = \frac{32{,}000{,}000\ p}{18{,}282{,}000} = 1.75\ p \text{ mg } O_2$$

$$\text{mg } O_2 \text{ uptake} = \left(\frac{V_g \frac{273}{293} + V_f}{10{,}000}\right) h \times 1.75\ p \qquad [30]$$

Dividing by vol. of sample = mg/L $O_2$ uptake.

The accumulated oxygen uptake value for the test compound is determined by subtracting the blank solution uptake from the test solution uptake.

$$BOD_u' \text{ (test compound)} = BOD_u' \text{ (test solution)} - BOD_u' \text{ (blank solution)} \quad [31]$$

The nitrification correction (designated $\Delta NO_3^-$) is a term applied to the $BOD_u$ value to adjust for nitrification nonuniformities (5). A diagram of the nitrification correction technique is illustrated in Figure 1.

$$BOD_u = BOD_u' + \Delta NO_3^- \quad [32]$$

$\Delta NO_3^-$ equals the difference in nitrification occurring in raw sewage plus test solution to the nitrification that has occurred in raw sewage alone. In cases where nitrogen is present in the test material and more nitrification is noted in the solution containing this material, the $\Delta NO_3^-$ value cannot be used unless its value is larger than the total concentration of chemically bound nitrogen as $O_2$ demand in the test material. In such cases, the difference is subtracted from $BOD_u'$.

# ITEM C

Warburg Respirometer Curves for Ultimate Biochemical Oxygen Demand and Refractory Index Determination

(In Alphabetical Order)

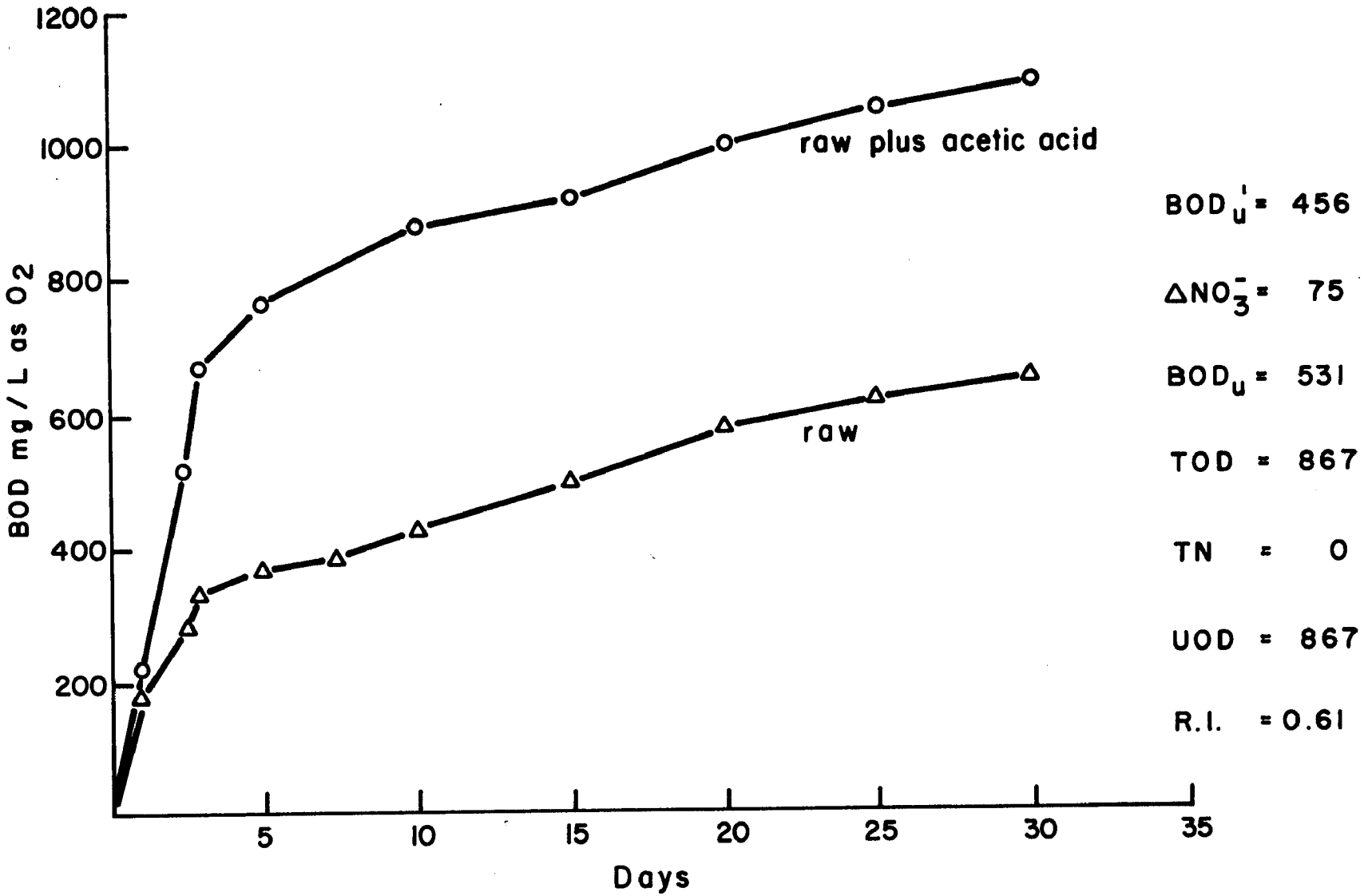



Figure C-1 OXYGEN UPTAKE FOR ACETIC ACID

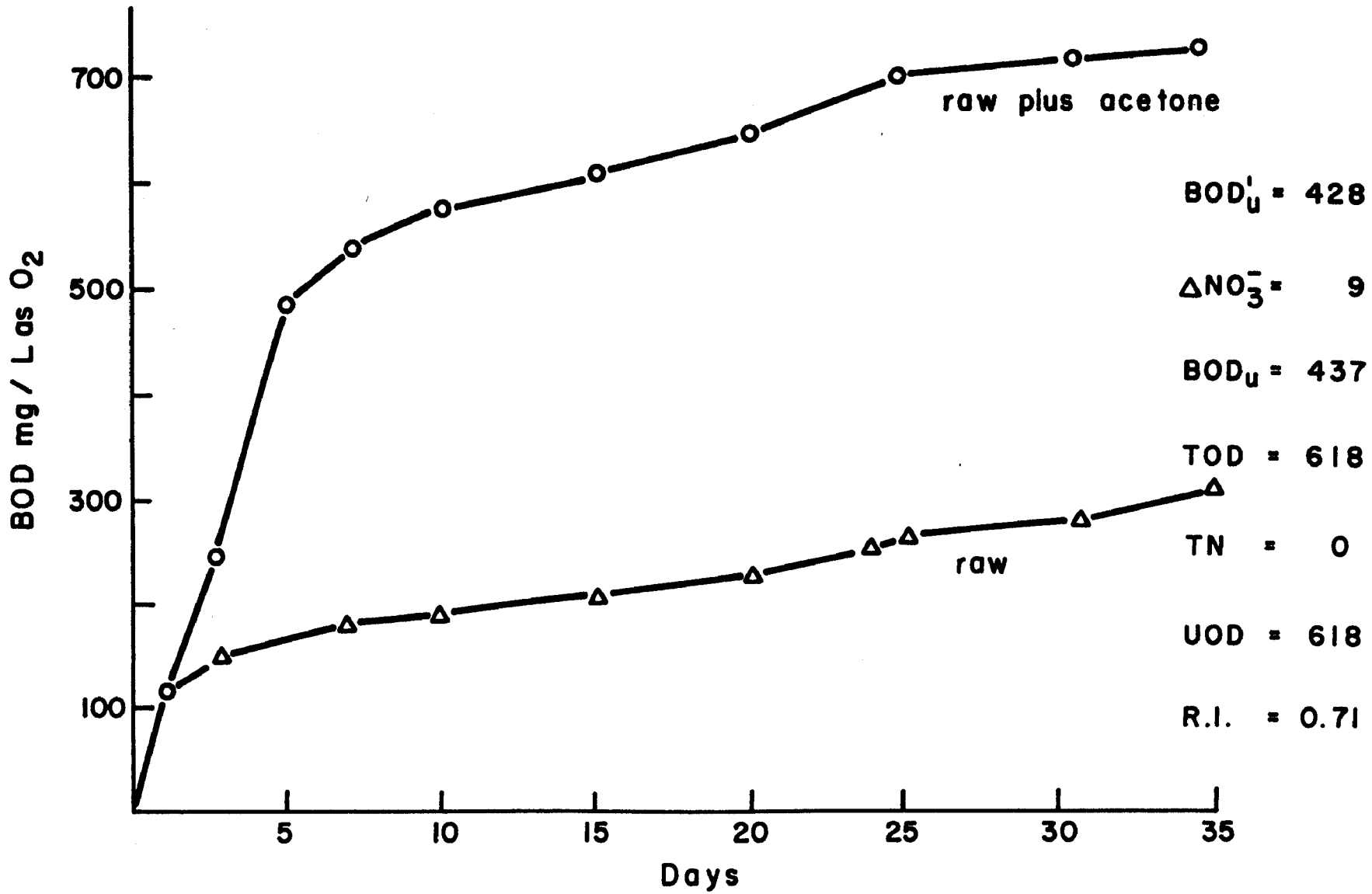



Figure C-2 OXYGEN UPTAKE FOR ACETONE

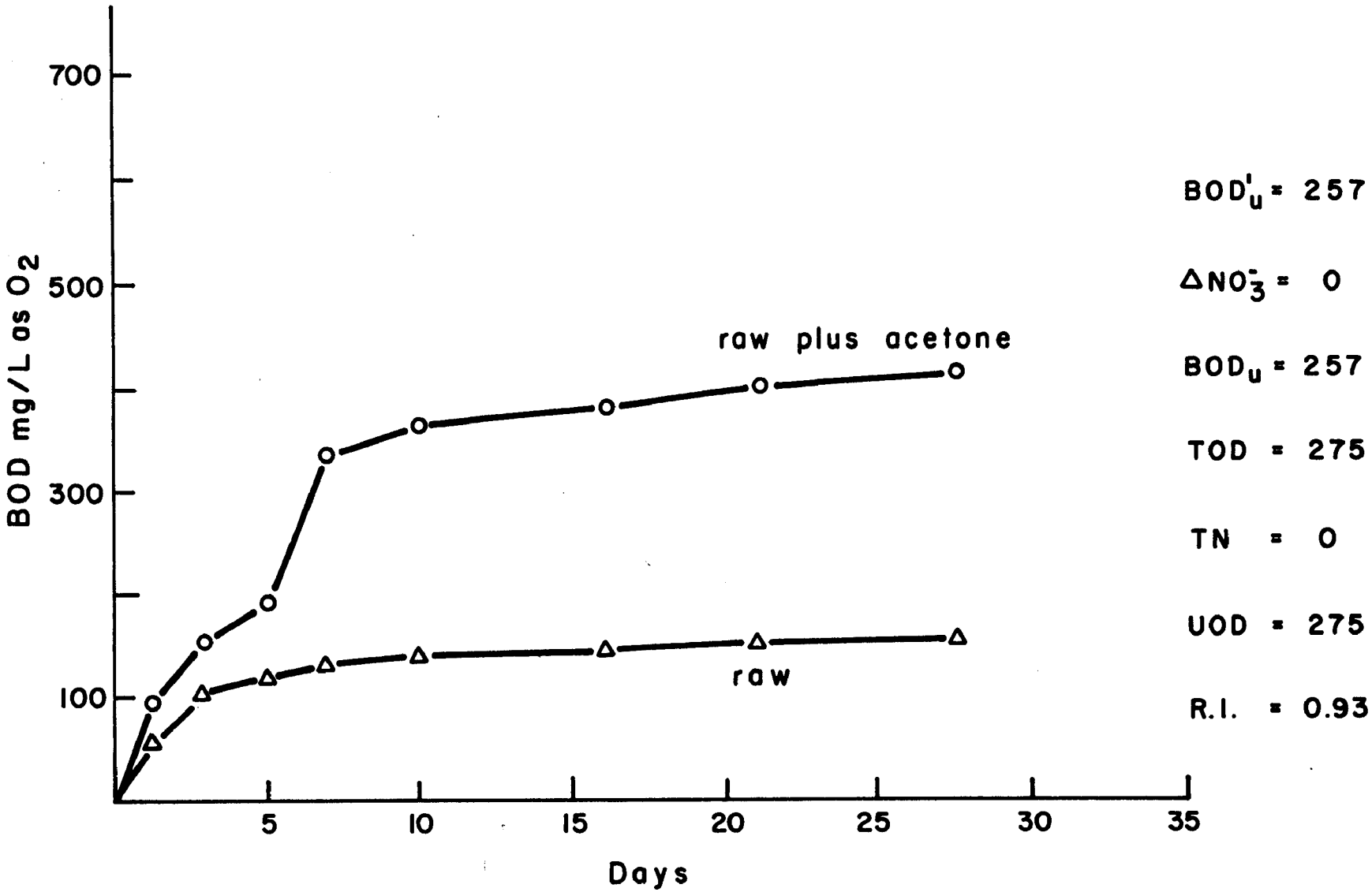



Figure C-3 OXYGEN UPTAKE FOR ACETONE

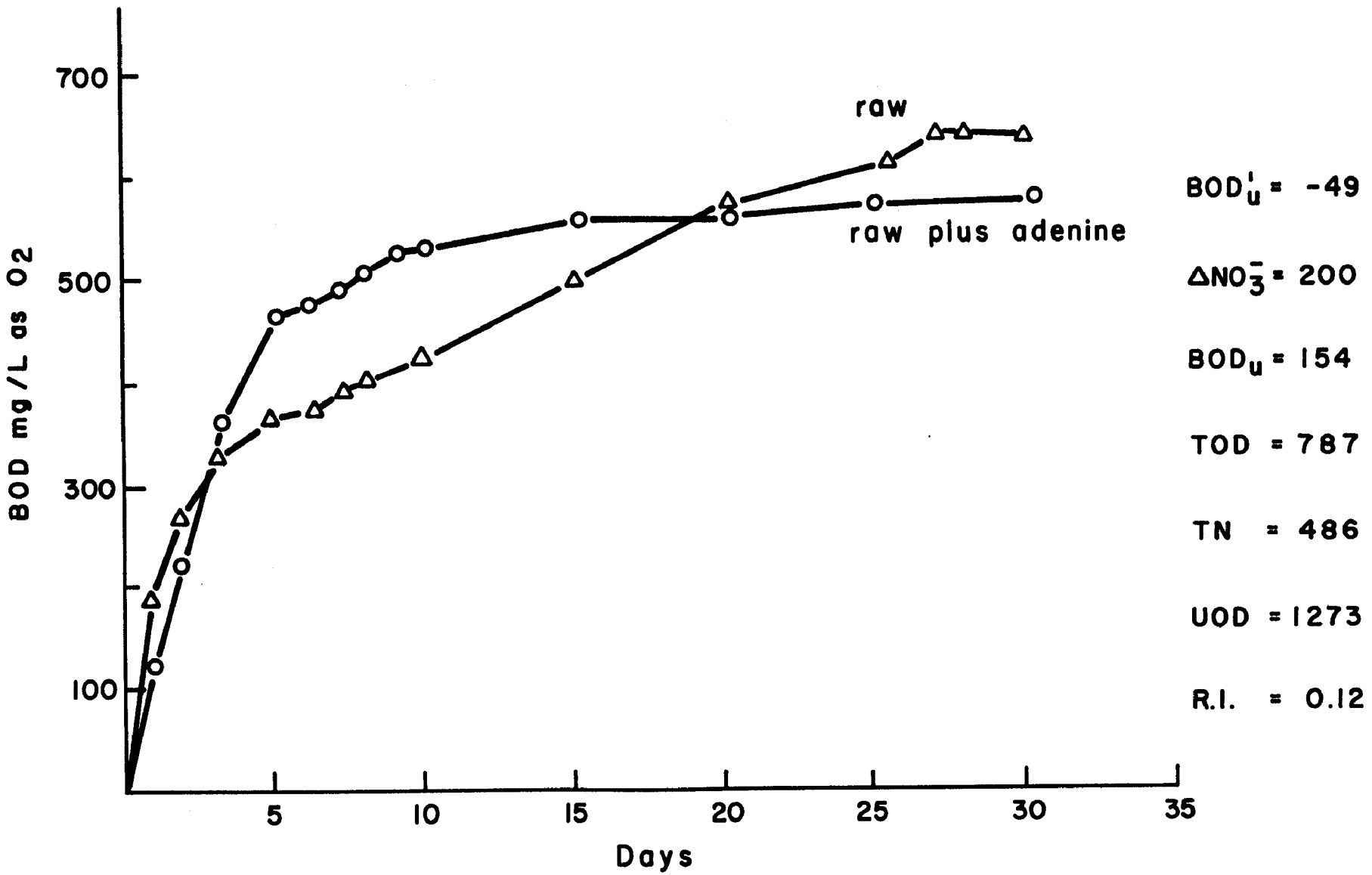



Figure C-4 OXYGEN UPTAKE FOR ADENINE

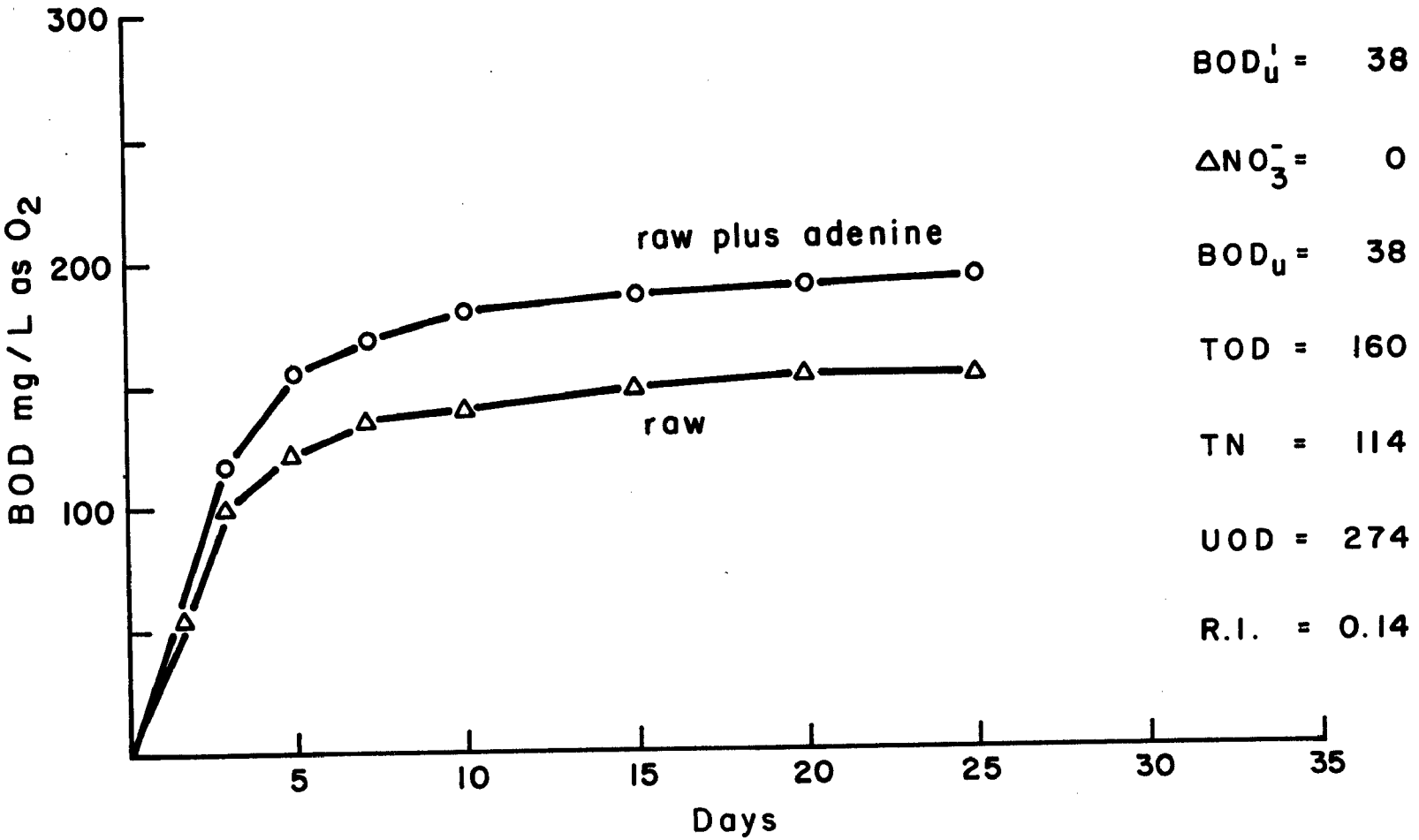



Figure C-5 OXYGEN UPTAKE FOR ADENINE

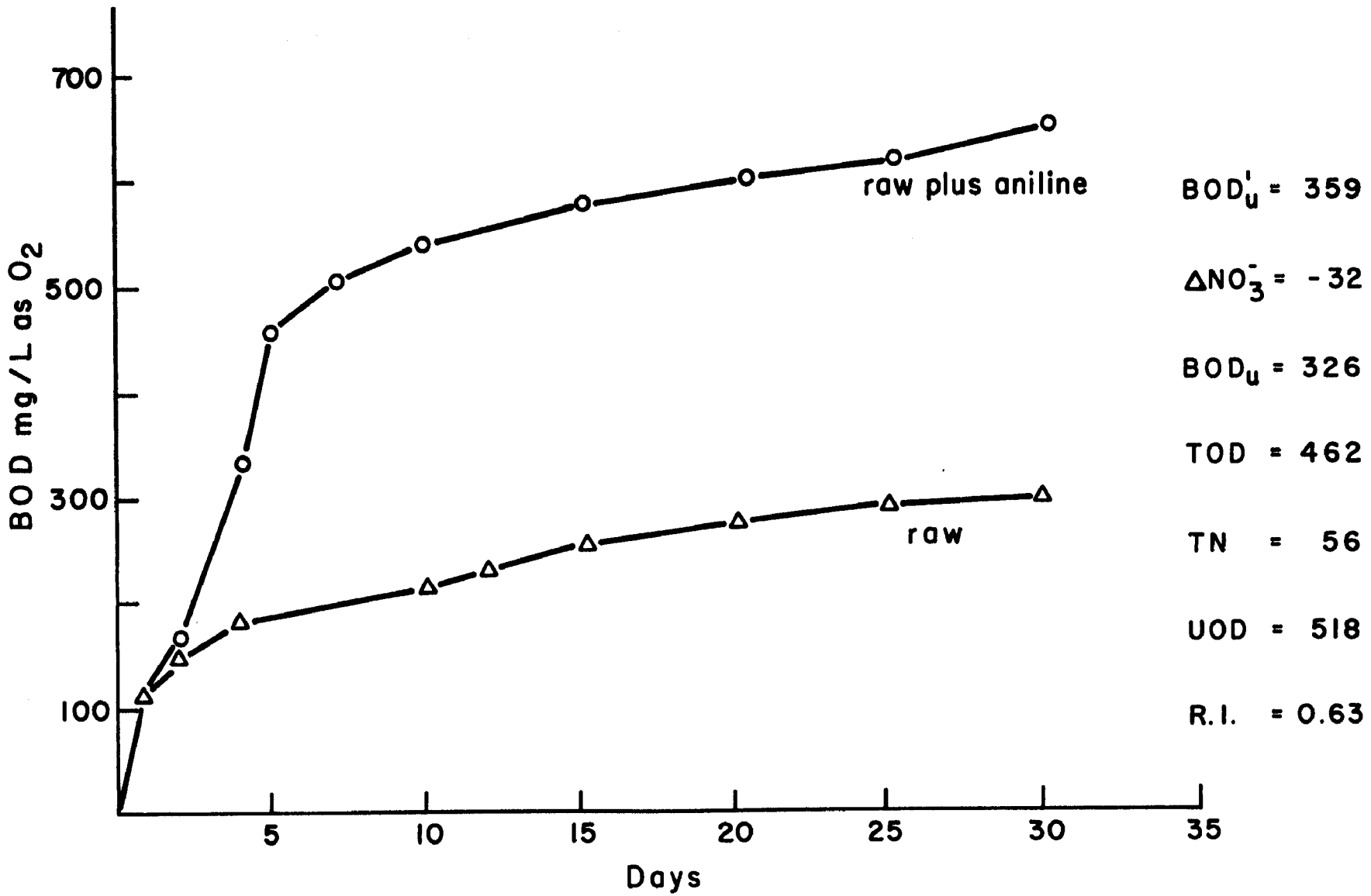



Figure C-6 OXYGEN UPTAKE FOR ANILINE

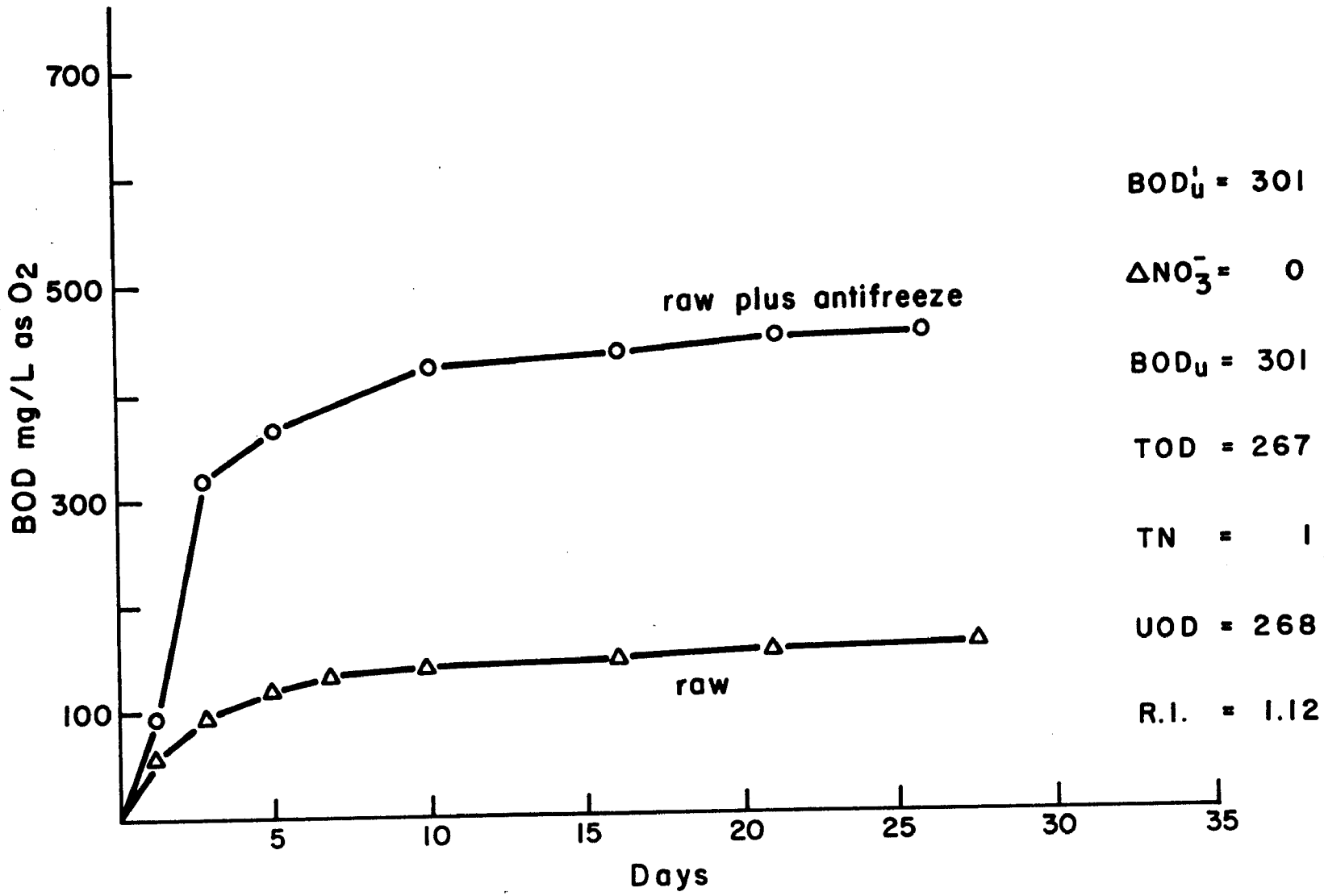



Figure C-7 OXYGEN UPTAKE FOR ANTIFREEZE

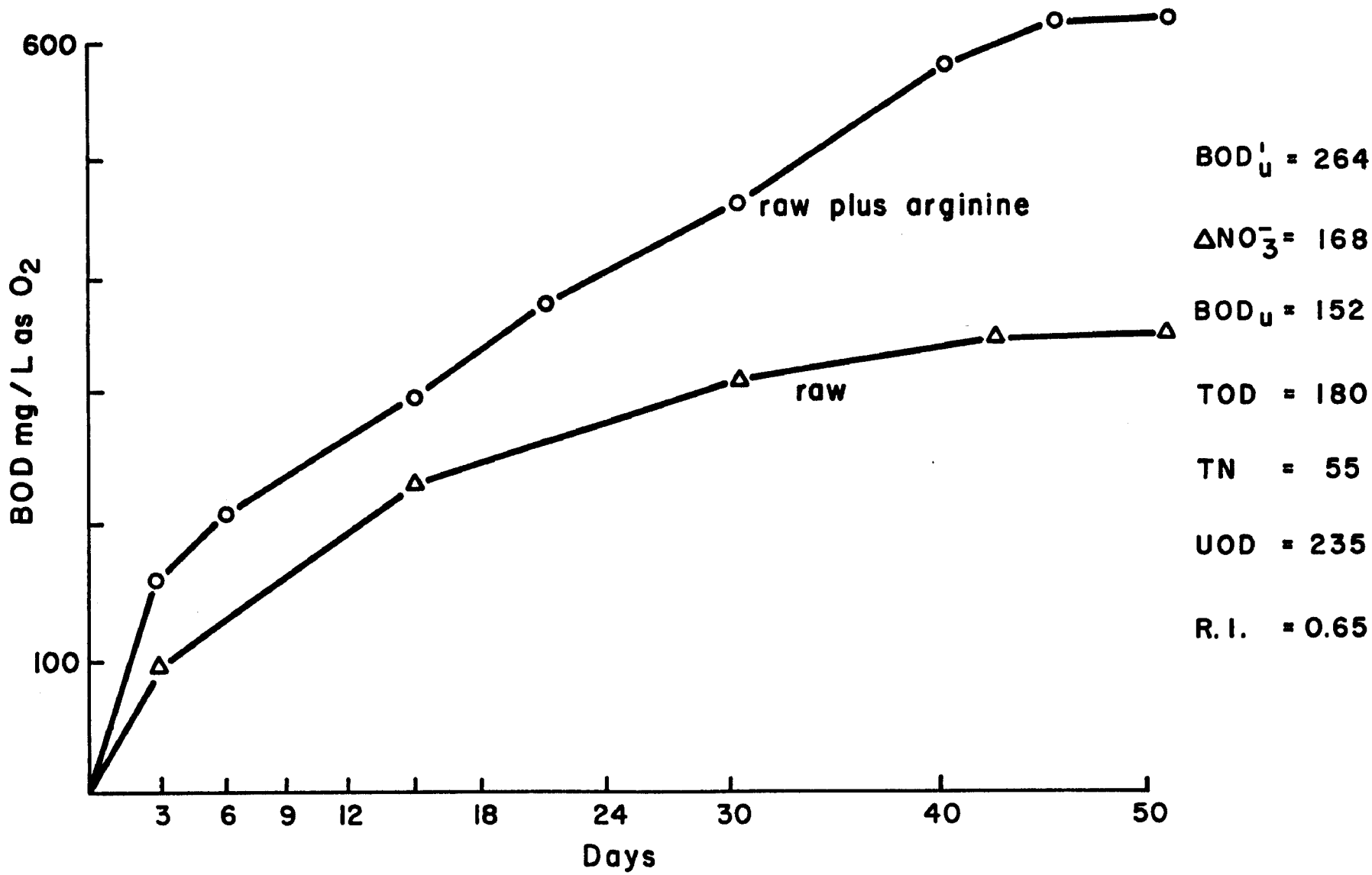



Figure C-8 OXYGEN UPTAKE FOR ARGININE

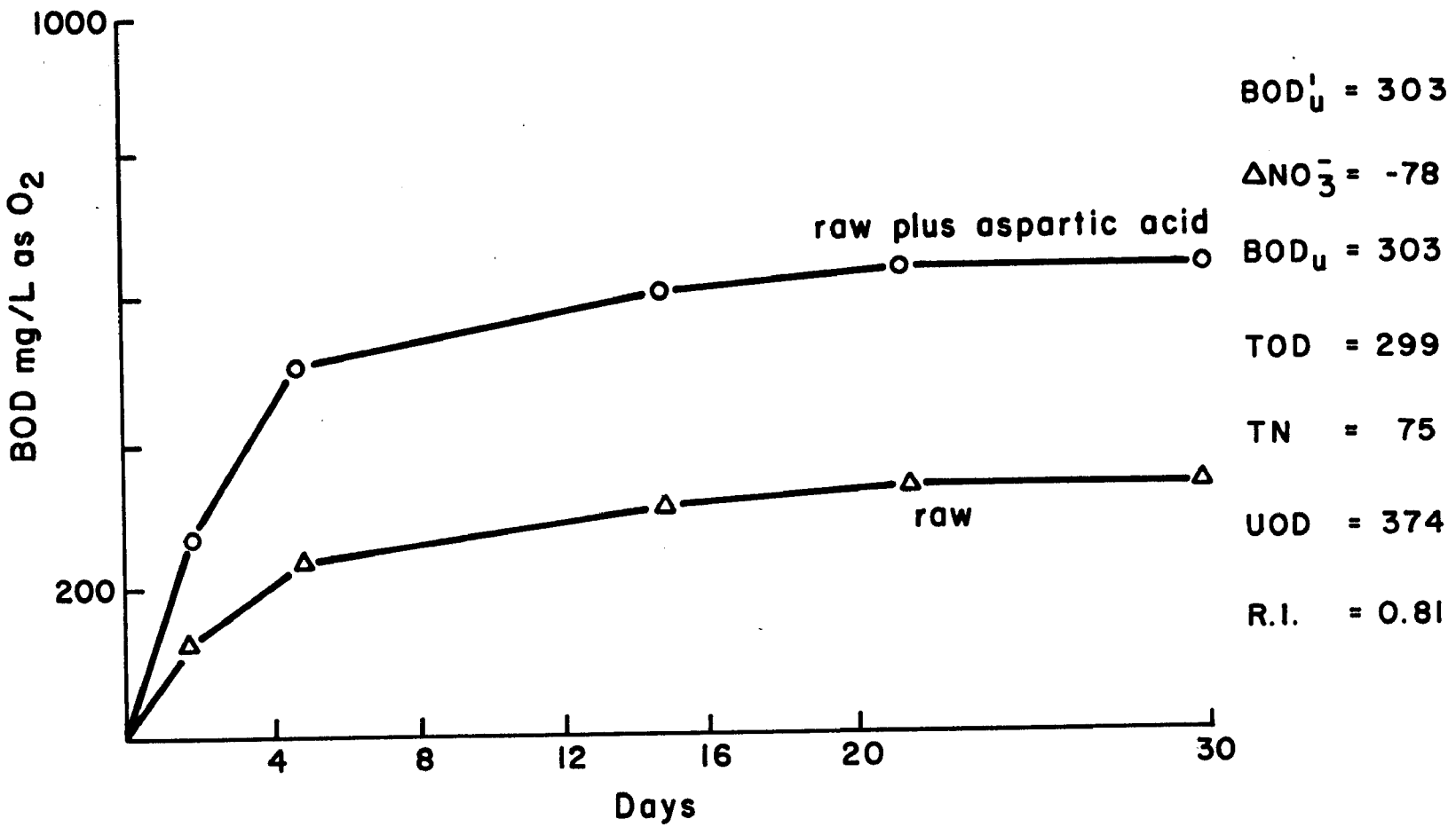



Figure C-9 OXYGEN UPTAKE FOR ASPARTIC ACID

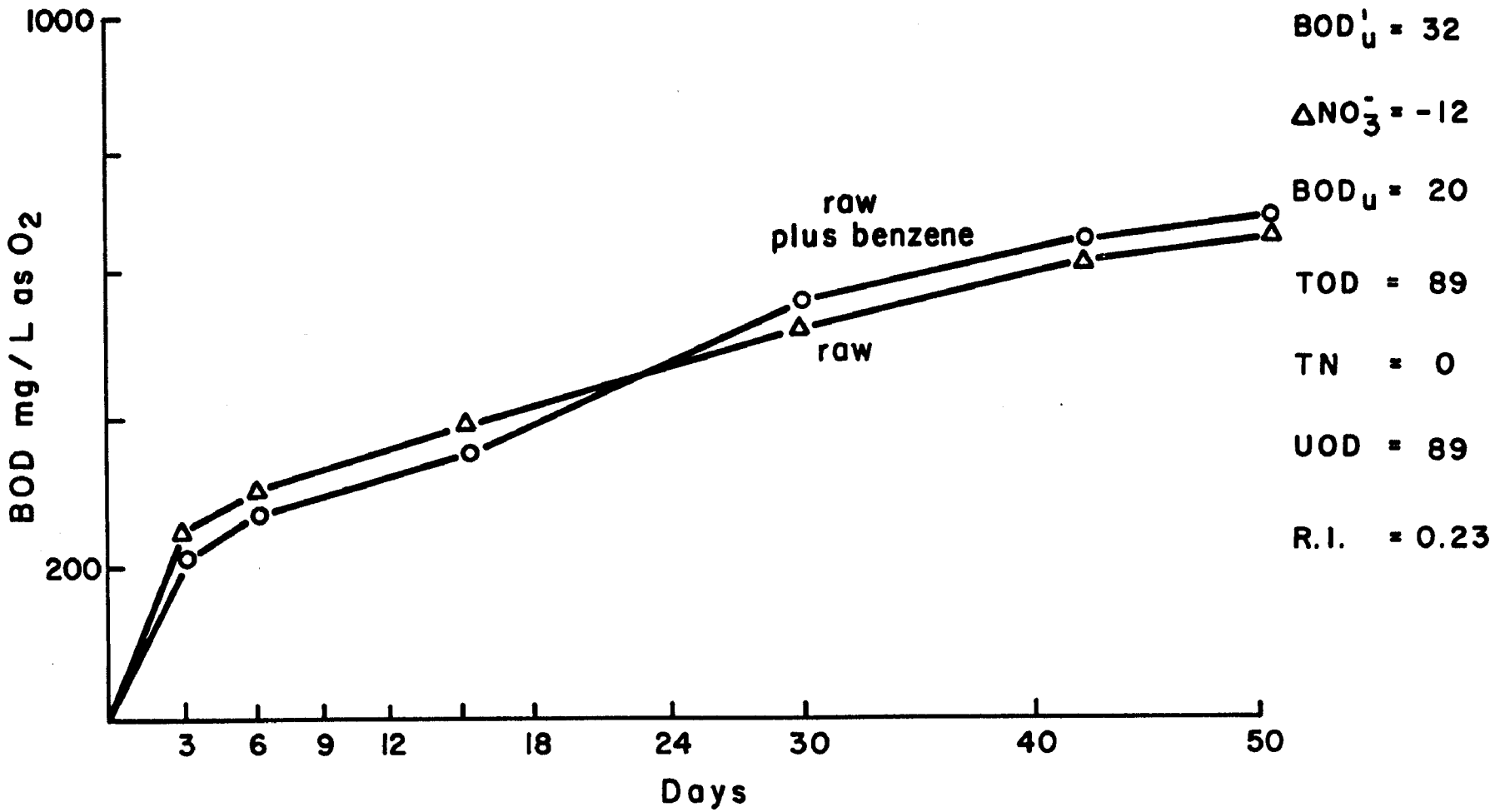



Figure C-10 OXYGEN UPTAKE FOR BENZENE

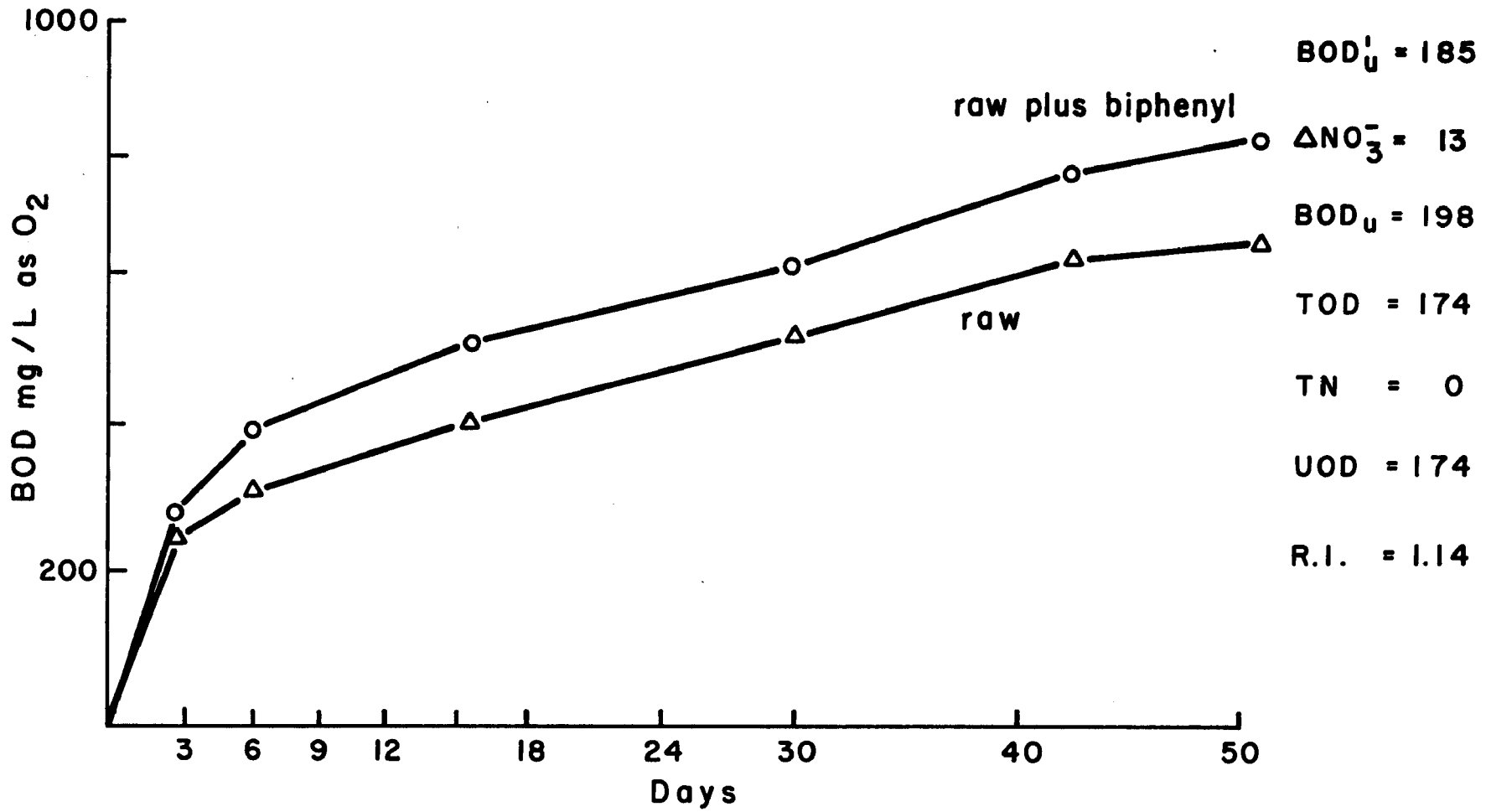



Figure C - 11 OXYGEN UPTAKE FOR BIPHENYL

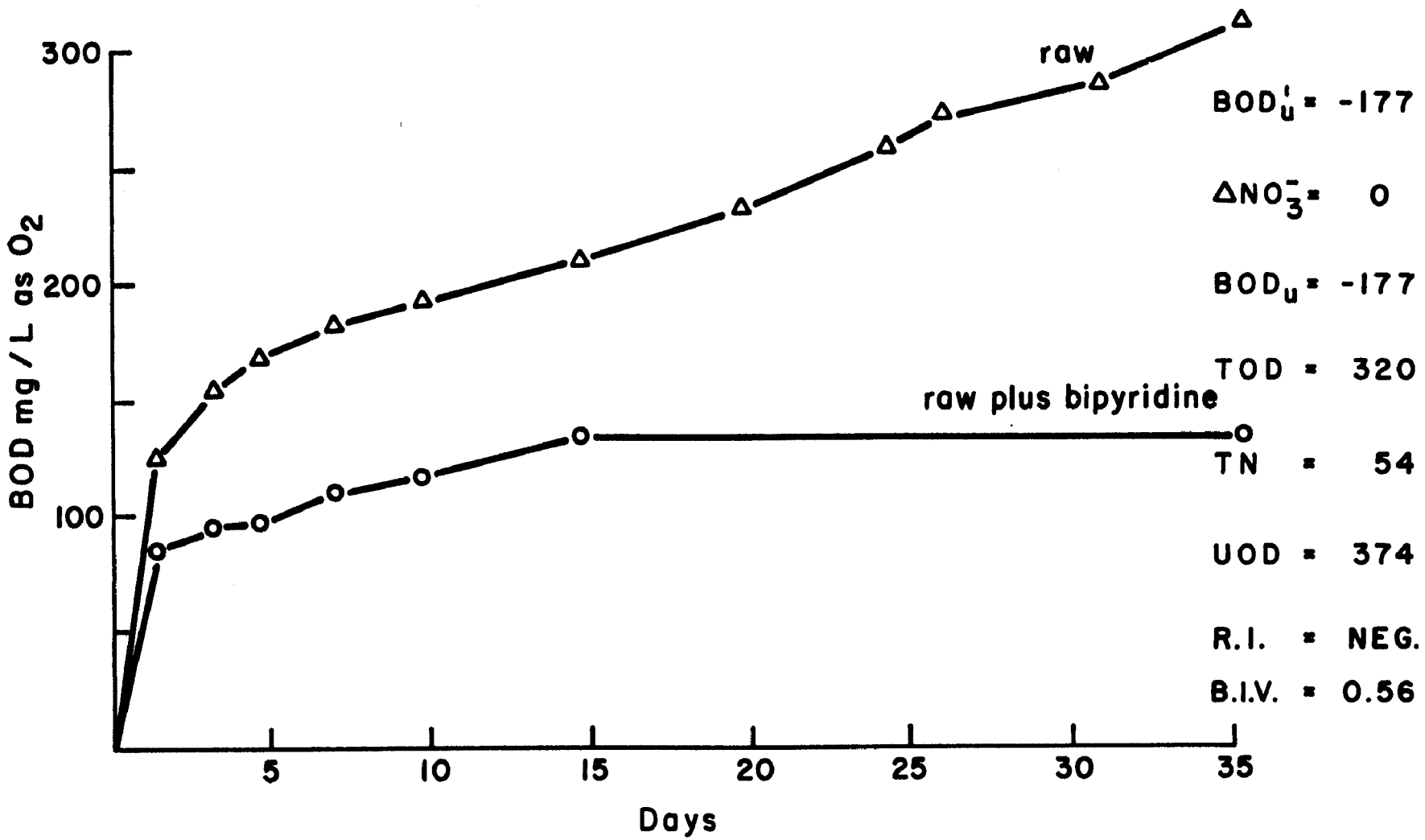



Figure C-12 OXYGEN UPTAKE FOR BIPYRIDINE

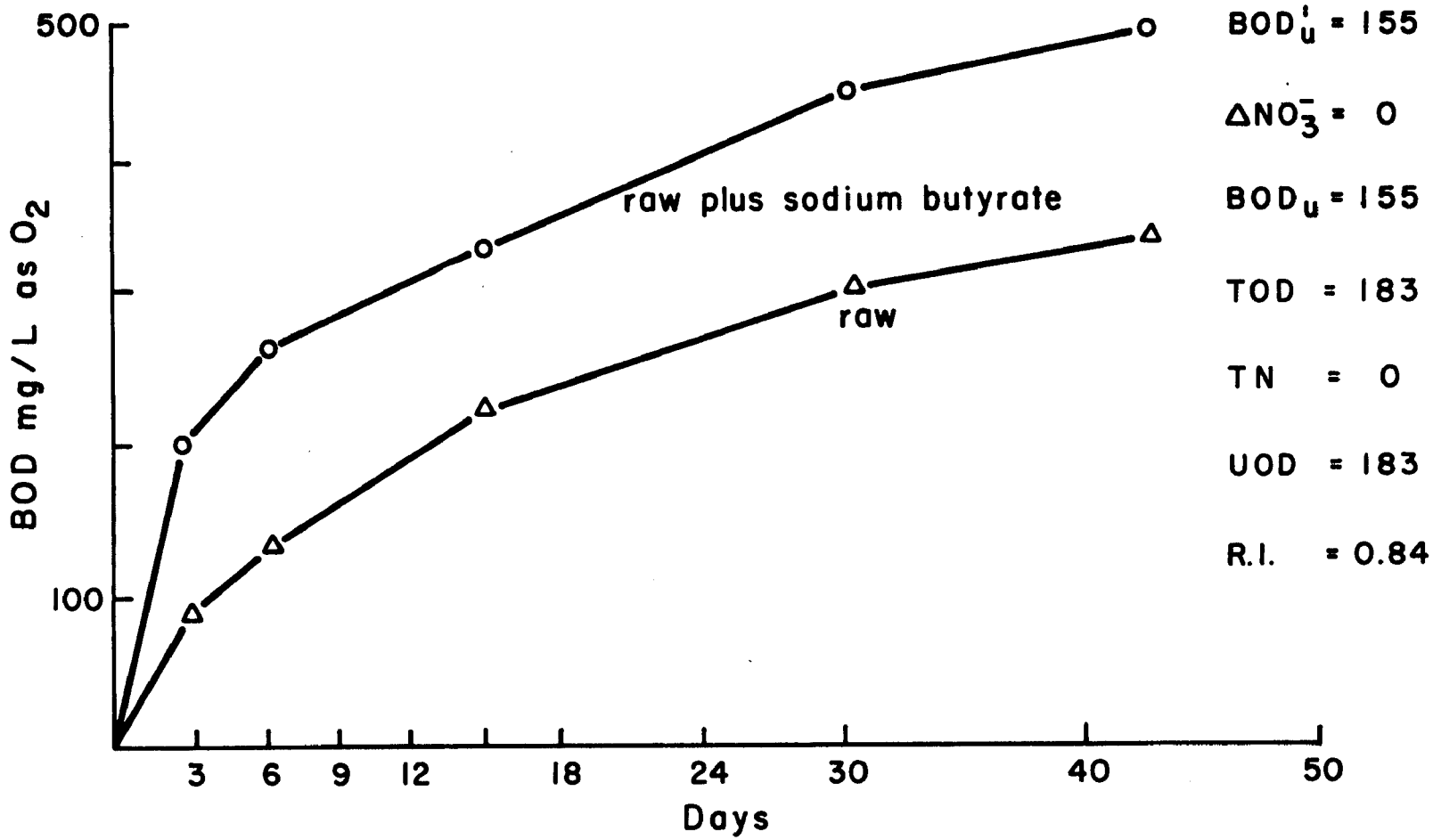



Figure C-13 OXYGEN UPTAKE FOR SODIUM BUTYRATE

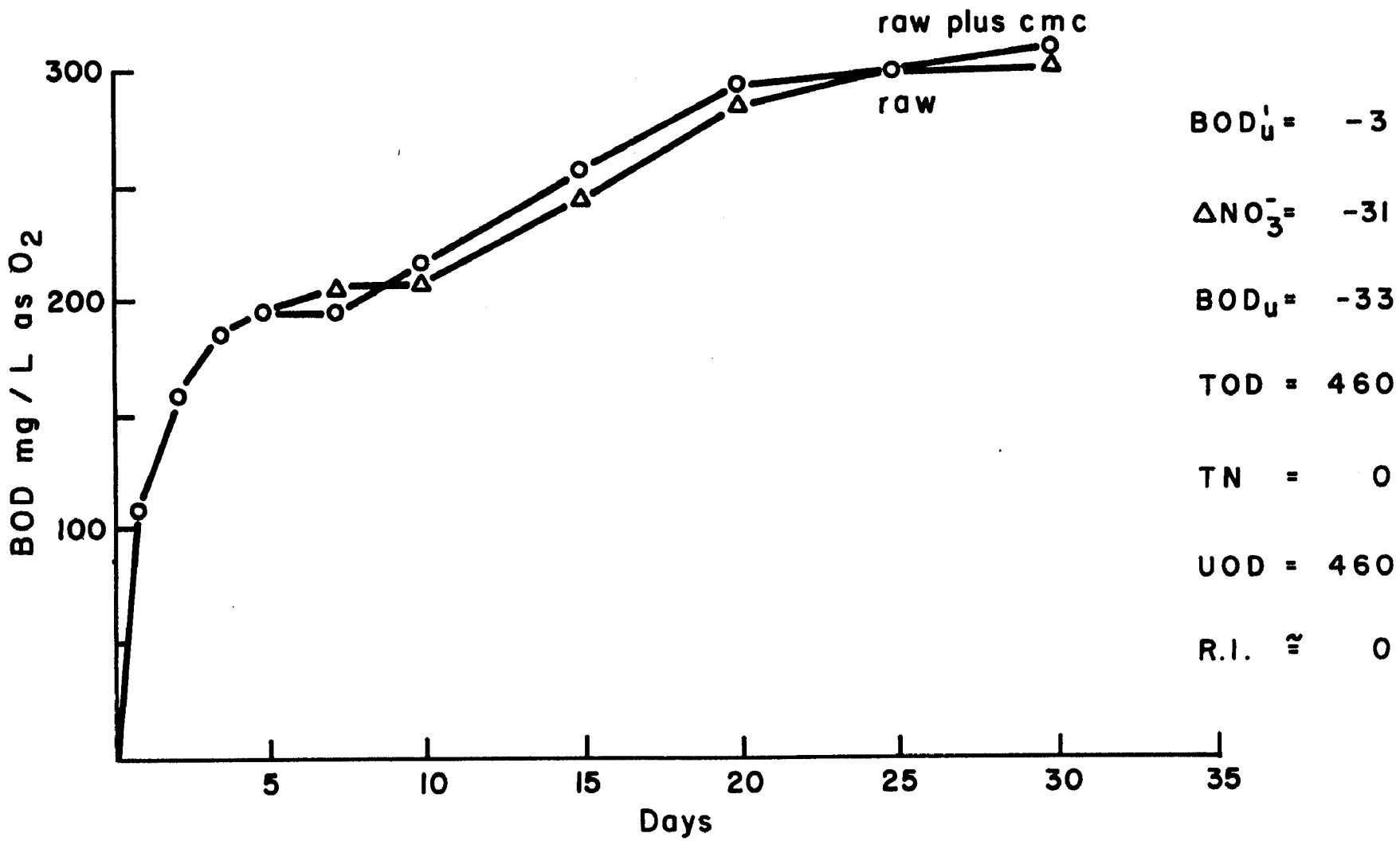



Figure C-14 OXYGEN UPTAKE FOR CARBOXYMETHYL CELLULOSE

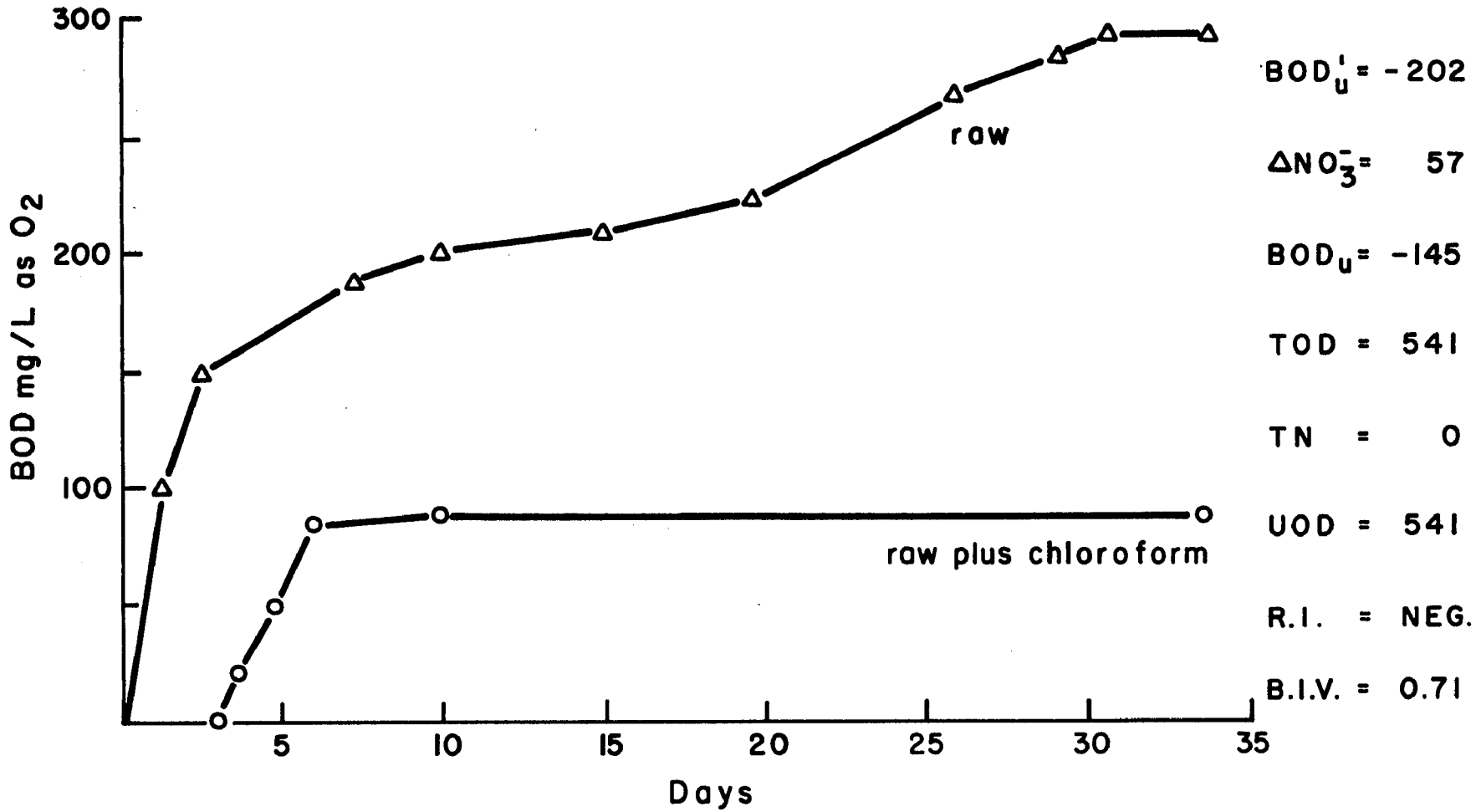



Figure C-15 OXYGEN UPTAKE FOR CHLOROFORM

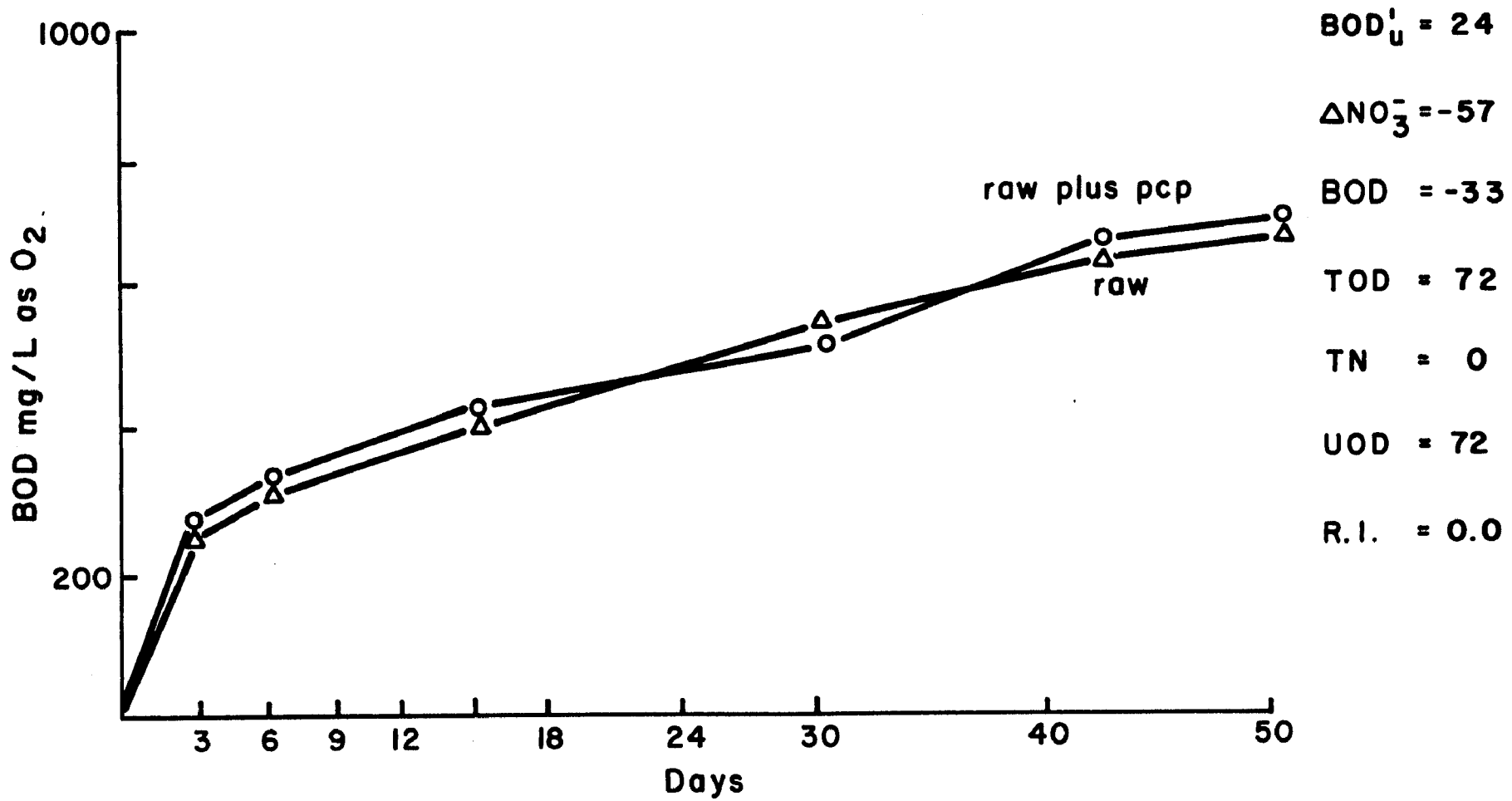



Figure C-16 OXYGEN UPTAKE FOR p-CHLOROPHENOL

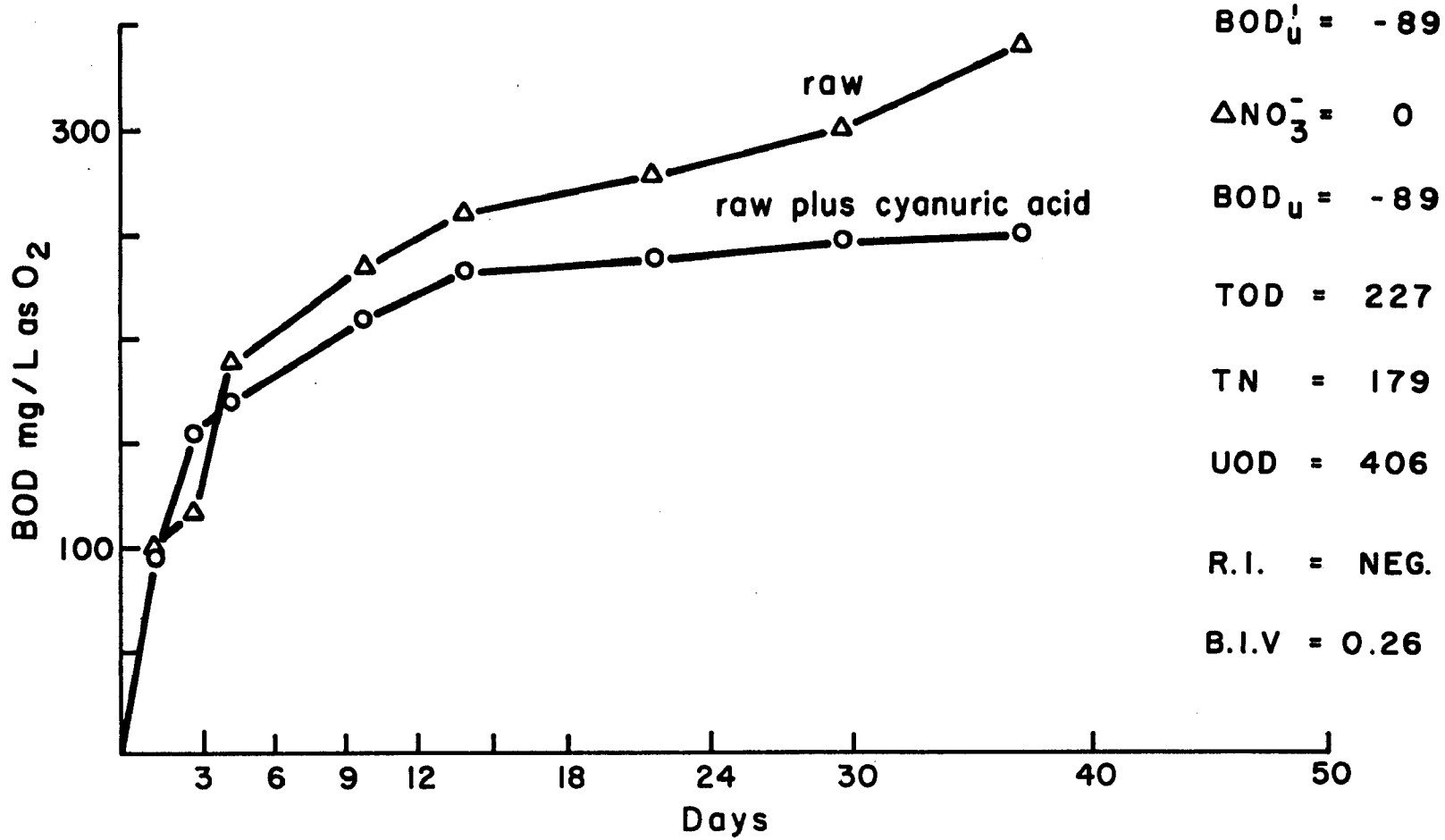



Figure C - 17 OXYGEN UPTAKE FOR CYANURIC ACID

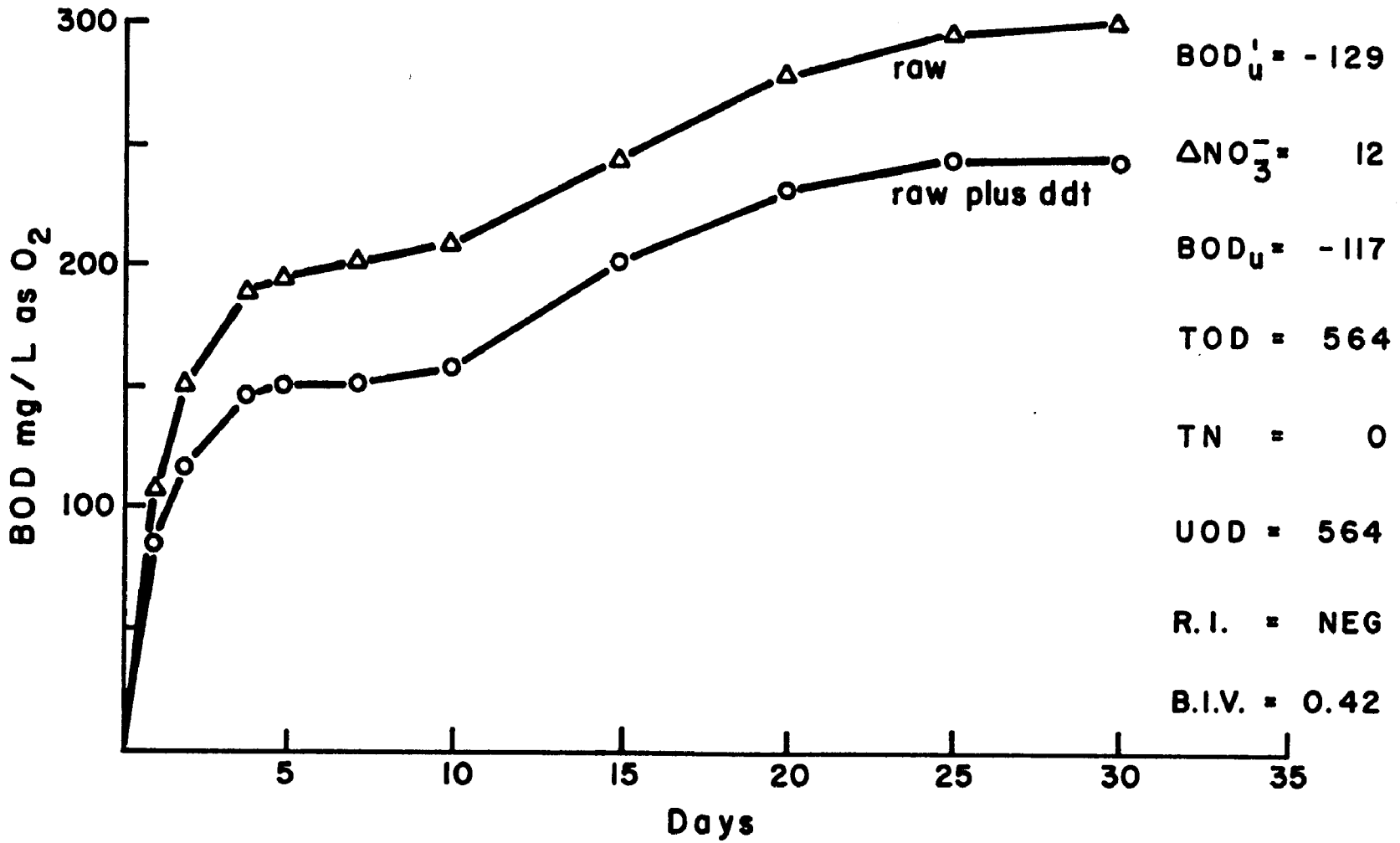



Figure C - 18 OXYGEN UPTAKE FOR DDT

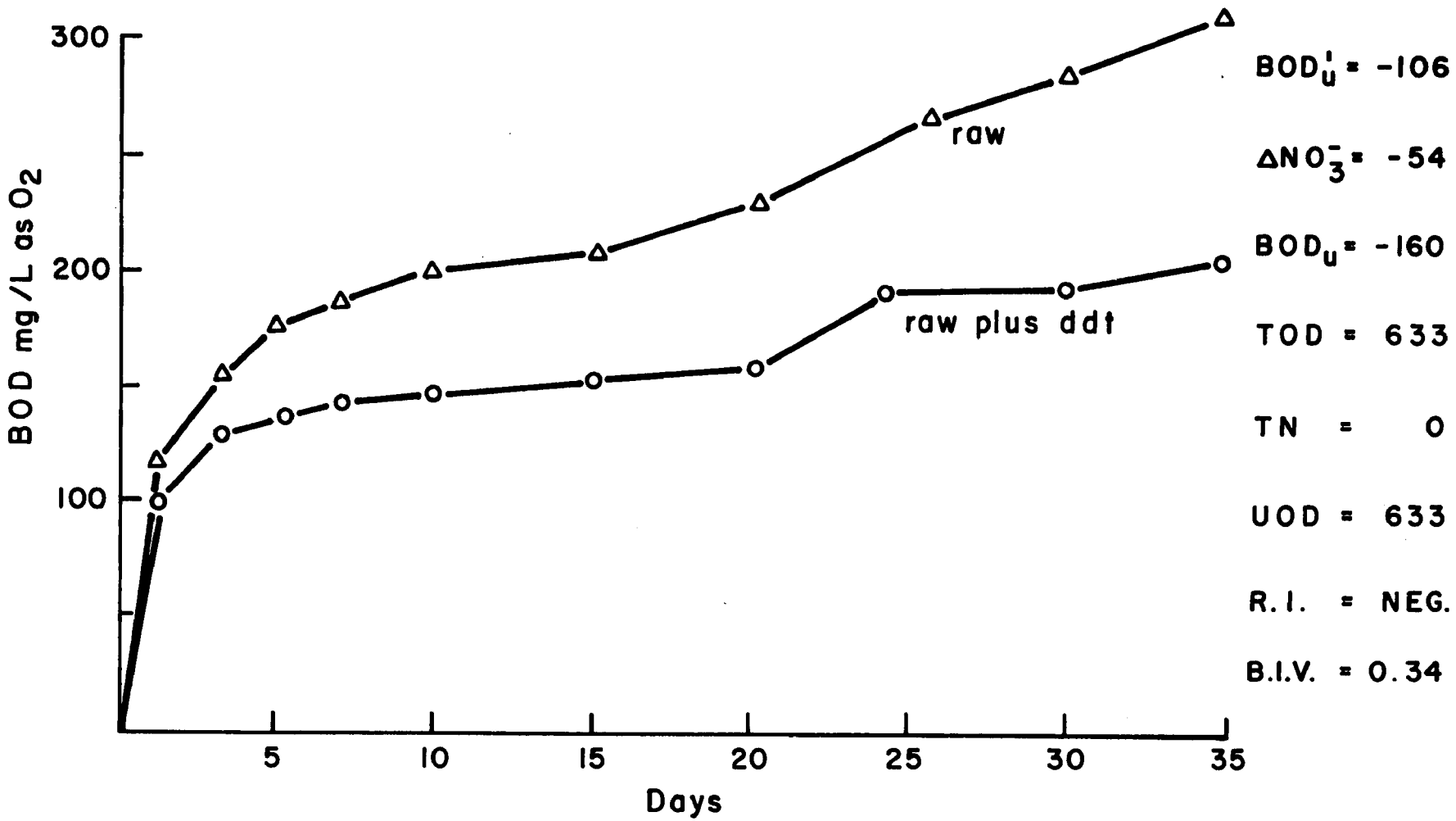



Figure C-19 OXYGEN UPTAKE FOR DDT WITH CARRIER

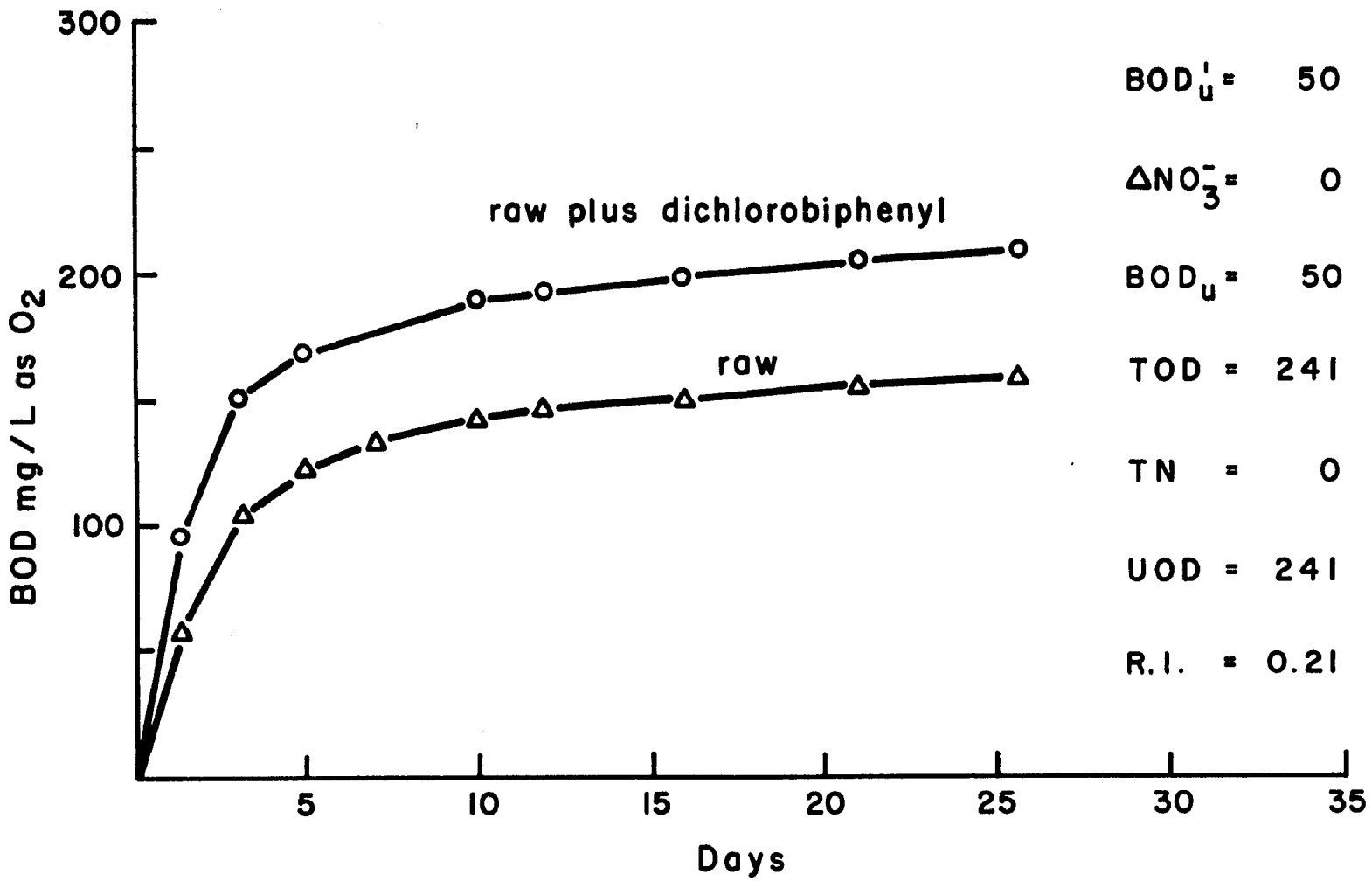



Figure C-20 OXYGEN UPTAKE FOR DICHLOROBIPHENYL

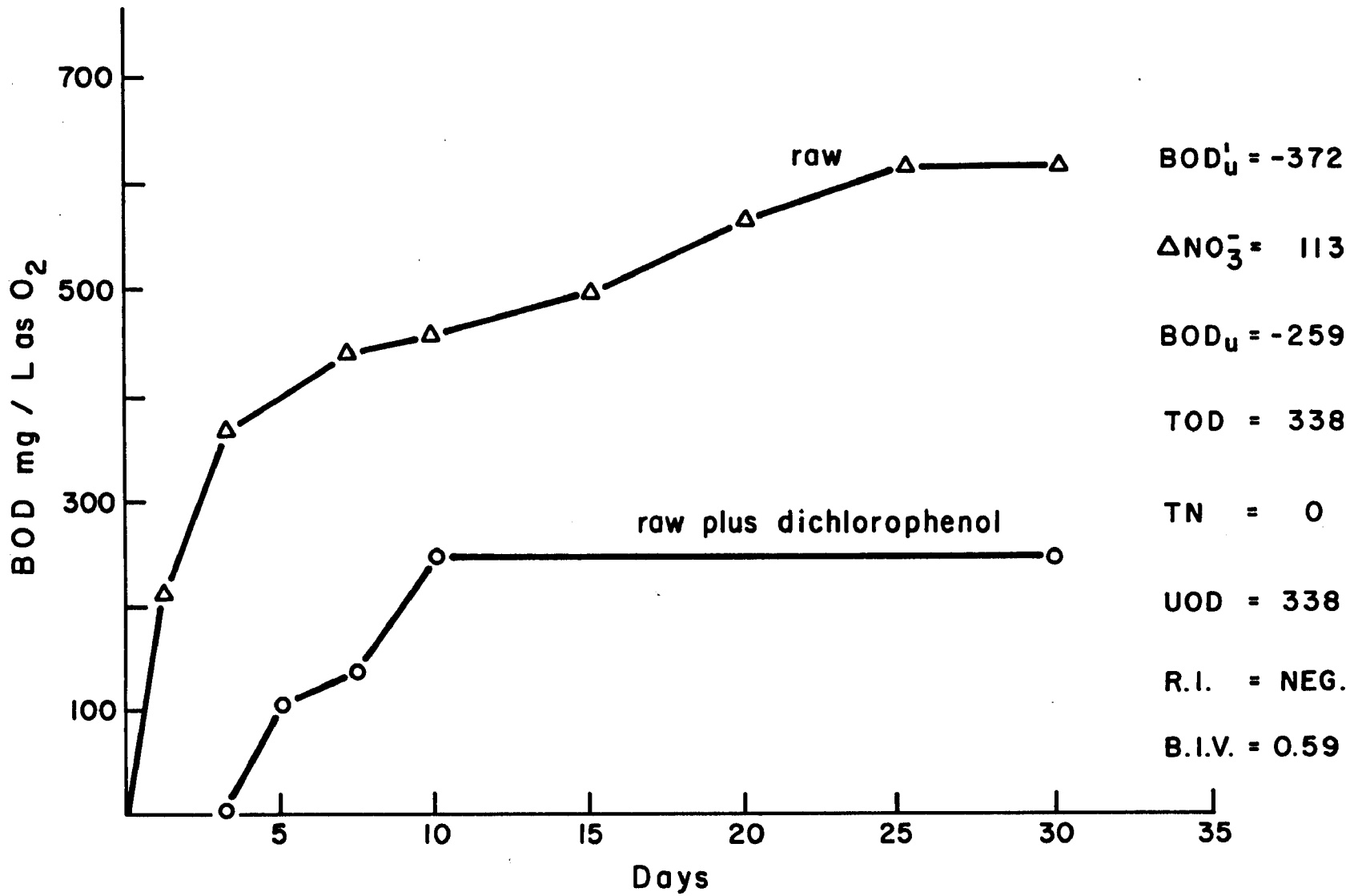



Figure C-21 OXYGEN UPTAKE FOR DICHLOROPHENOL

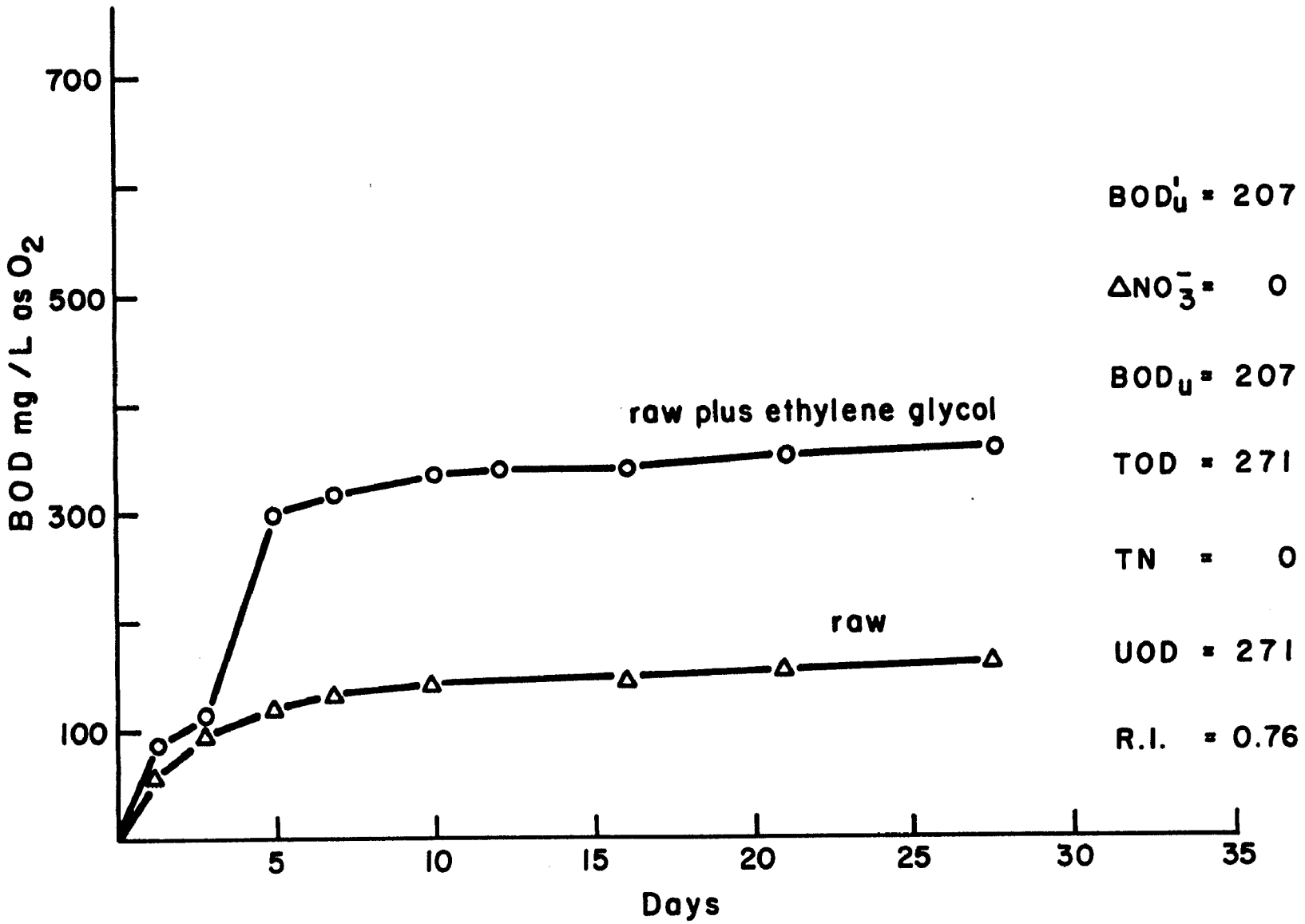



Figure C - 22 OXYGEN UPTAKE FOR ETHYLENE GLYCOL

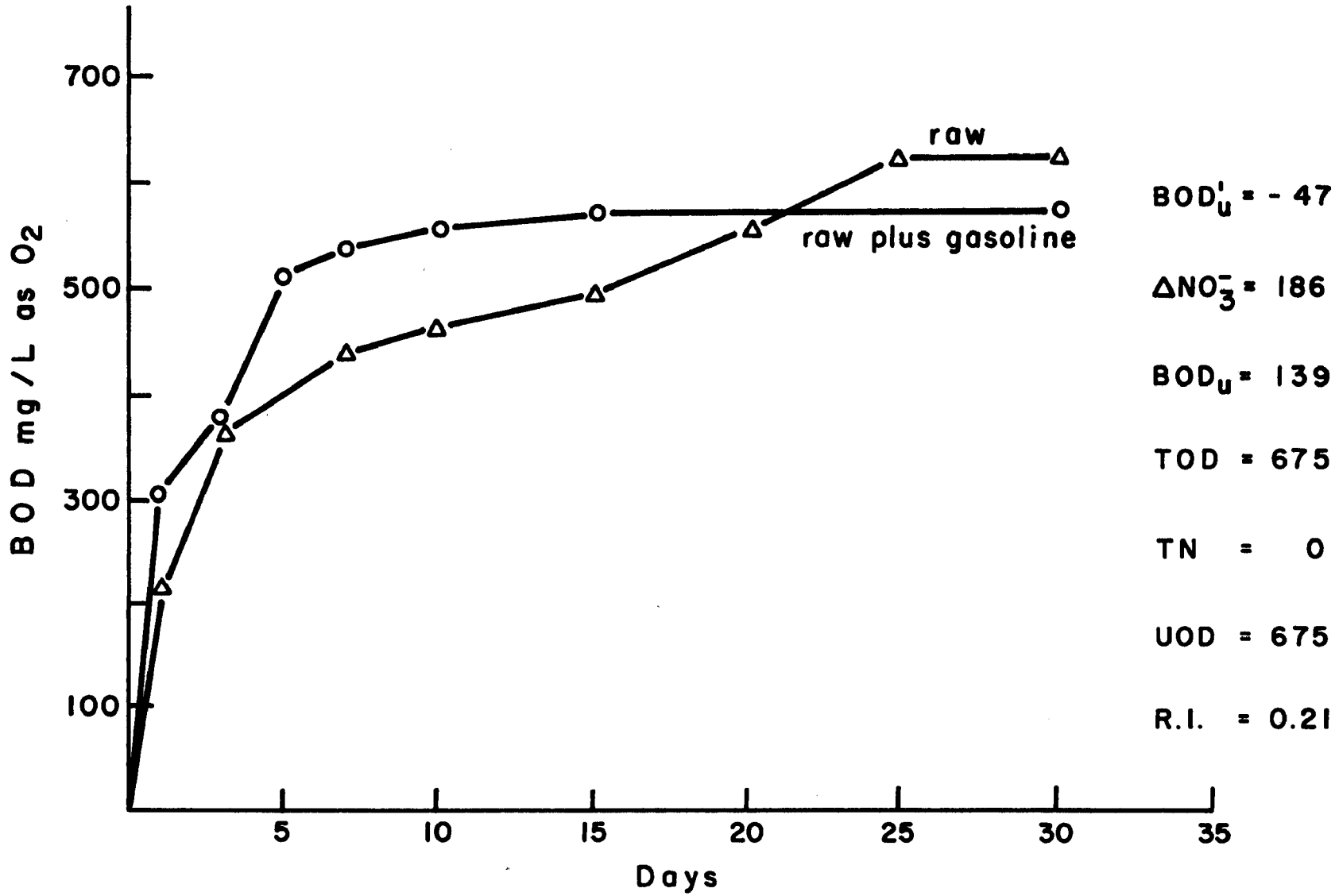



Figure C - 23 OXYGEN UPTAKE FOR GASOLINE

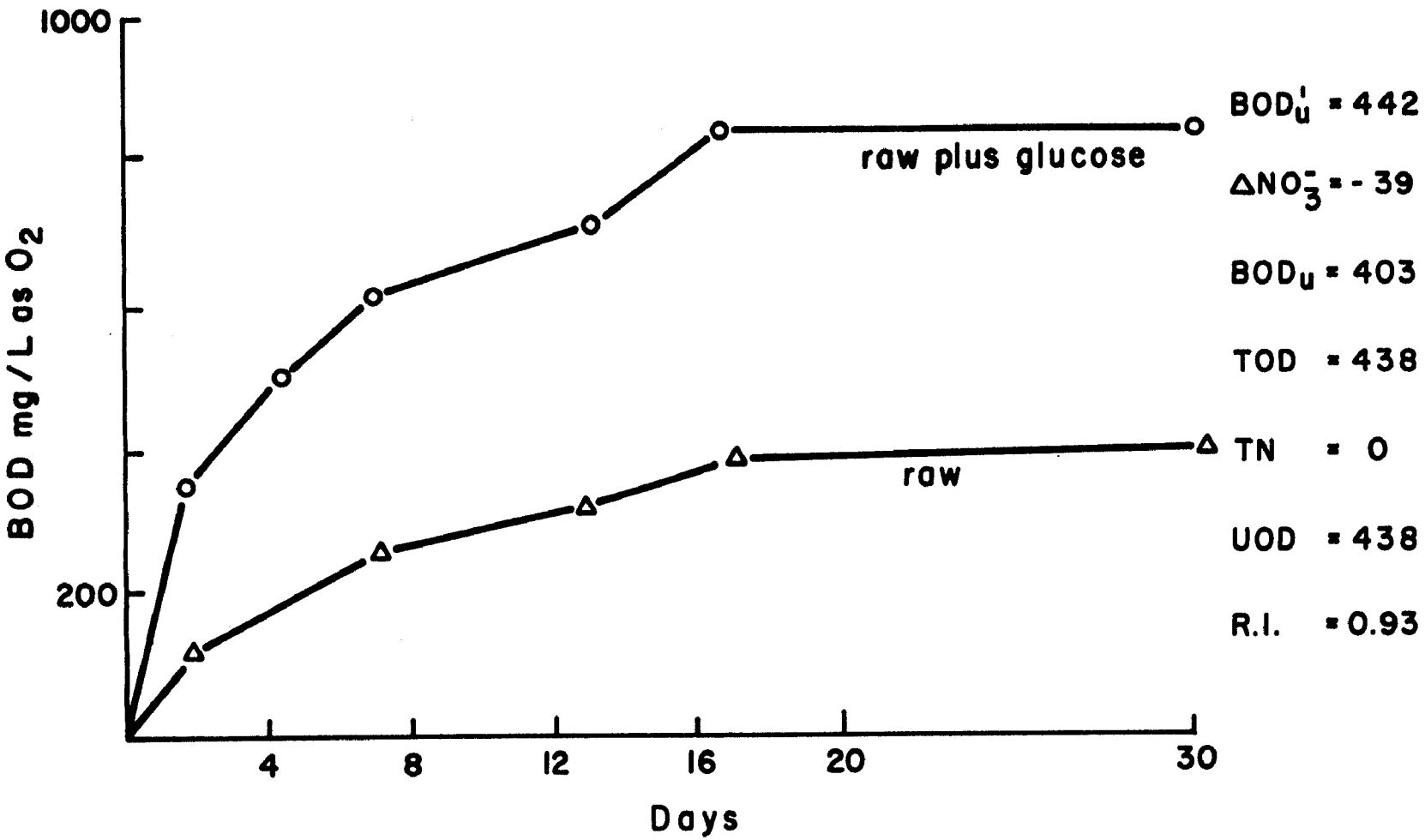



Figure C-24 OXYGEN UPTAKE FOR GLUCOSE

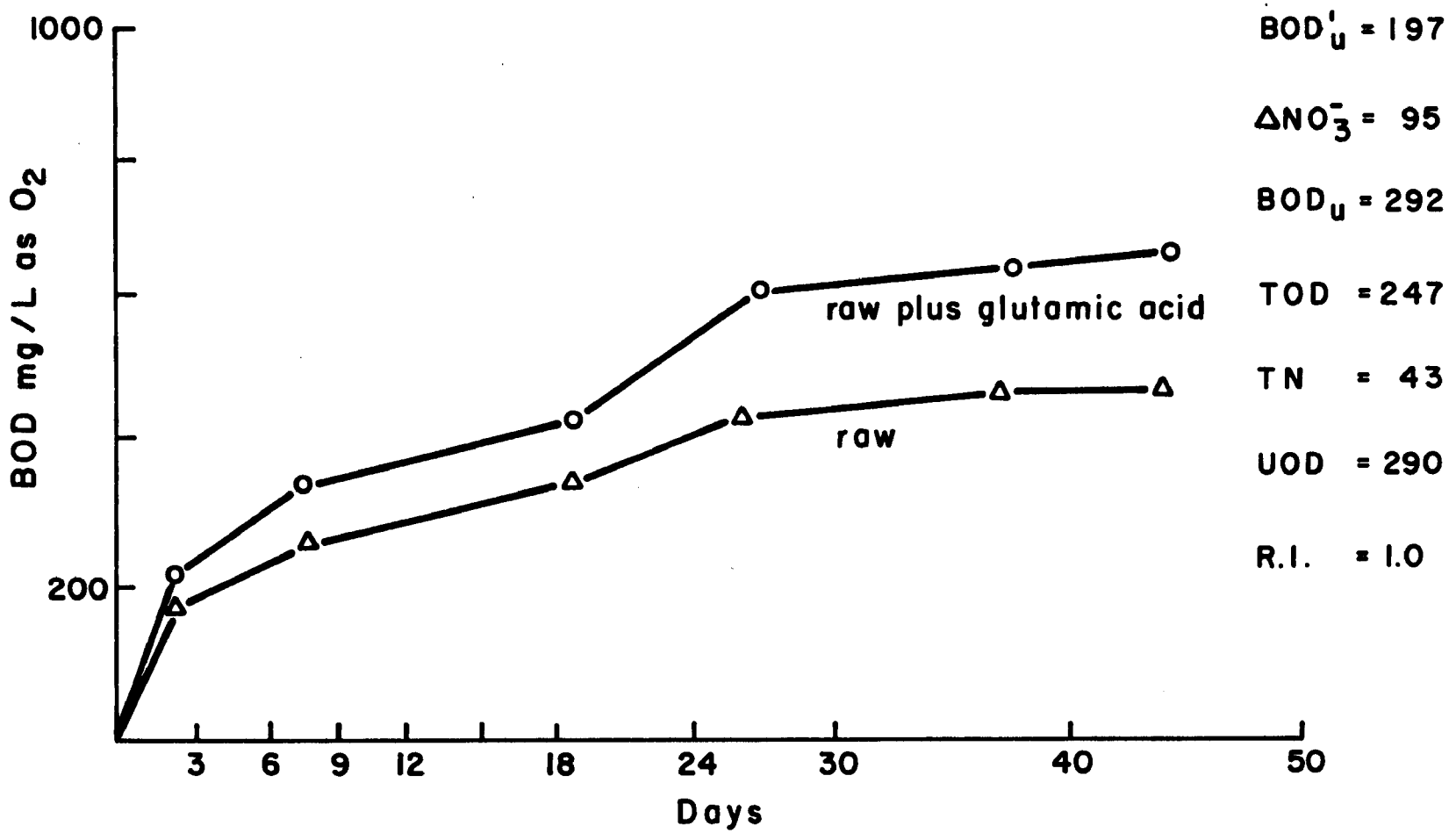



Figure C-25 OXYGEN UPTAKE FOR GLUTAMIC ACID

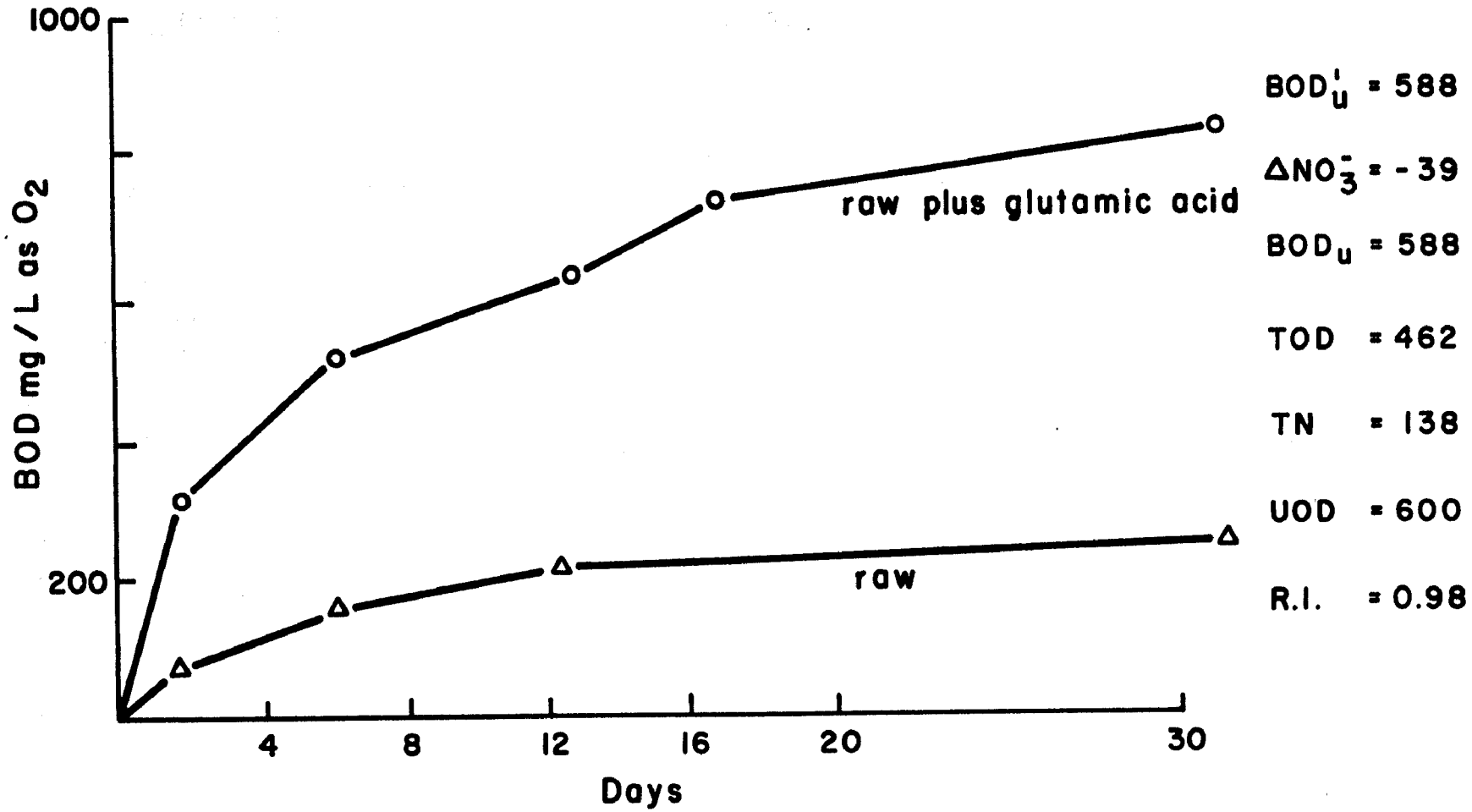



Figure C-26 OXYGEN UPTAKE FOR GLUTAMIC ACID

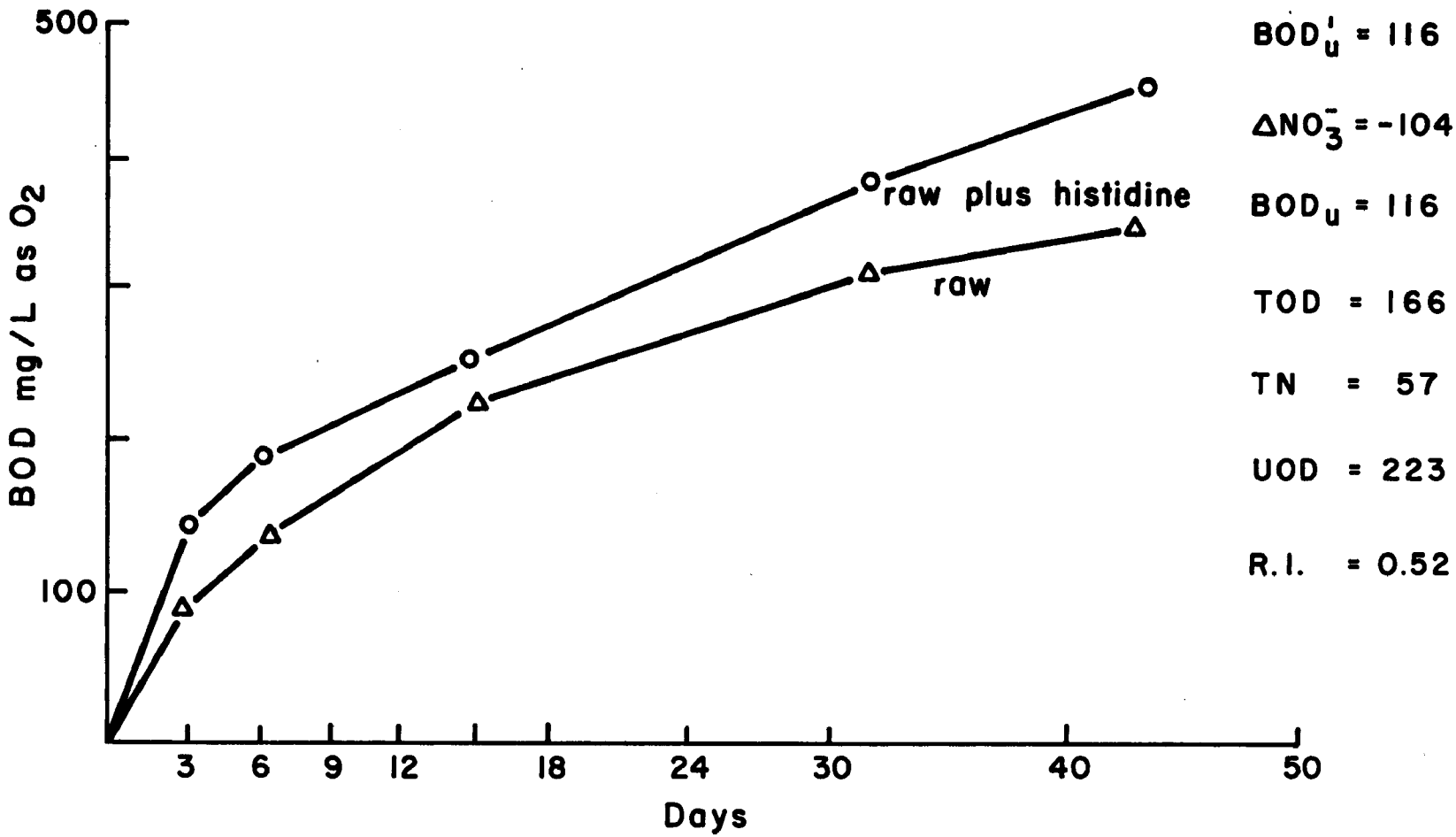



Figure C-27 OXYGEN UPTAKE FOR HISTIDINE

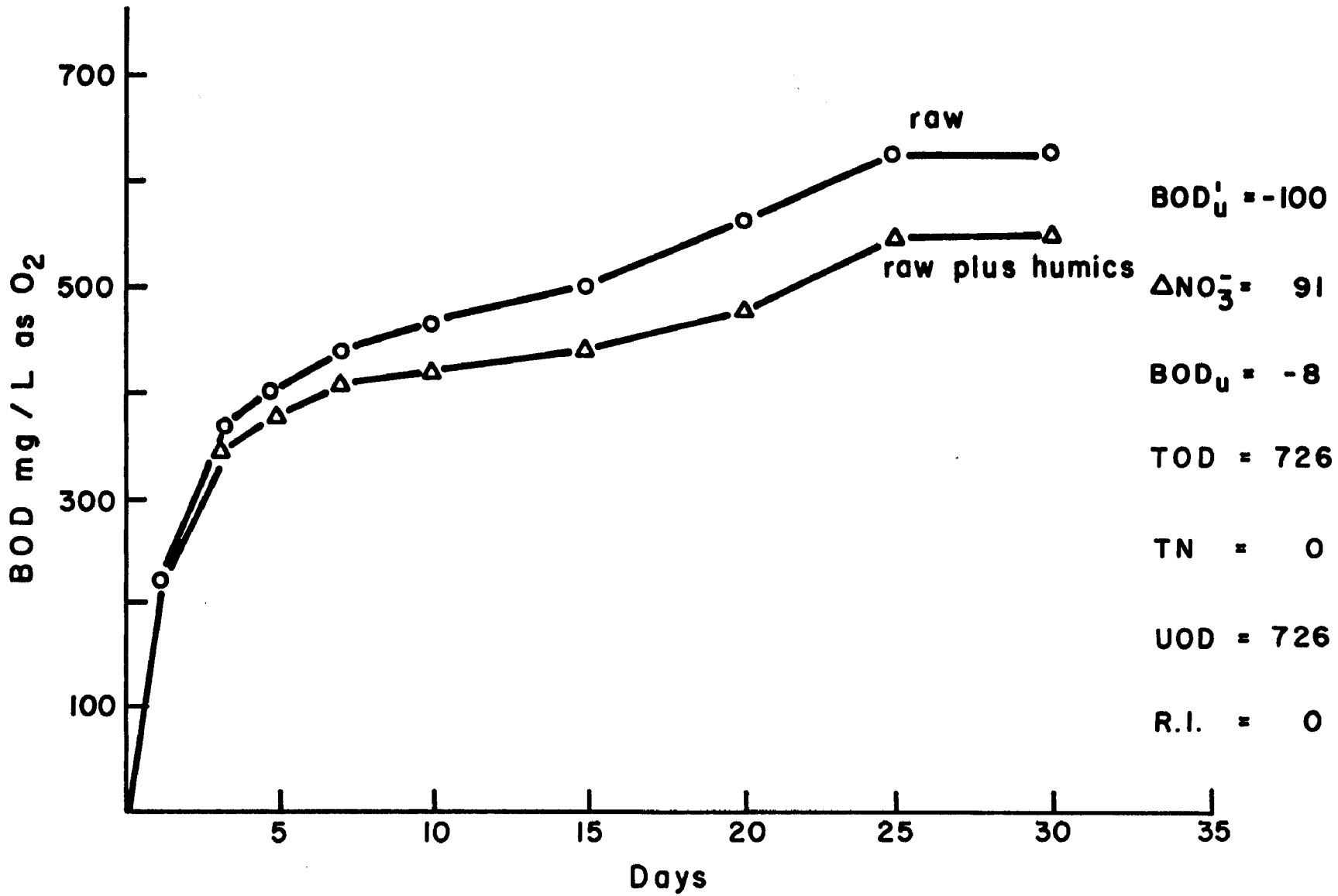



Figure C-28 OXYGEN UPTAKE FOR HUMICS

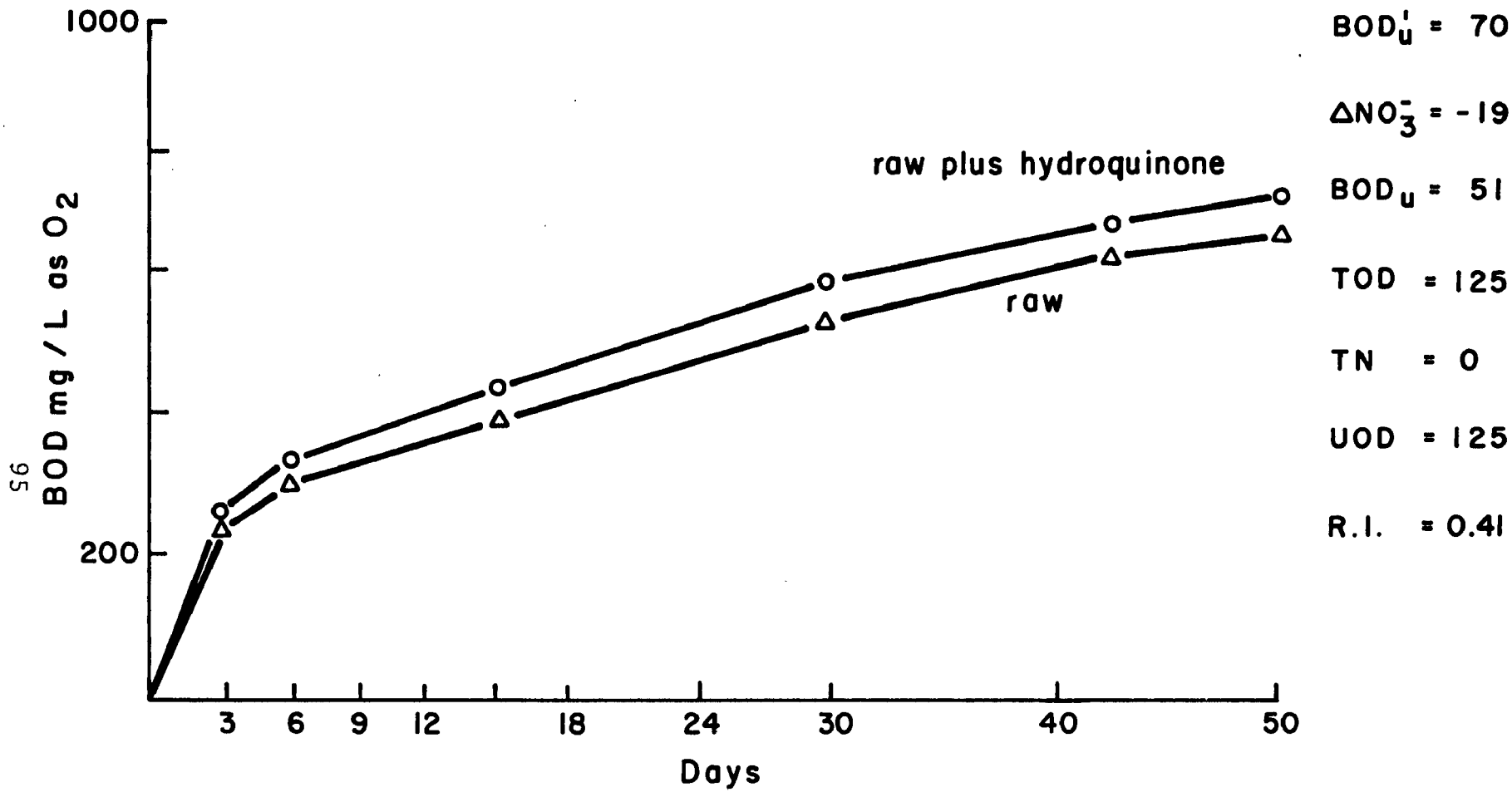



Figure C - 29 OXYGEN UPTAKE FOR HYDROQUINONE

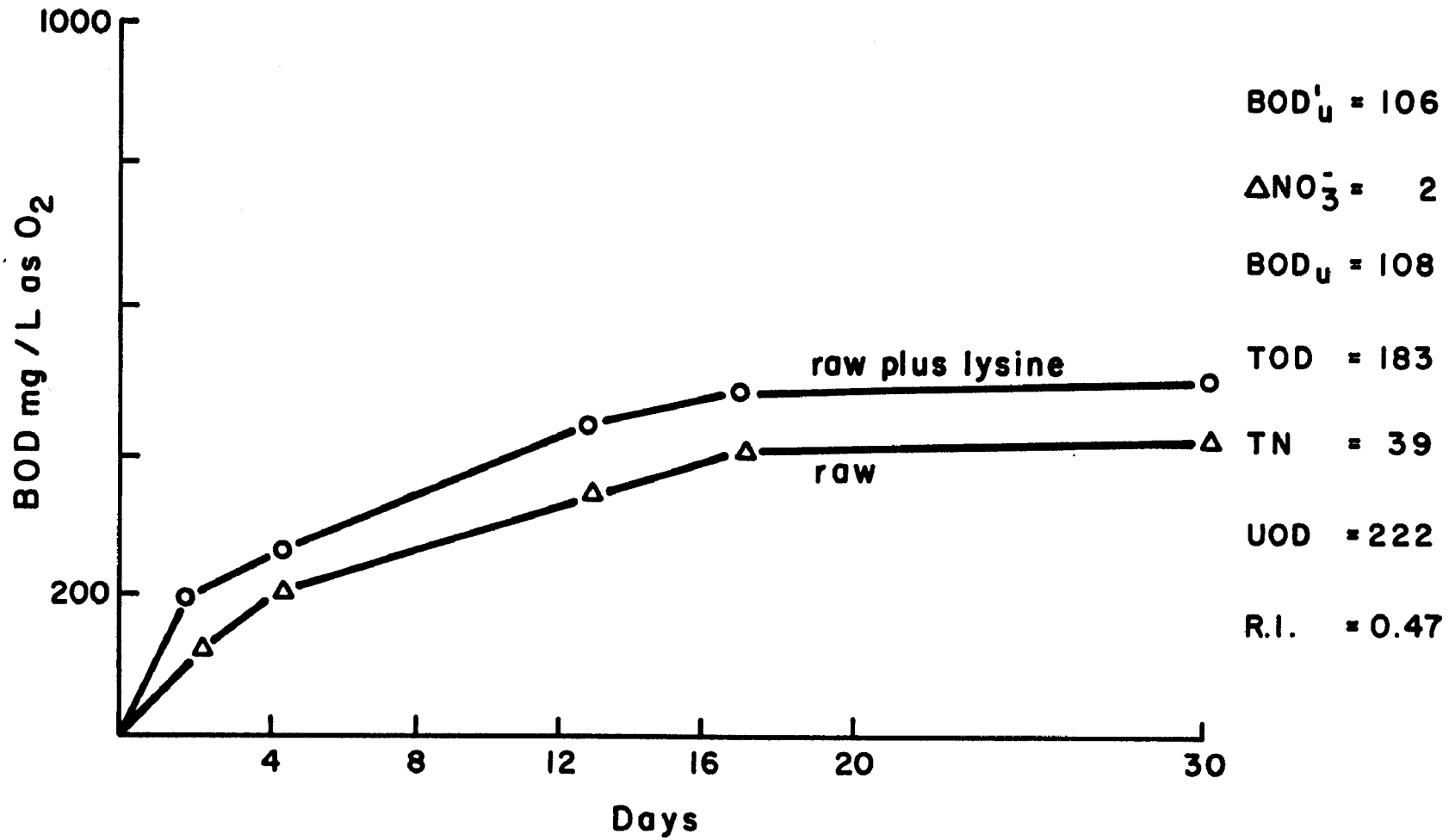



Figure C - 30 OXYGEN UPTAKE FOR LYSINE

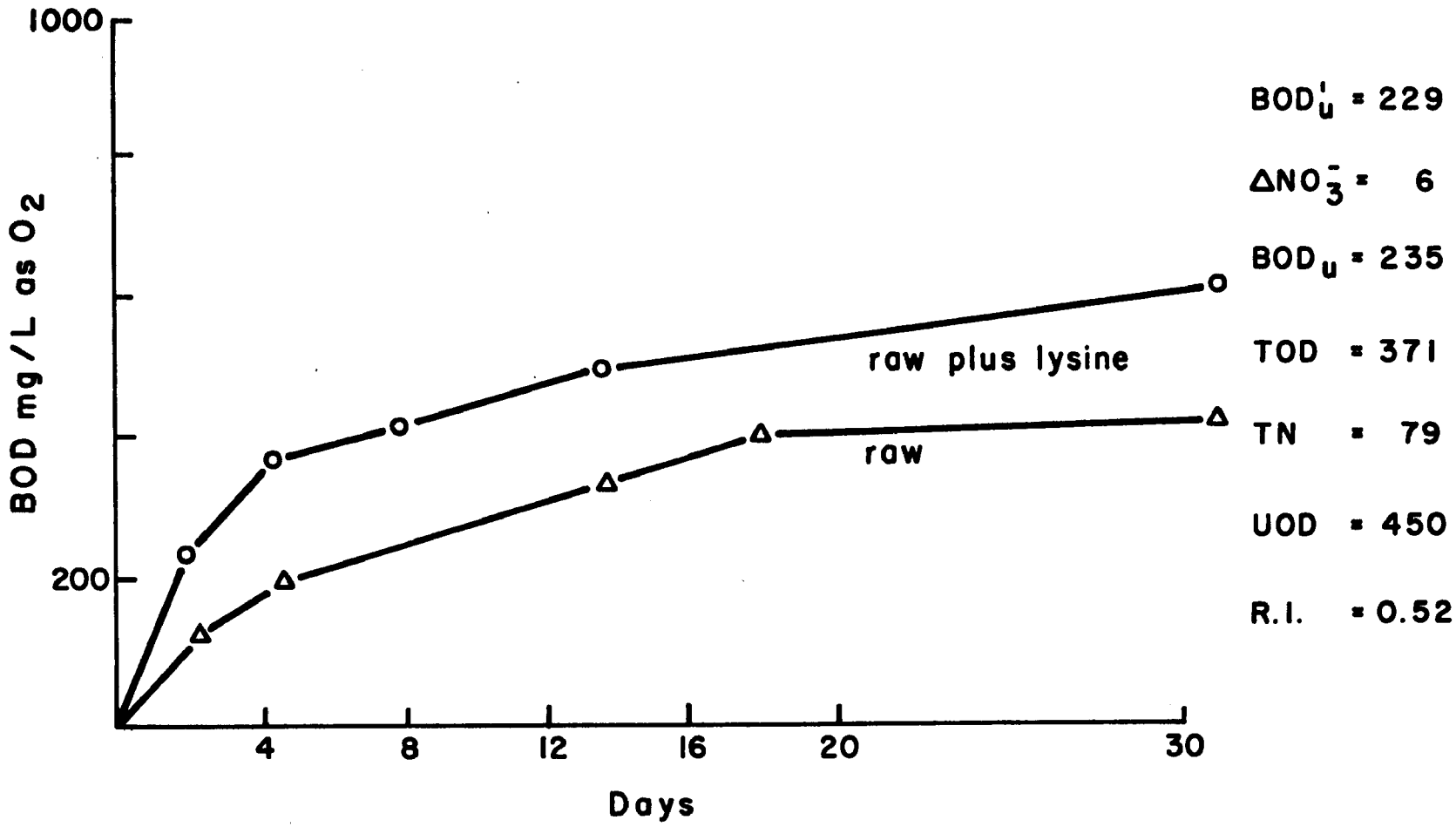



Figure C - 31 OXYGEN UPTAKE FOR LYSINE

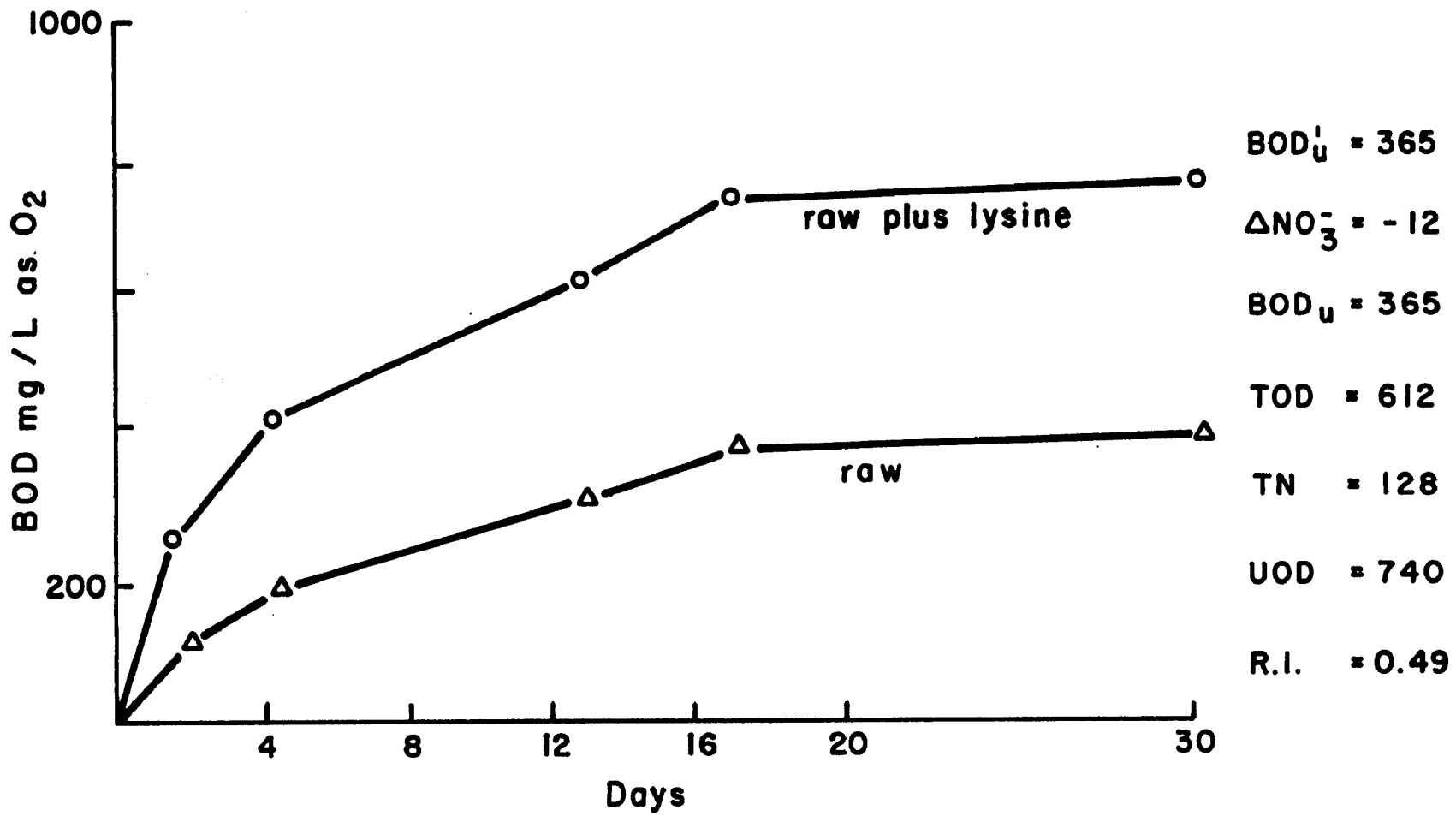



Figure C-32 OXYGEN UPTAKE FOR LYSINE

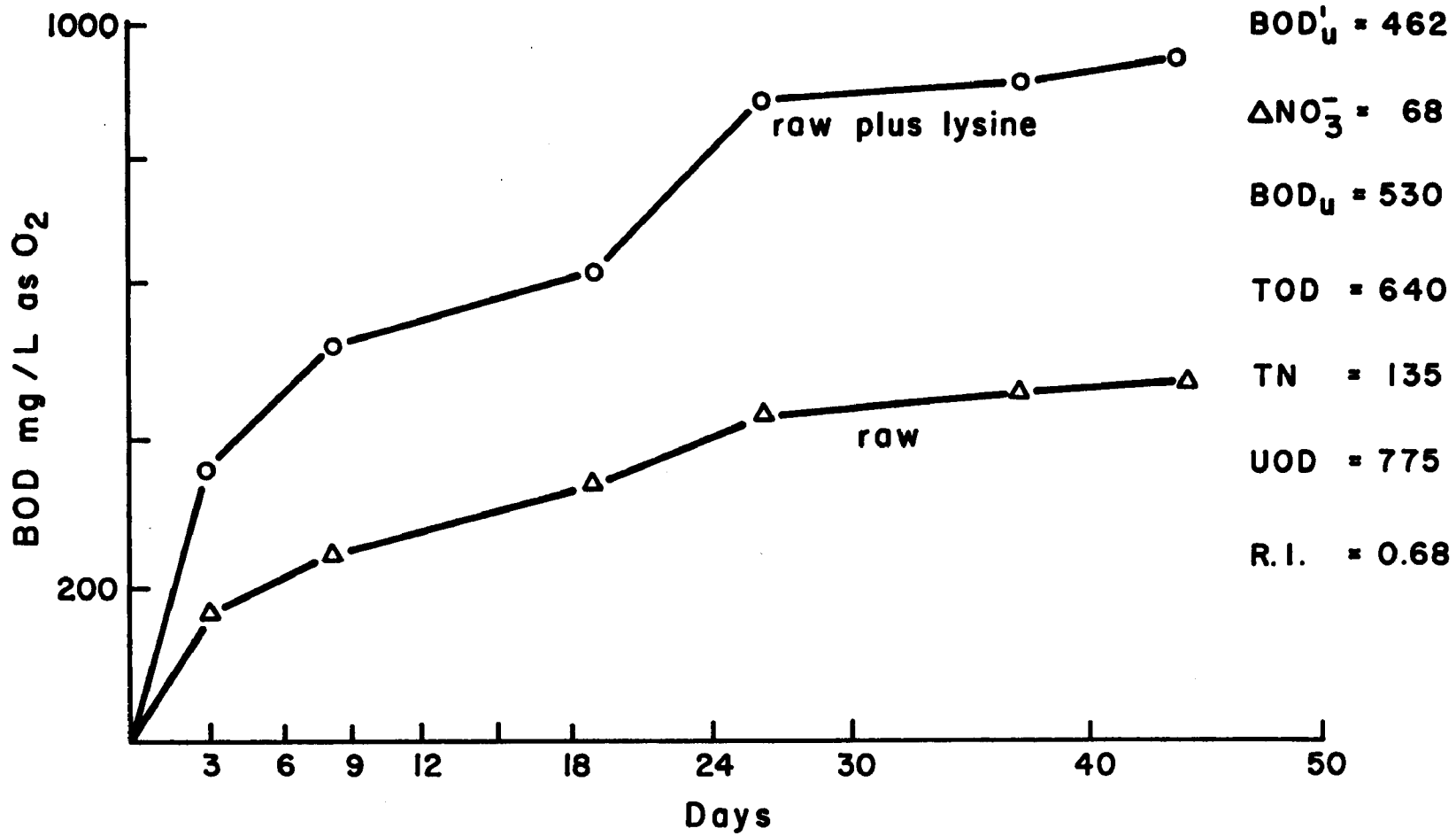



Figure C - 33 OXYGEN UPTAKE FOR LYSINE

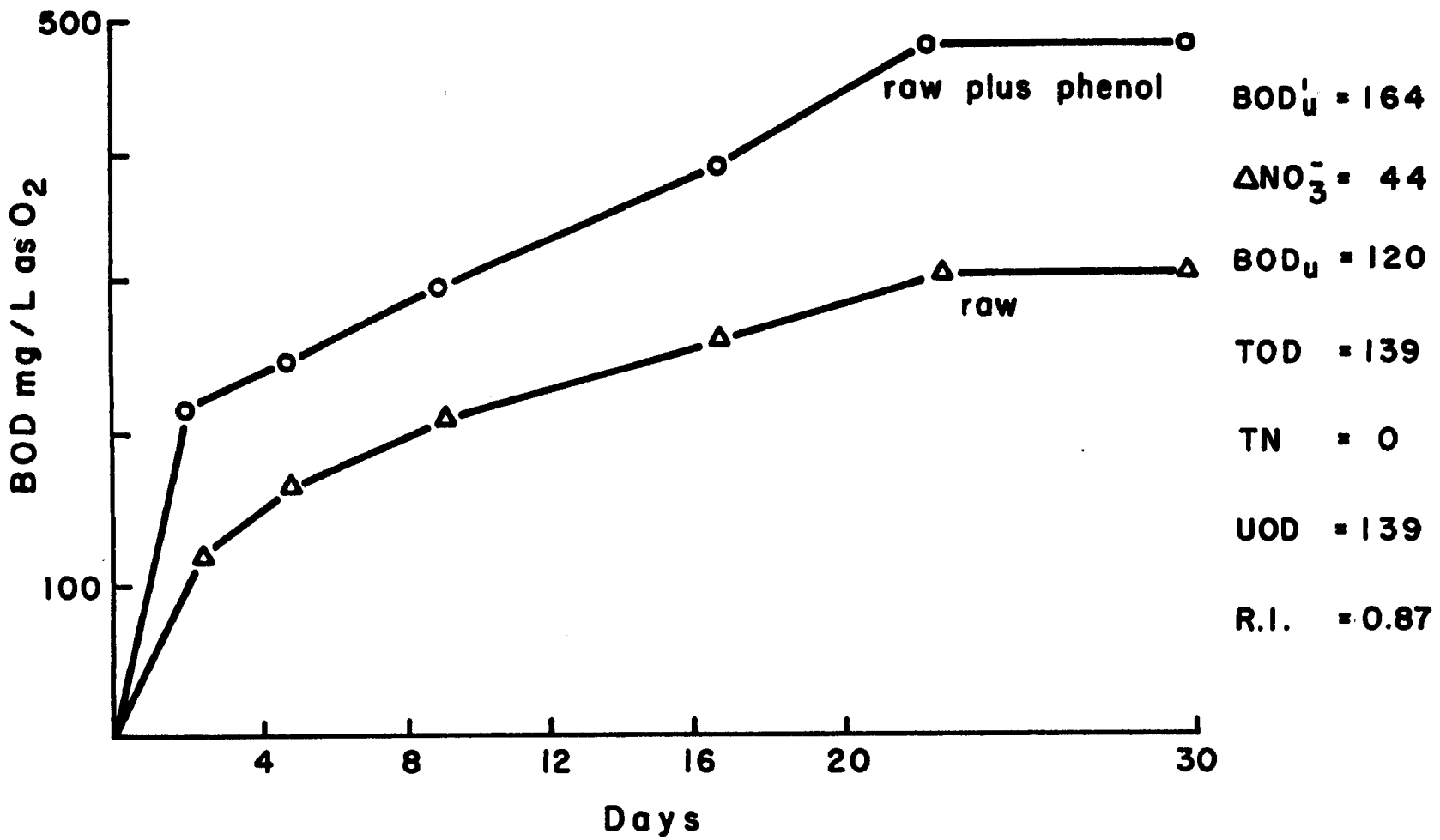



Figure C- 34 OXYGEN UPTAKE FOR PHENOL

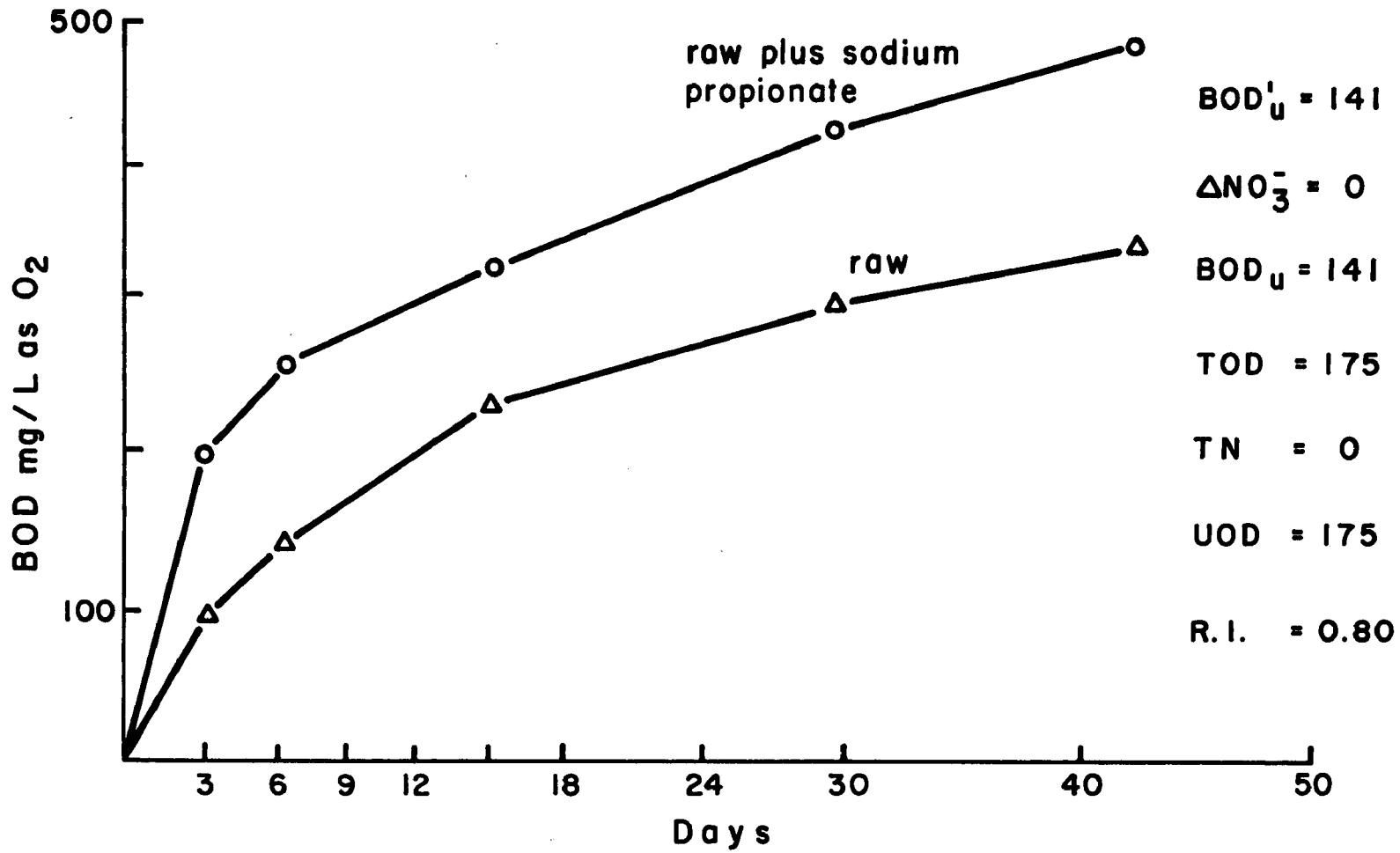



Figure C-35 OXYGEN UPTAKE FOR SODIUM PROPIONATE

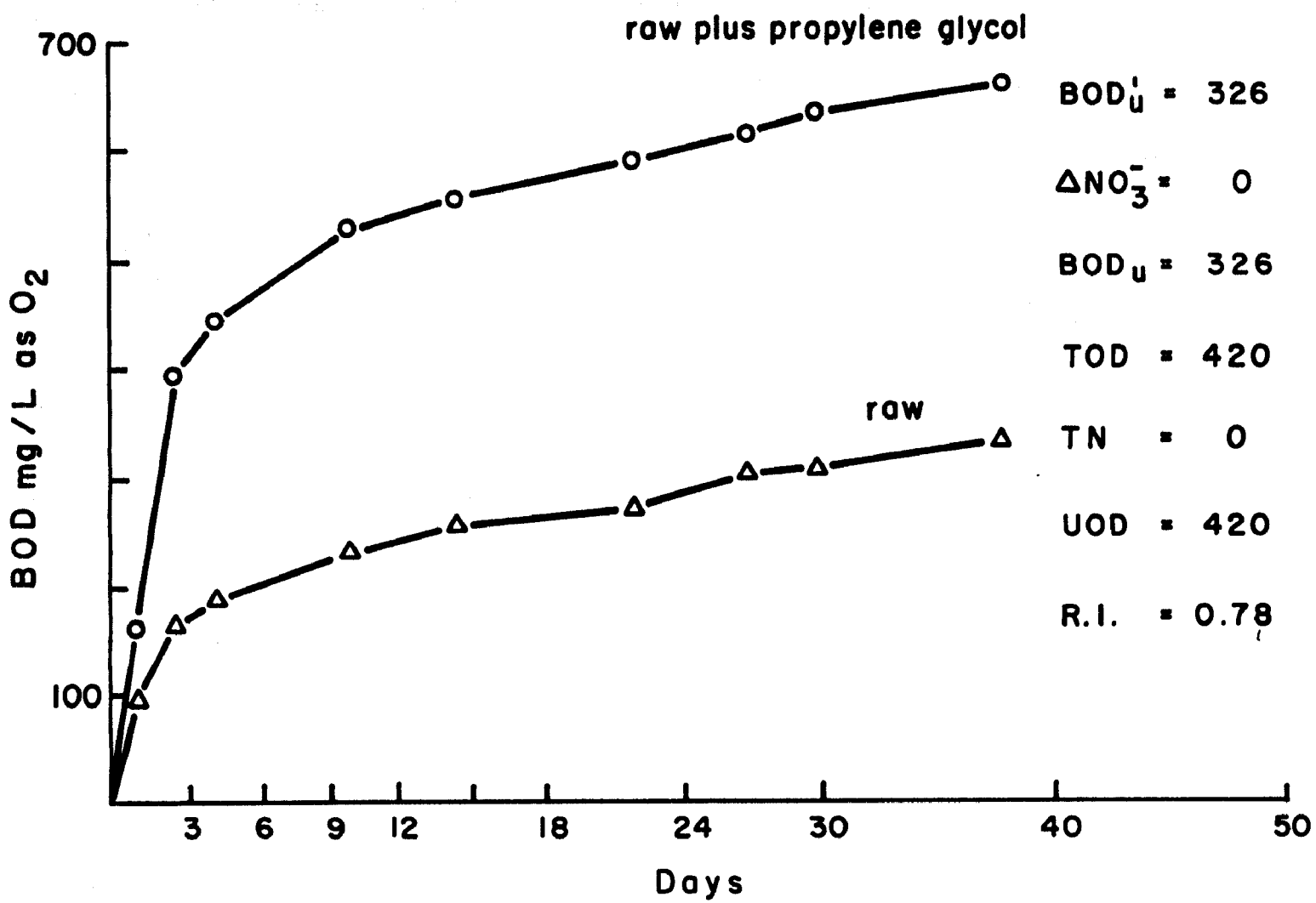



Figure C - 36 OXYGEN UPTAKE FOR PROPYLENE GLYCOL

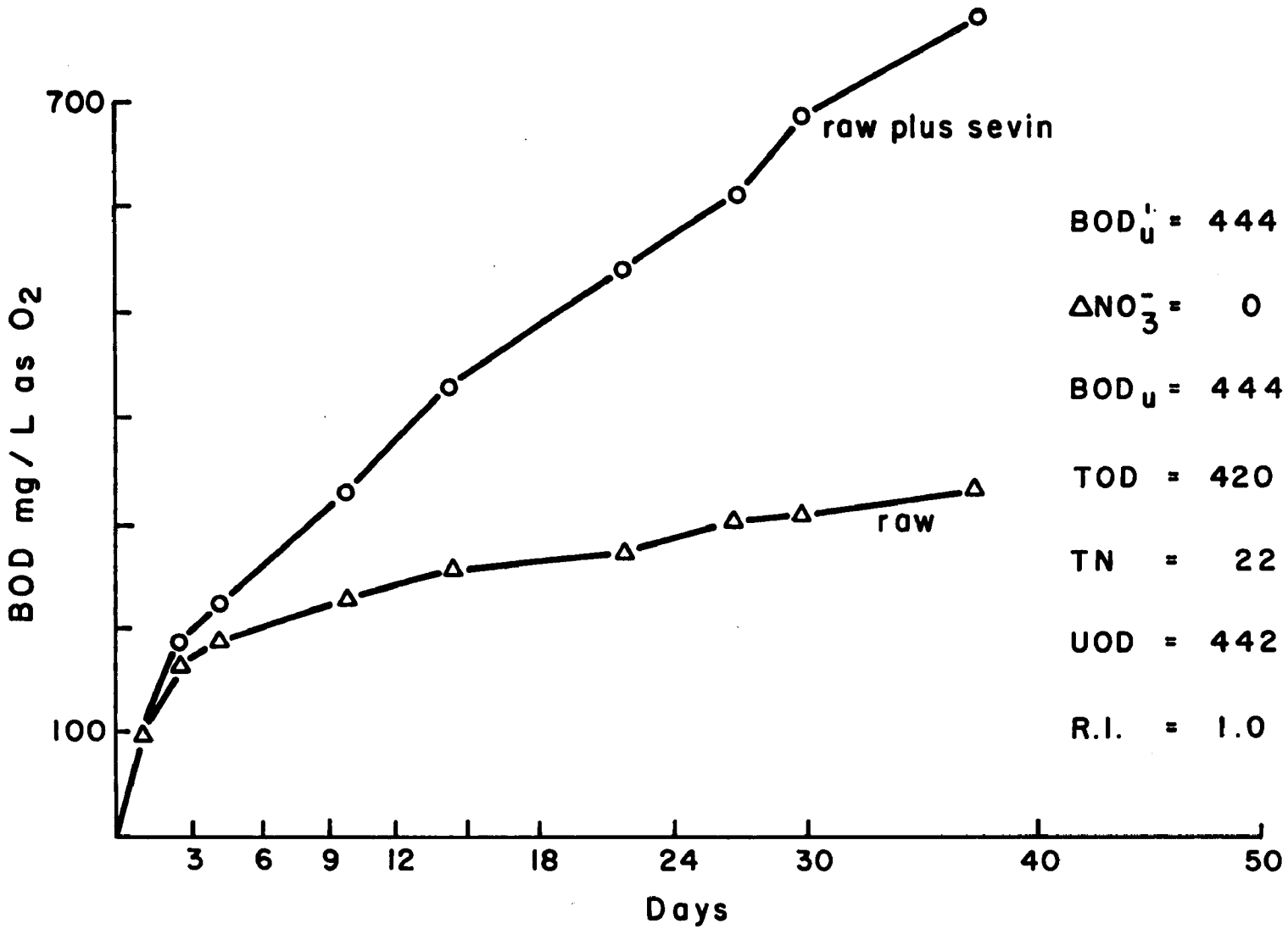



Figure C-37 OXYGEN UPTAKE FOR SEVIN WITH CARRIER

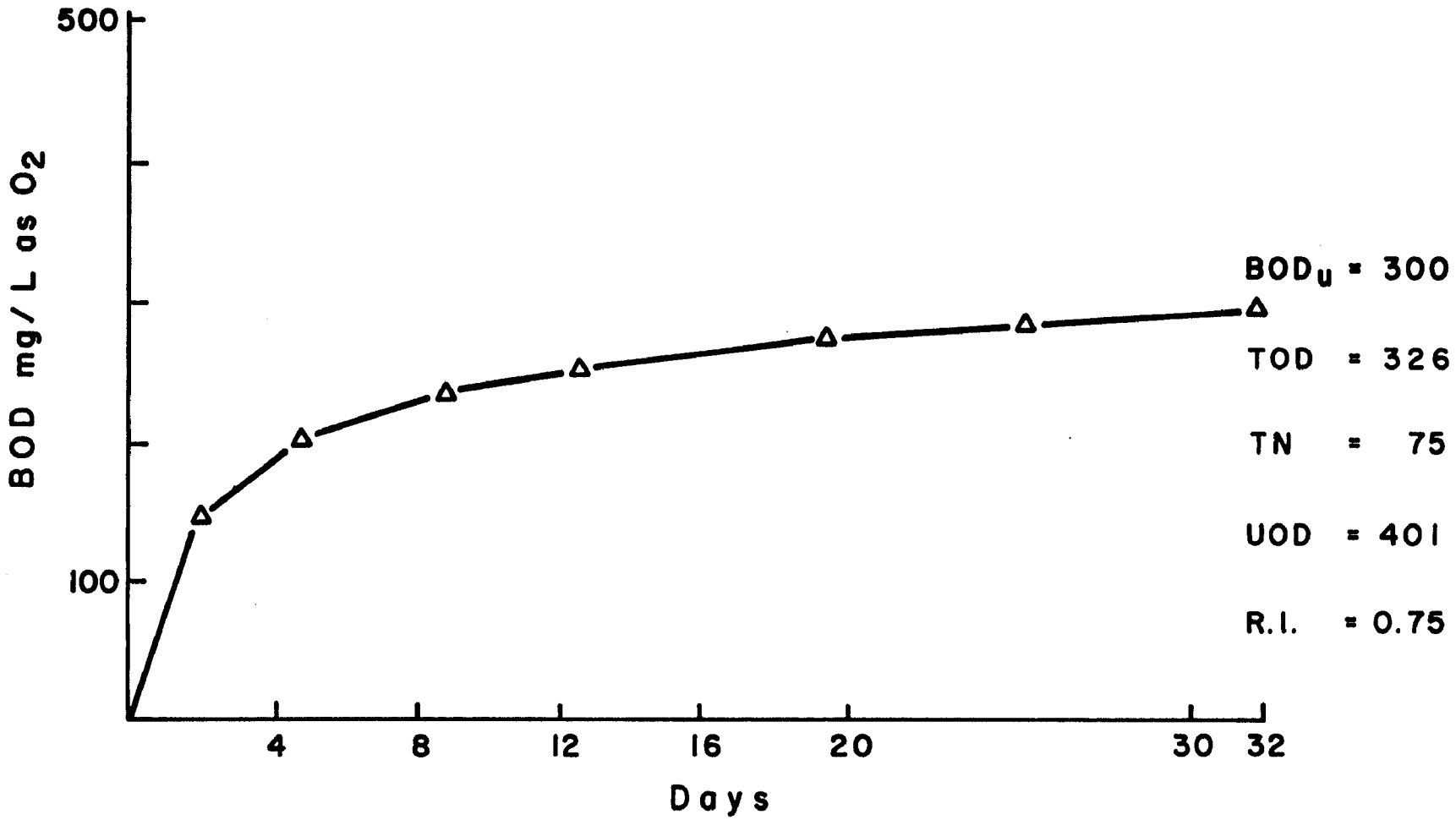



Figure C - 38 OXYGEN UPTAKE FOR RAW SEWAGE

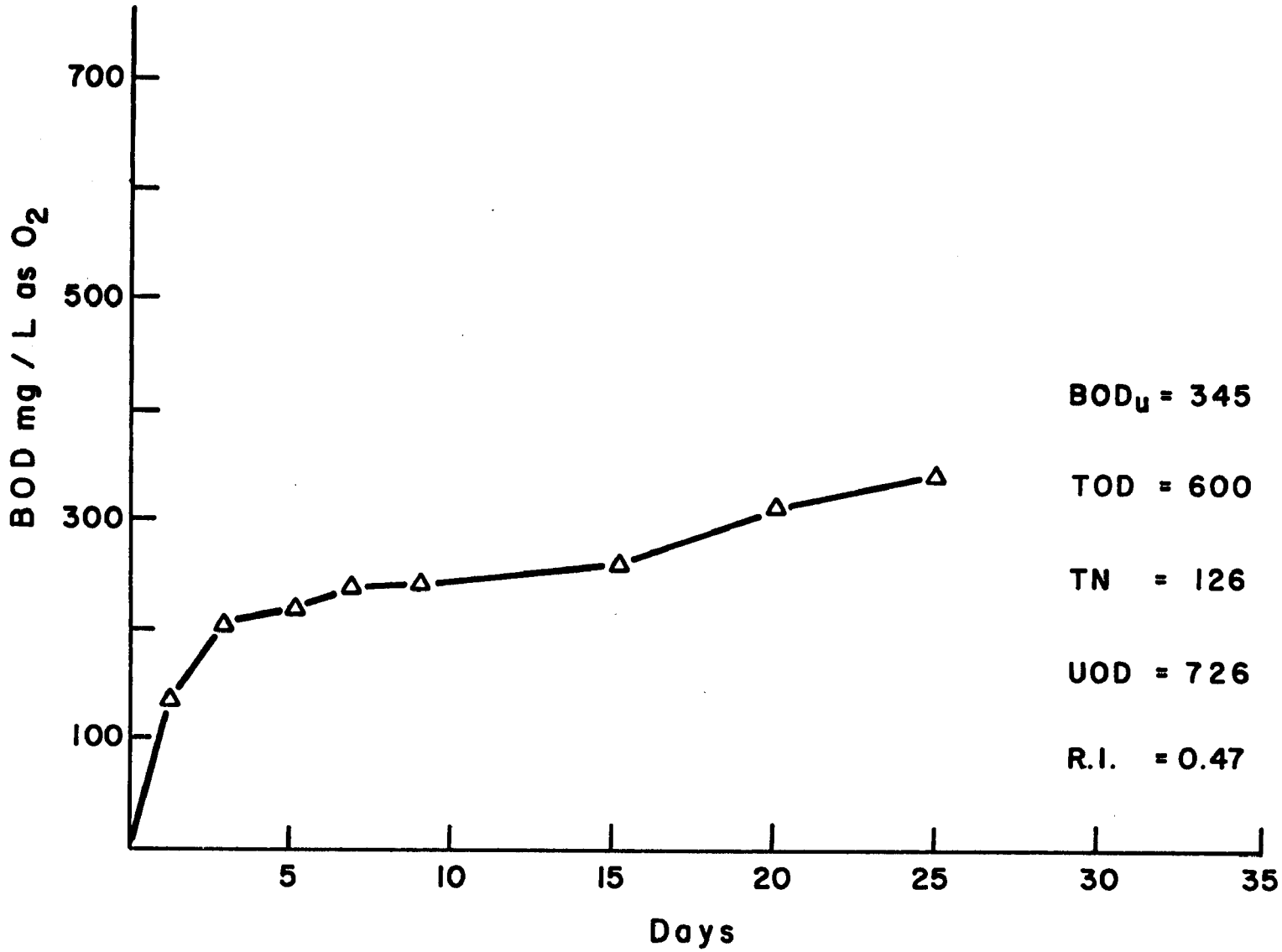



Figure C - 39 OXYGEN UPTAKE FOR RAW SEWAGE

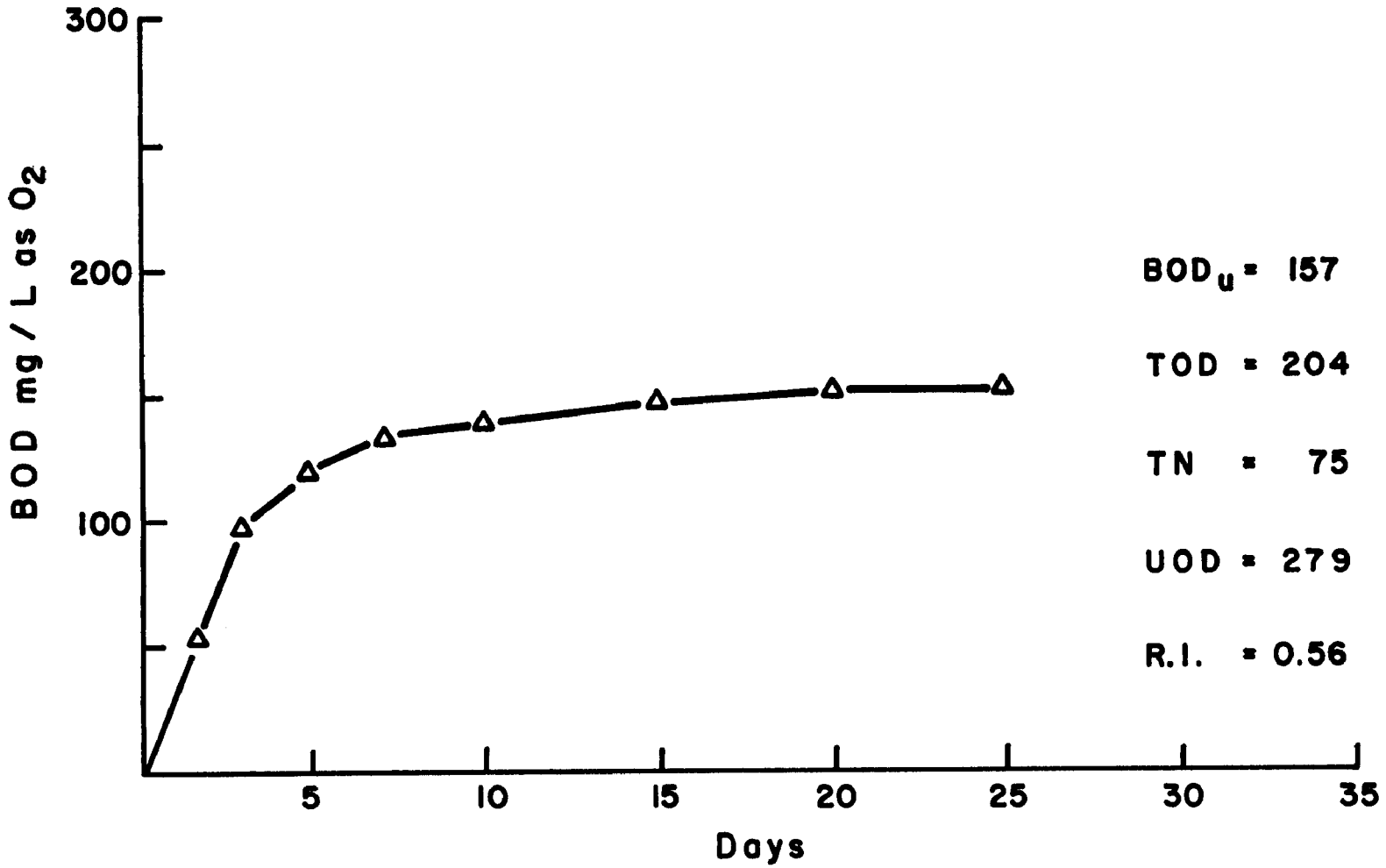



Figure C-40 OXYGEN UPTAKE FOR RAW SEWAGE

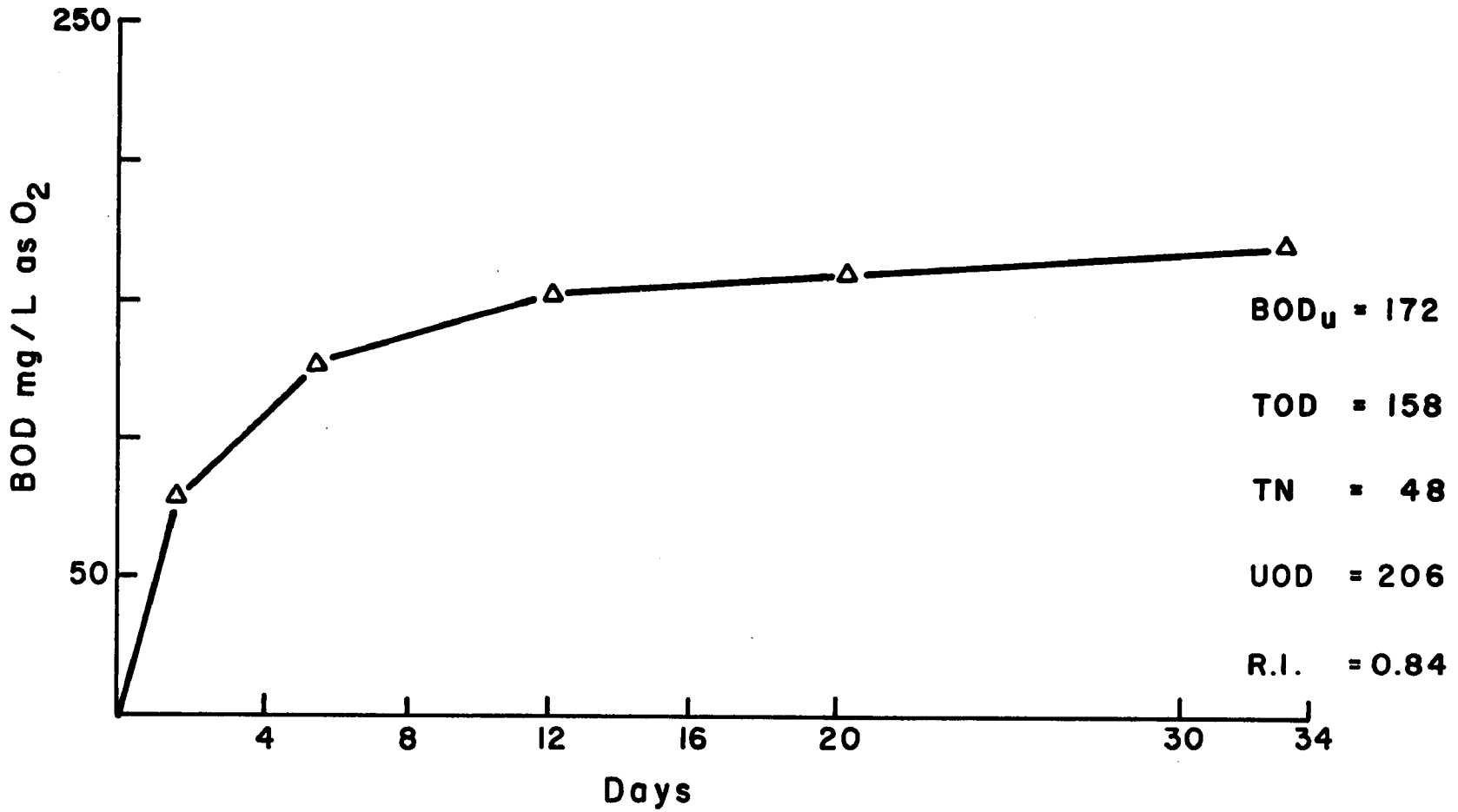



Figure C - 41 OXYGEN UPTAKE FOR RAW SEWAGE

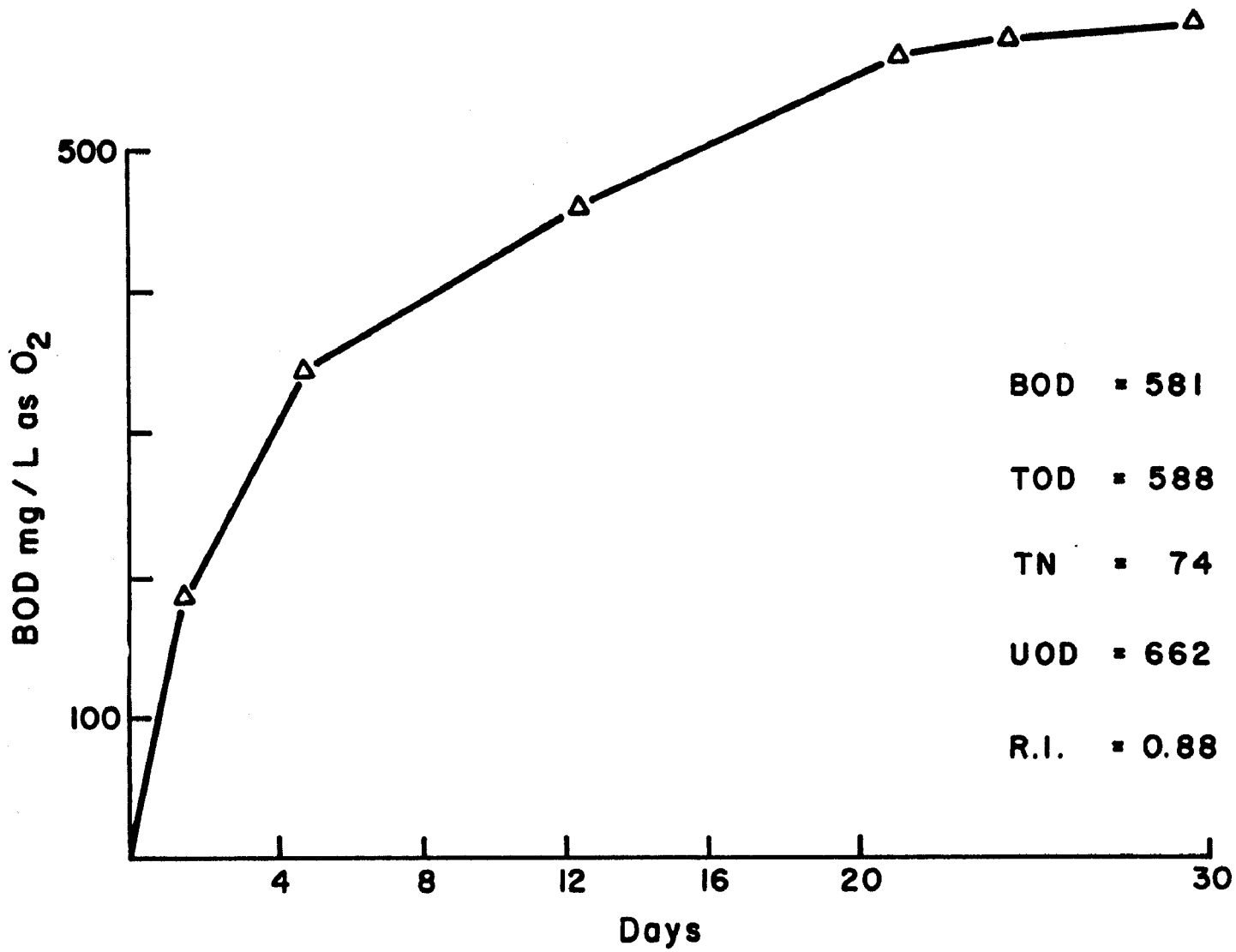



Figure C - 42 OXYGEN UPTAKE FOR RAW SEWAGE

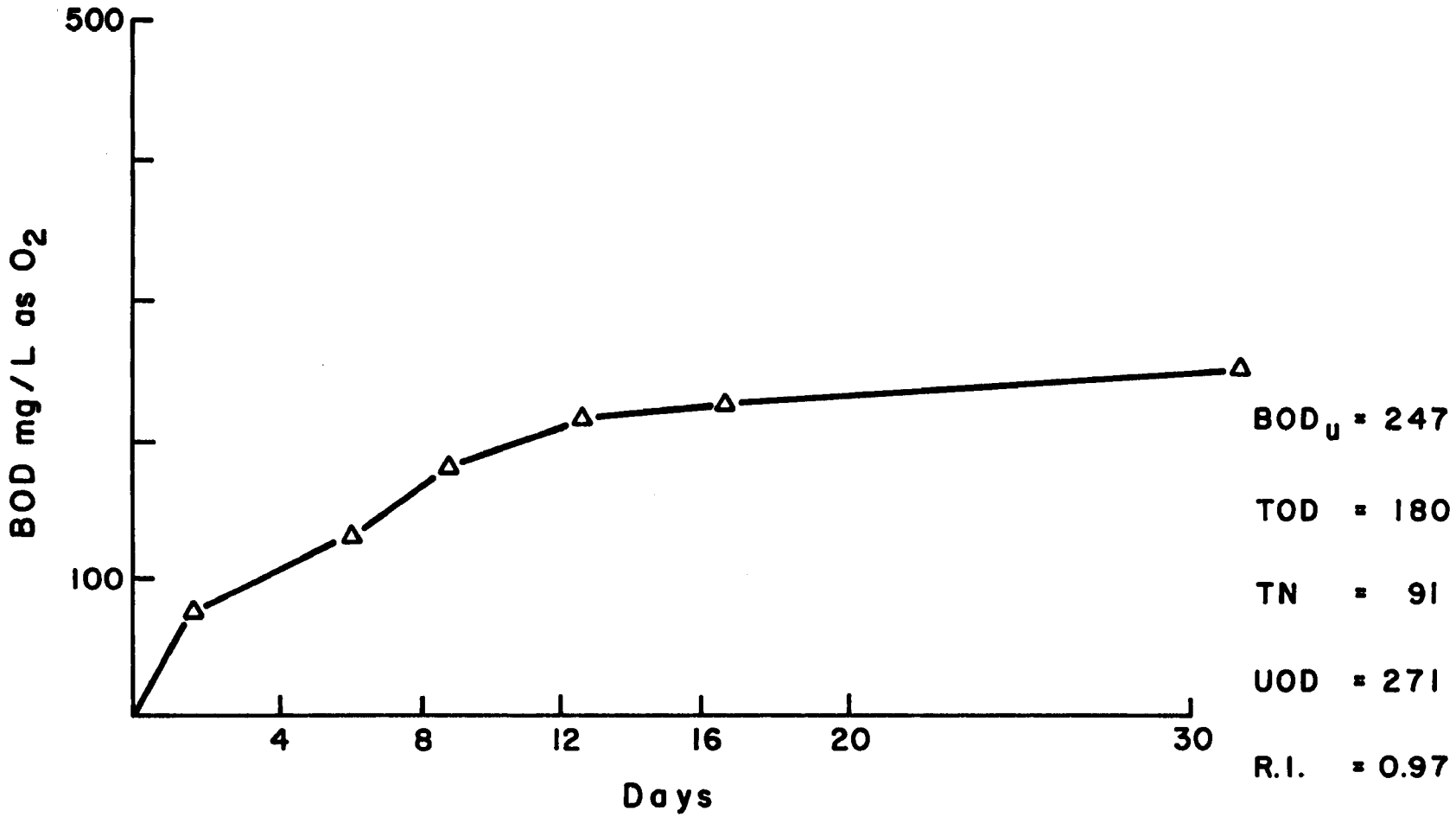



Figure C-43 OXYGEN UPTAKE FOR RAW SEWAGE

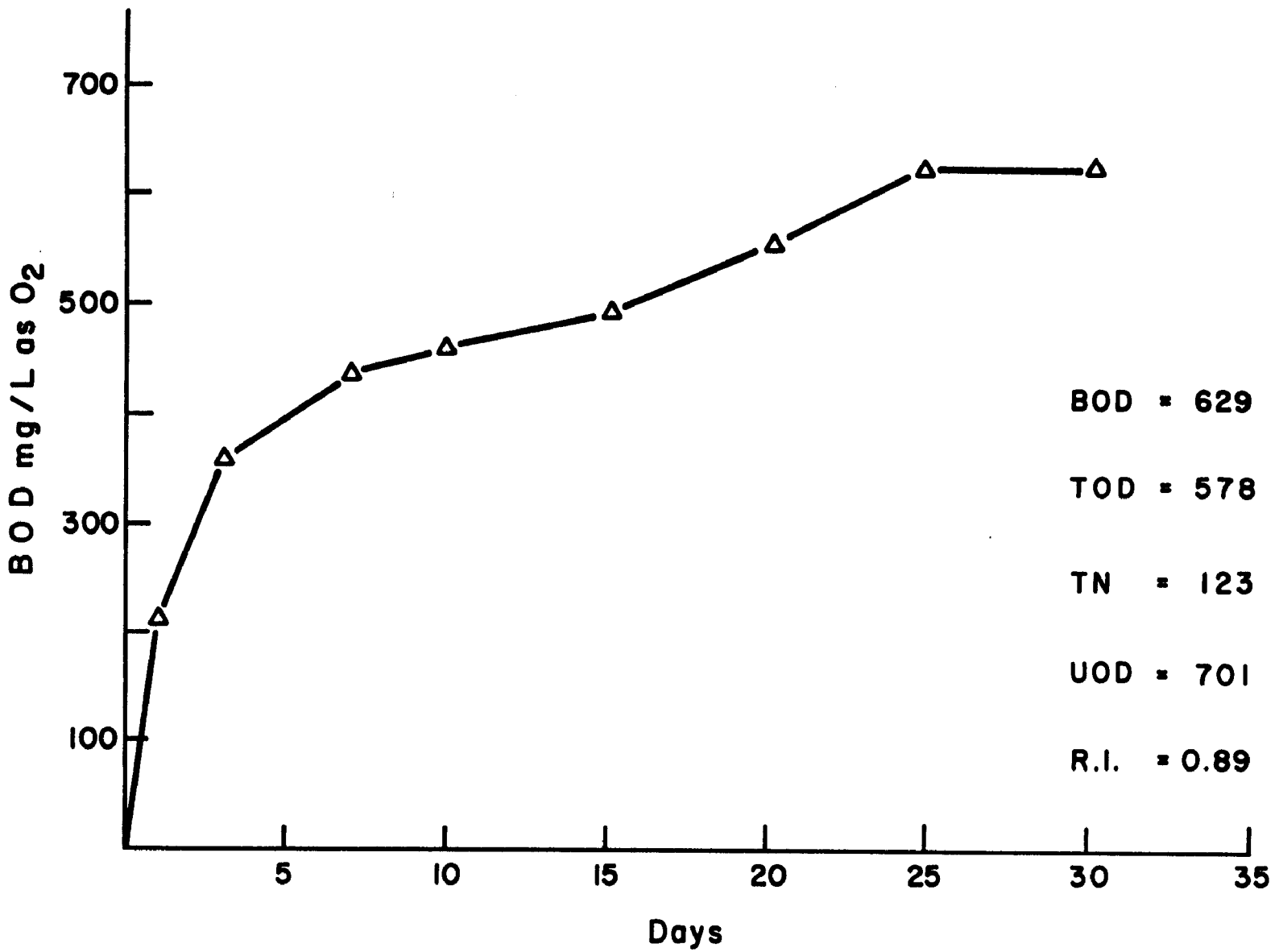



Figure C - 44 OXYGEN UPTAKE FOR RAW SEWAGE

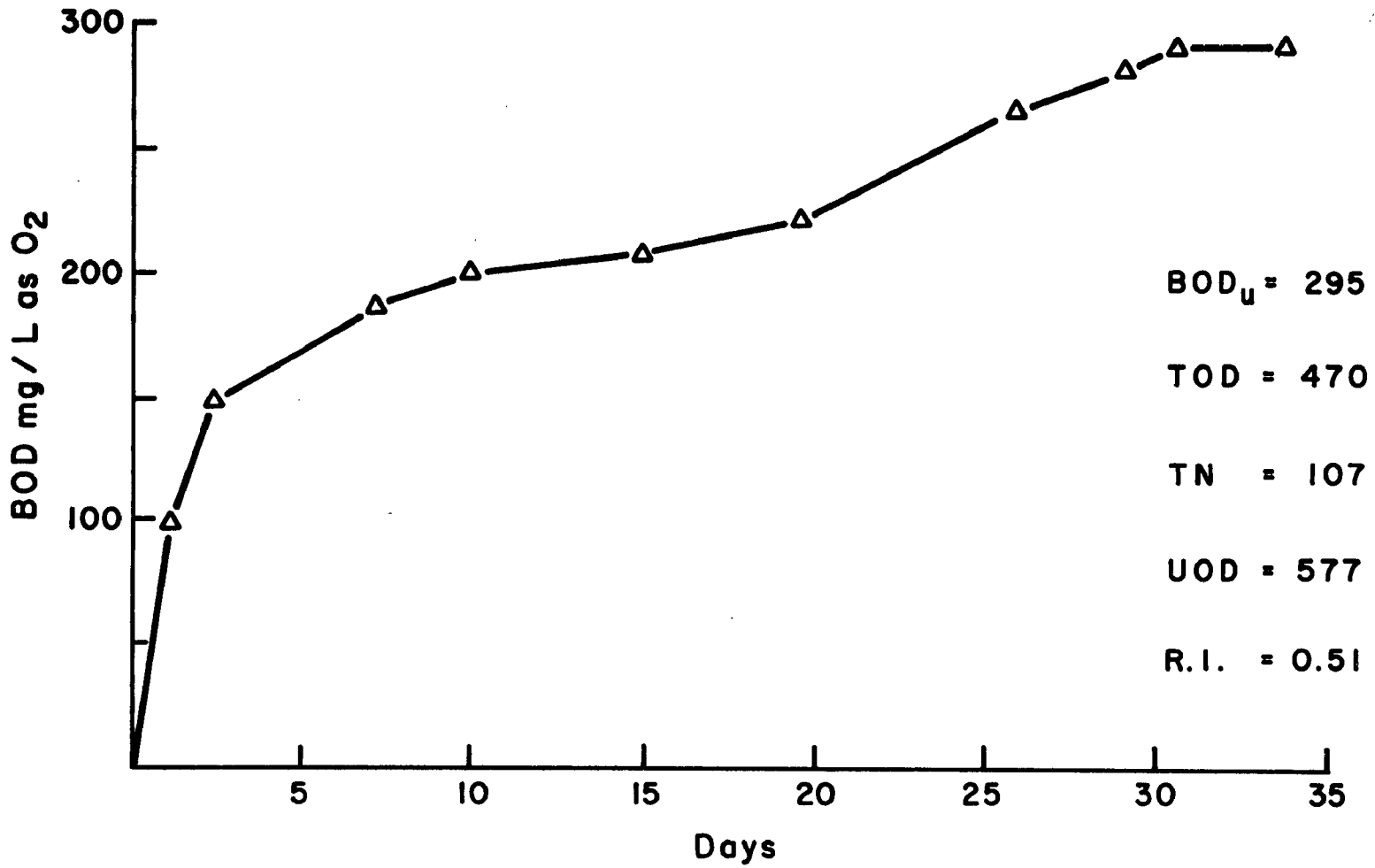



Figure C - 45 OXYGEN UPTAKE FOR RAW SEWAGE

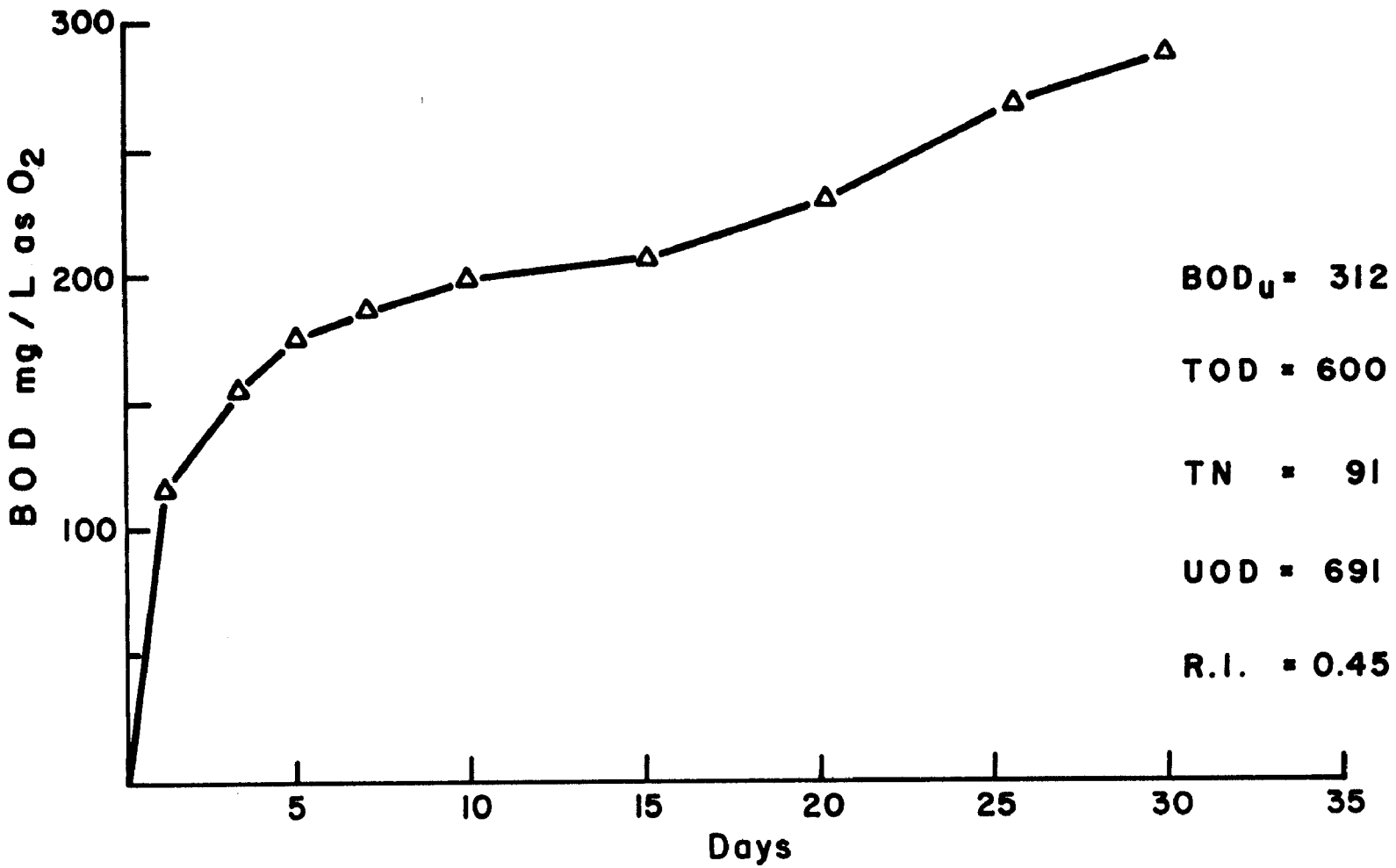



Figure C - 46 OXYGEN UPTAKE FOR RAW SEWAGE

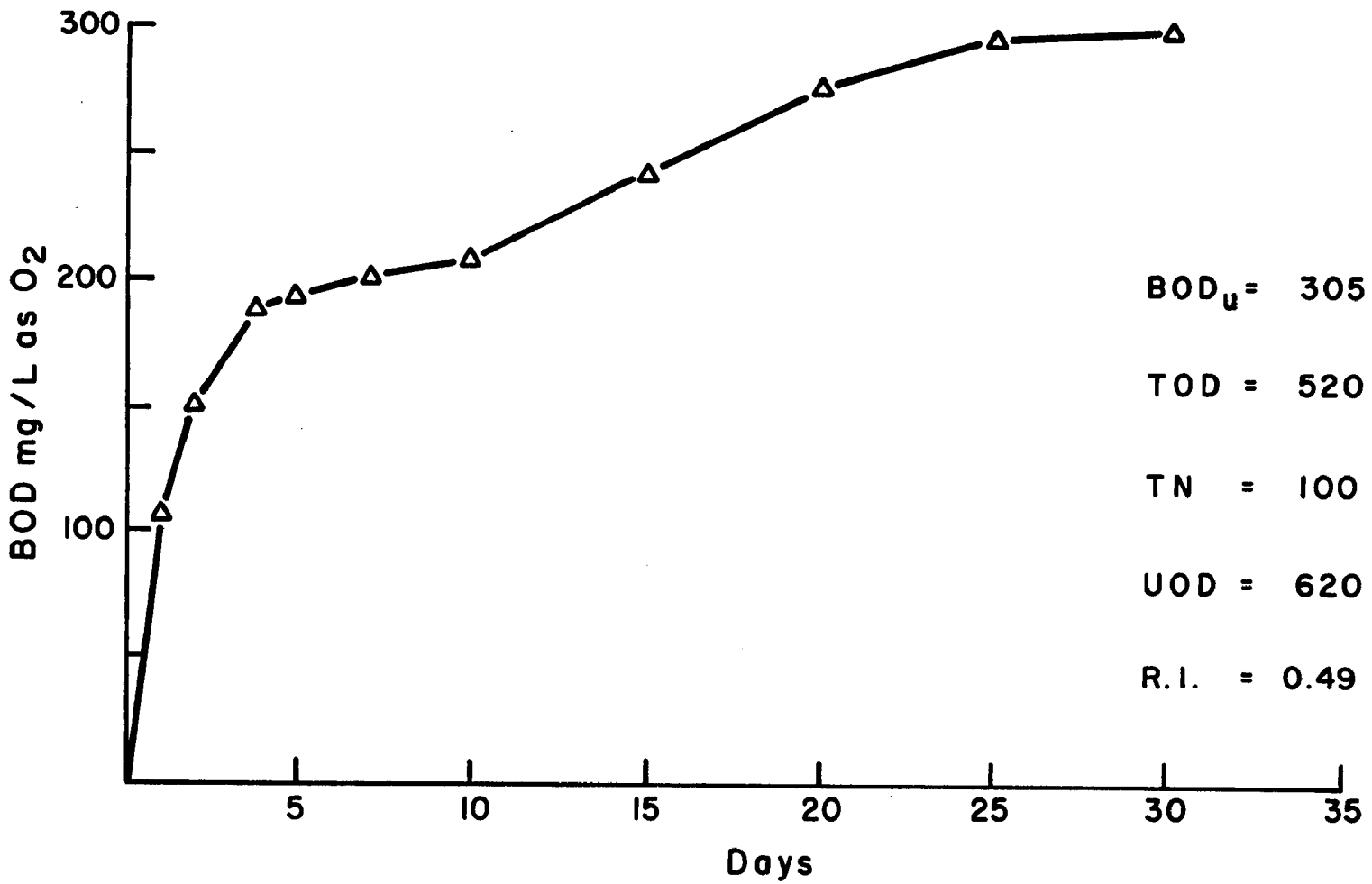



Figure C-47 OXYGEN UPTAKE FOR RAW SEWAGE

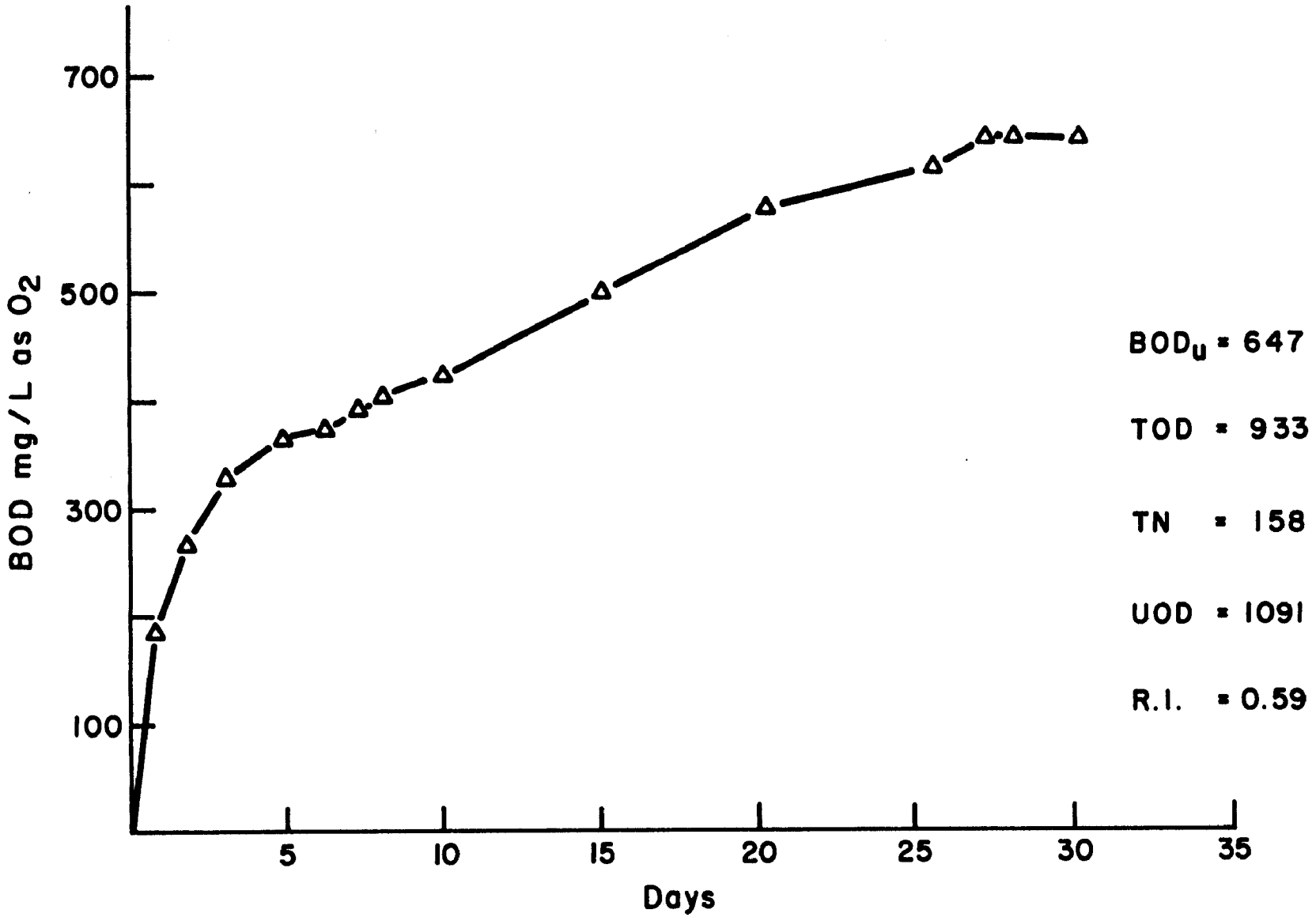



Figure C-48 OXYGEN UPTAKE FOR RAW SEWAGE

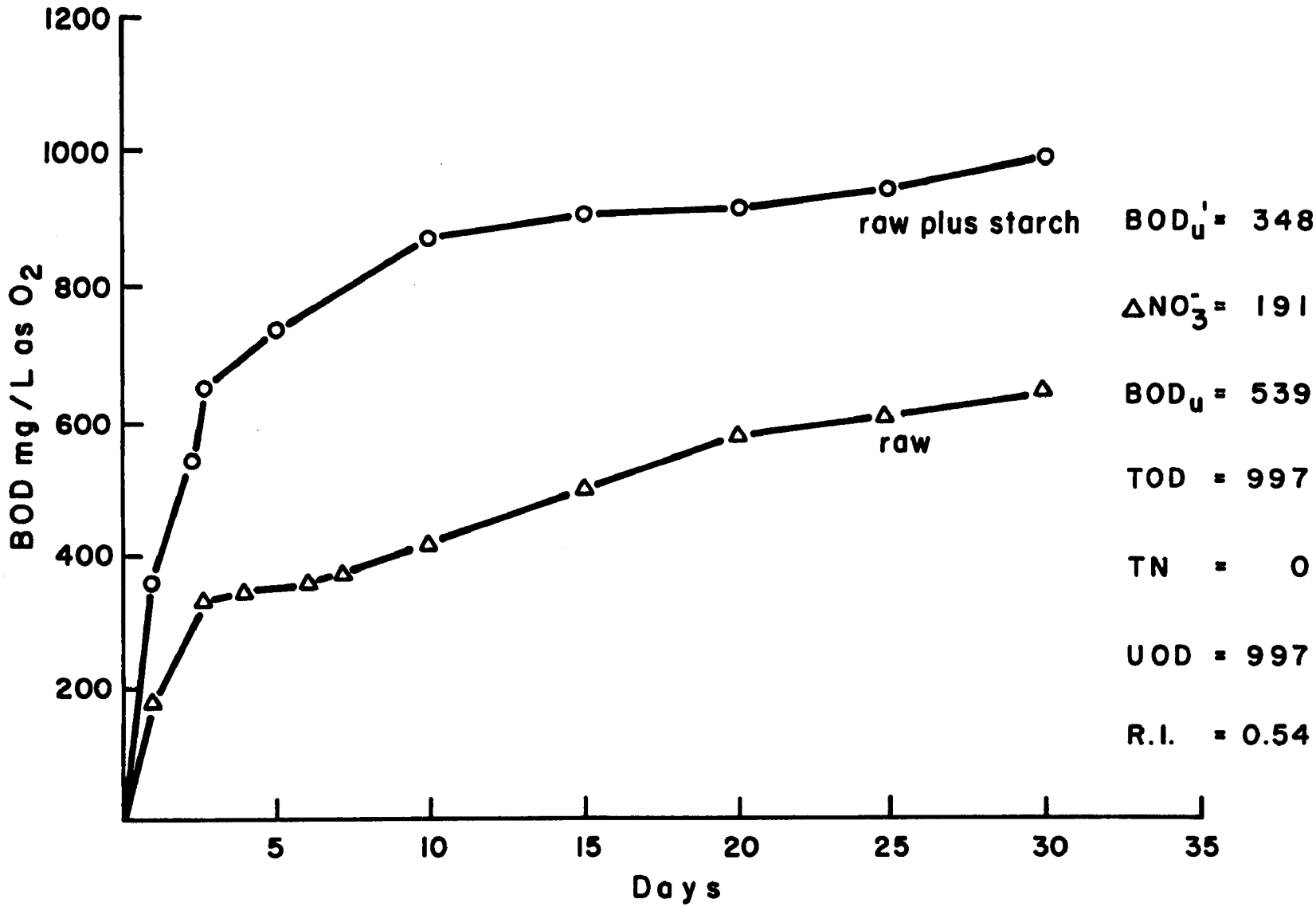



Figure C-49 OXYGEN UPTAKE FOR STARCH

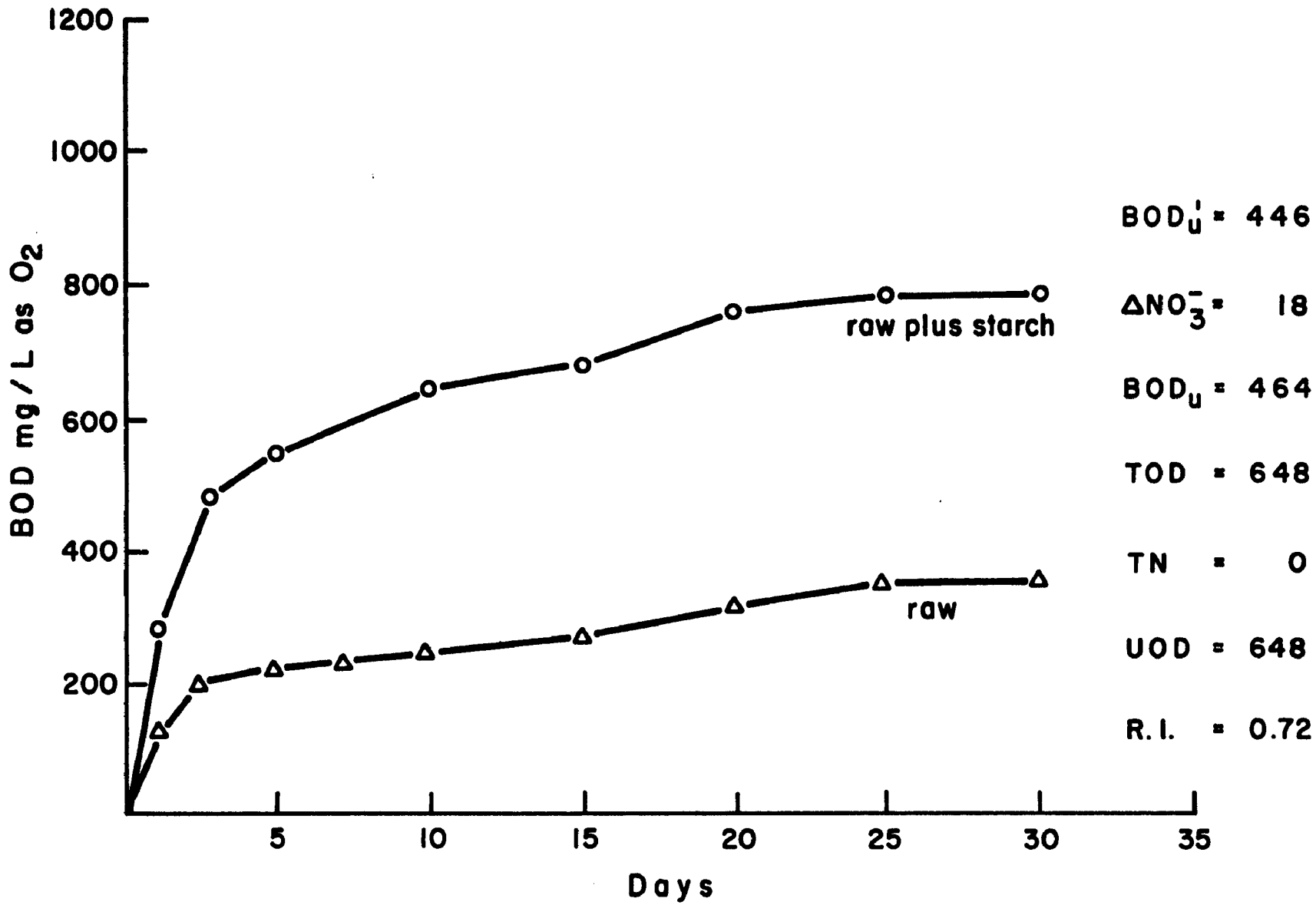



Figure C - 50 OXYGEN UPTAKE FOR STARCH

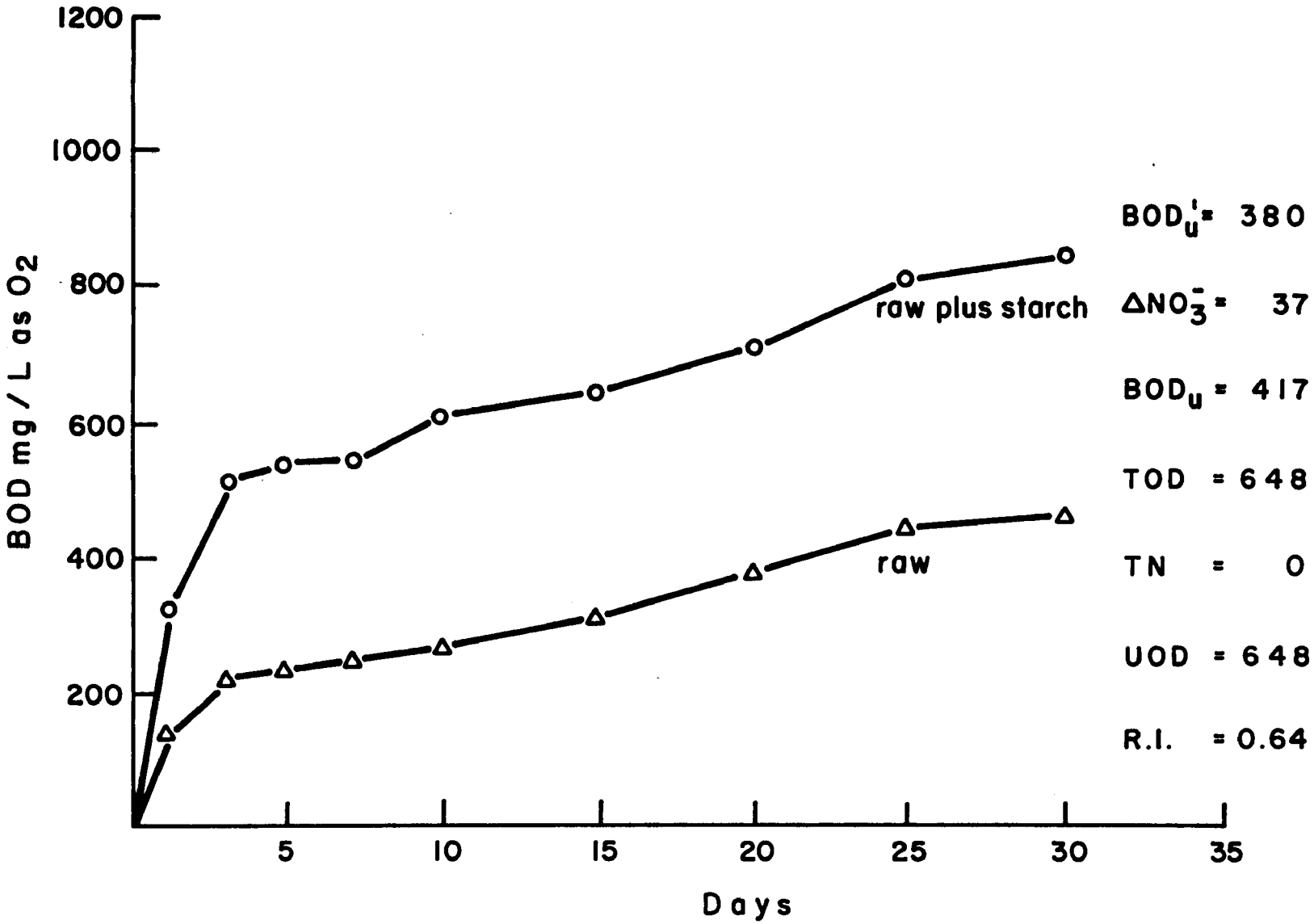



Figure C-51 OXYGEN UPTAKE FOR STARCH WITH ADDITIONAL AMMONIA

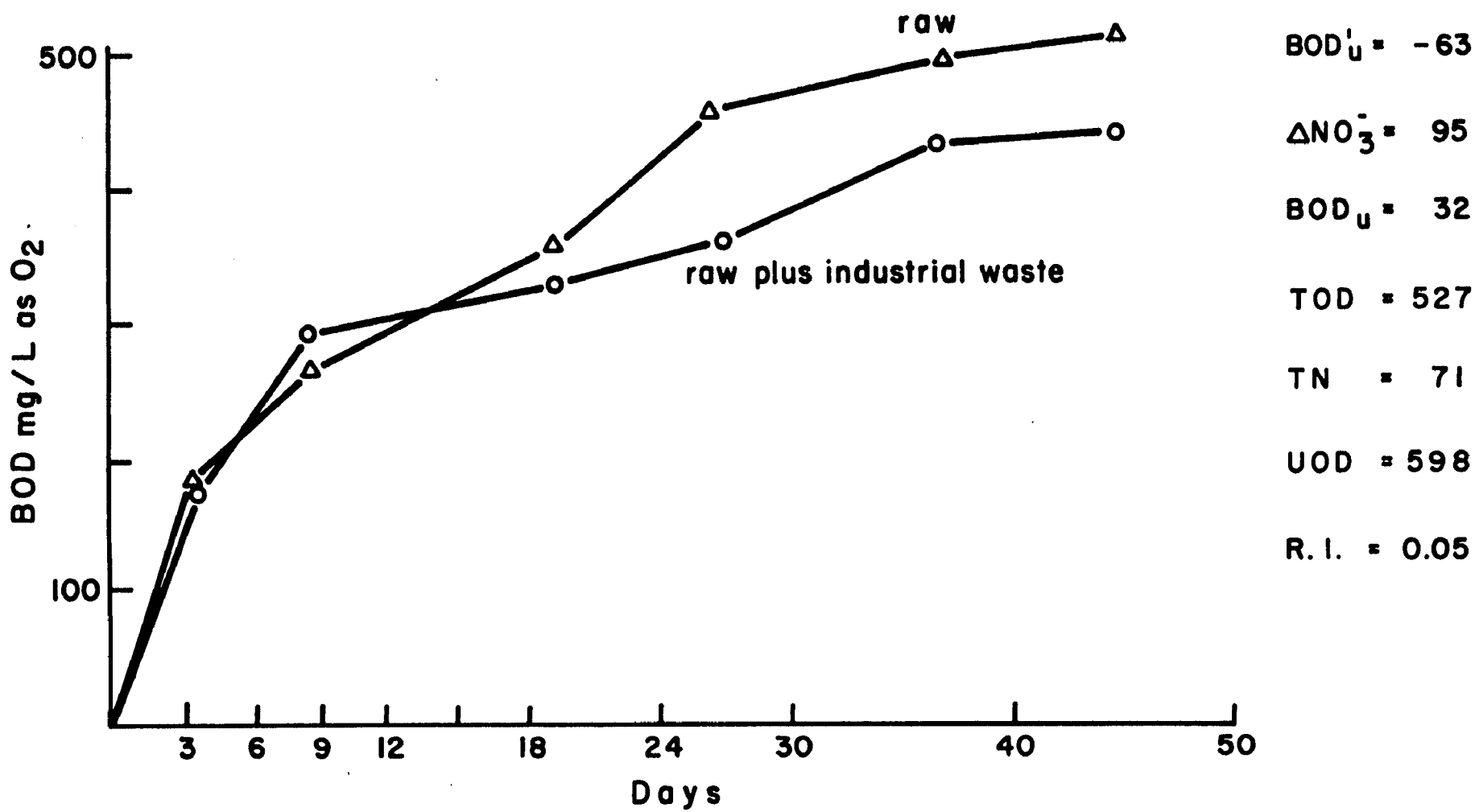



Figure C-52 OXYGEN UPTAKE FOR INDUSTRIAL WASTE
TEXTILE DYE

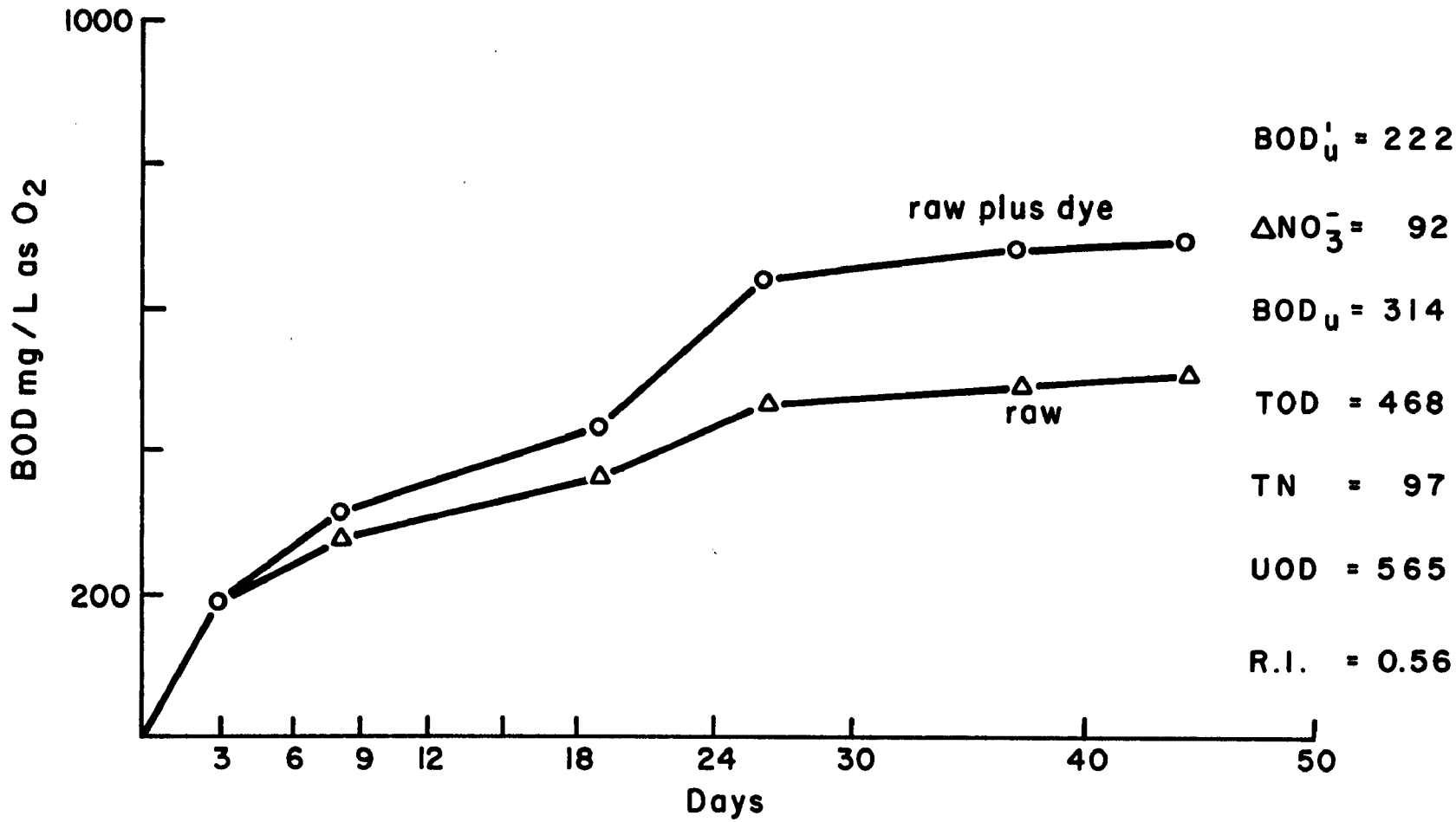
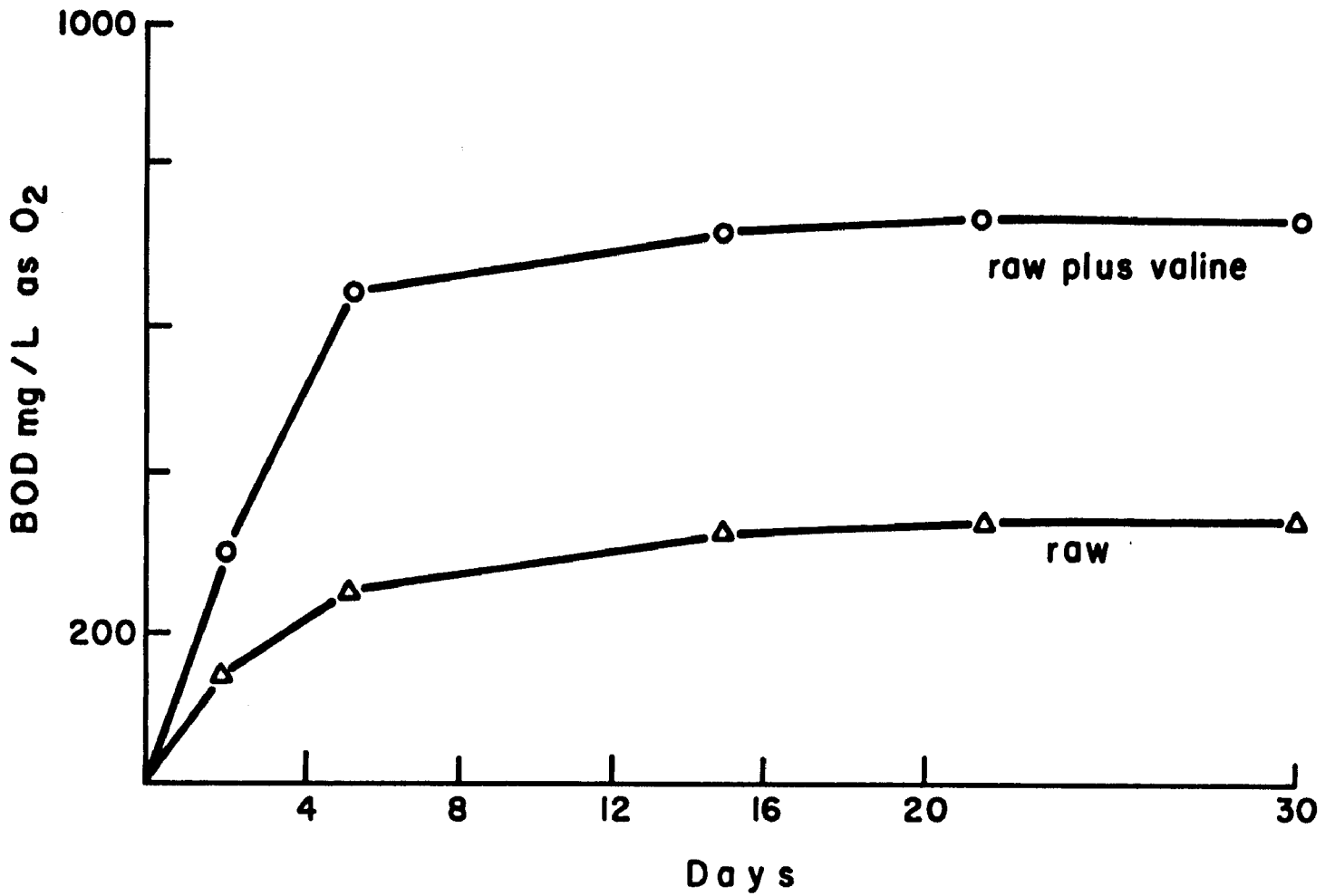



Figure C - 53 OXYGEN UPTAKE FOR DYE , TEXTILE




$BOD_u' = 392$

$\Delta NO_3^- = -46$

$BOD_u = 392$

TOD = 371

TN = 51

UOD = 422

R.I. = 0.93

Figure C -54 OXYGEN UPTAKE FOR VALINE

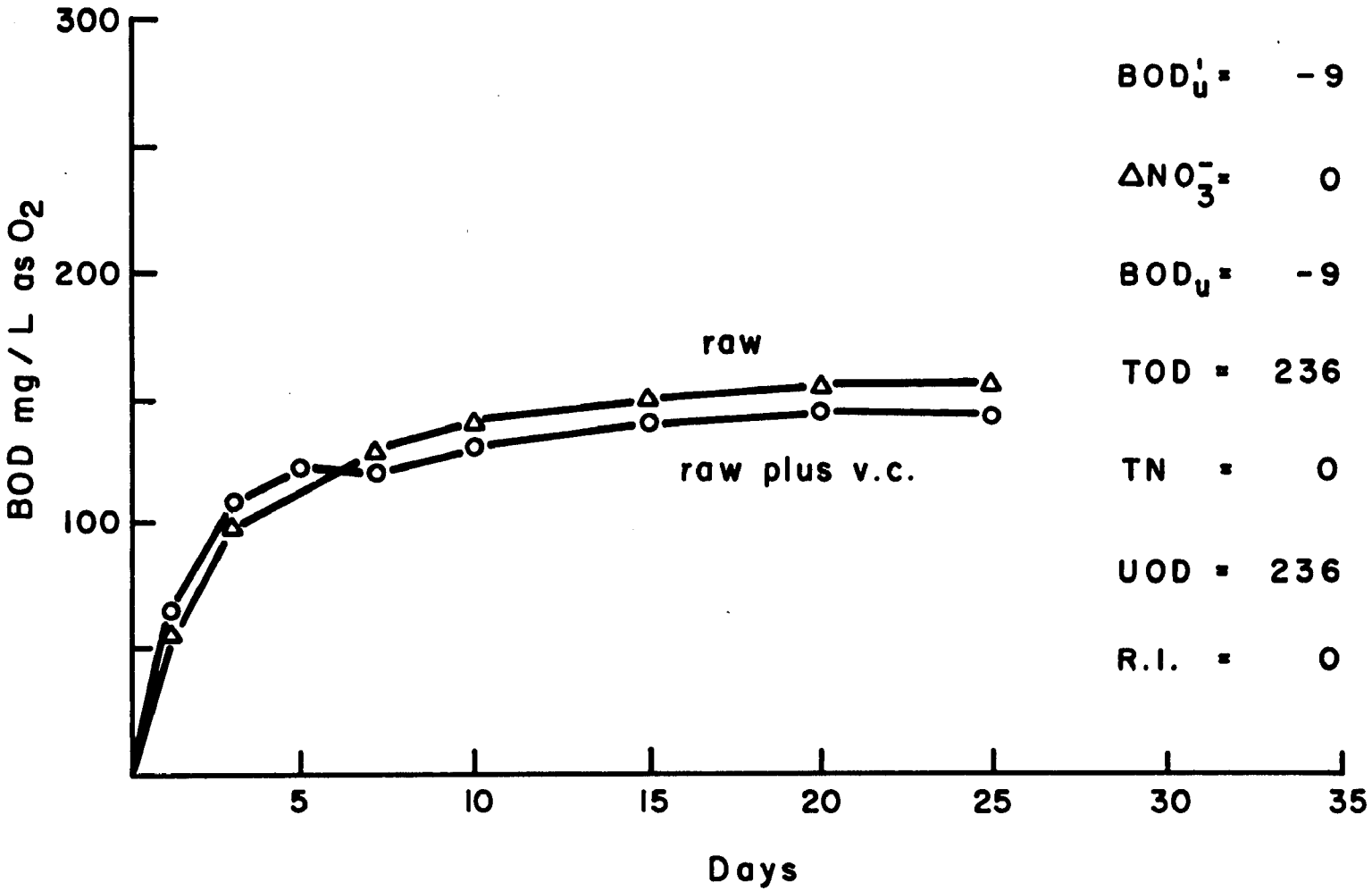



Figure C - 55 OXYGEN UPTAKE FOR VINYL CHLORIDE

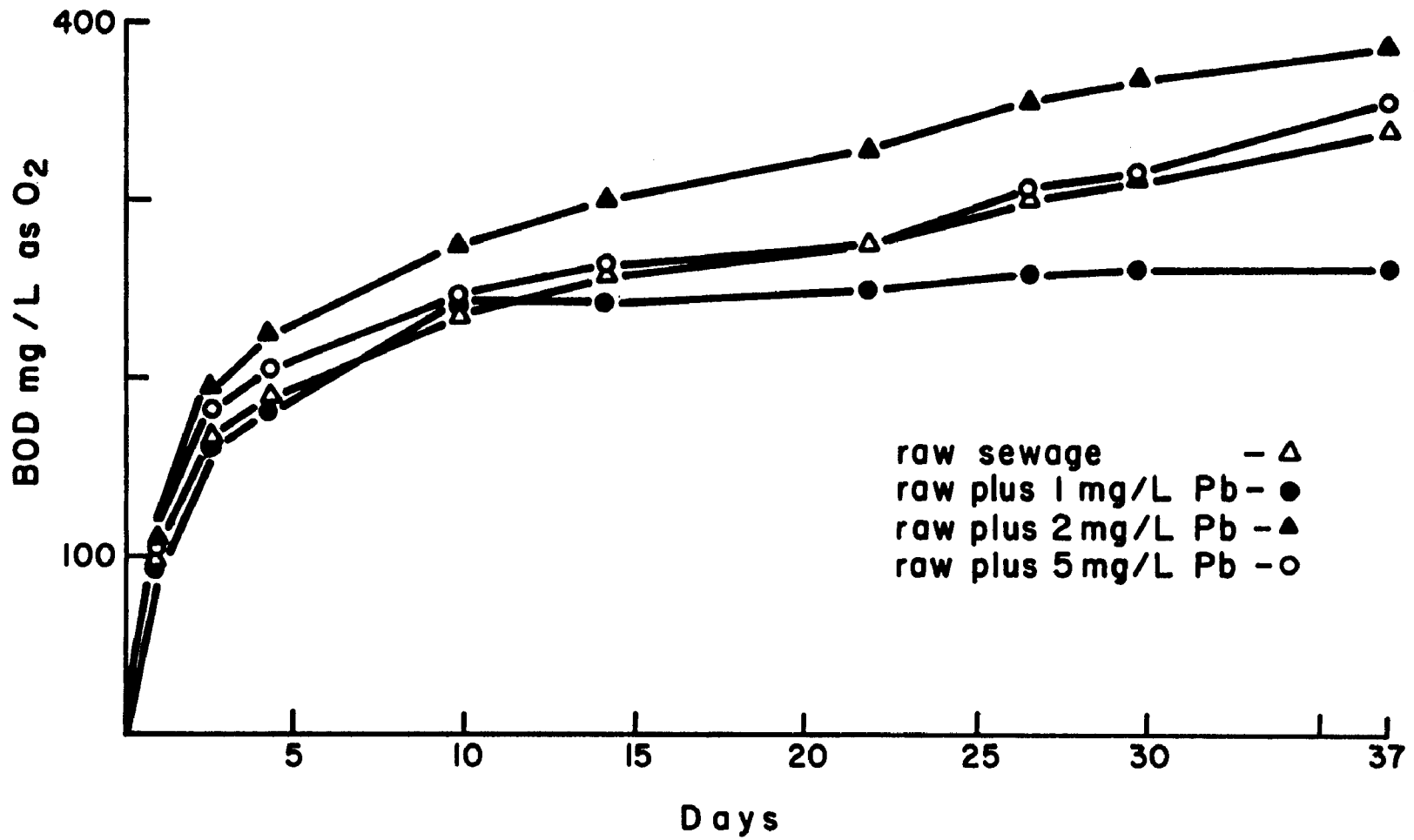



Figure C - 56 OXYGEN UPTAKE FOR LEAD

ITEM D

# SUGGESTED PRETREATMENT REQUIREMENTS FOR INDUSTRIAL WASTEWATERS PREREQUISITE TO DISCHARGE INTO MUNICIPAL SEWAGE TREATMENT PLANTS

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## 1. PREFACE

Before certain industrial wastes can be admitted into conventional sewage treatment plants, they need, in most cases, to be pretreated. This is especially true if the municipal treatment has a biological reactor (trickling filters or activated sludge which is even more sensitive to inhibitory materials) as part of the train of unit operations and processes used to treat the wastewater to achieve required standards. The various forms of biological treatment are easily disrupted. However, fixed media bioreactors and completely mixed activated sludge are relatively more stable.

When biological treatments do not operate at high efficiency, they act as vehicles for the transport rather than the degradation of certain refractory materials, discharging these pollutants into the receiving aquatic environment. Not all wastes are amenable to treatment by any one unit step. Usually a series of unit operations and processes are necessary for managing the heterogenous constituents found in almost all wastewaters. Two other alternatives should be noted:

a. There are a few industrial wastewaters of relatively simple and invariant composition, but these are the exceptions.

b. Physical-chemical treatment trains of unit steps can often manage heterogenous wastewaters more effectively than conventional treatment with its sensitive biological reactors.

Conventional plants are too often used as pipelines from the factory to the receiving waters. Restraints based on the classical parameters of $BOD_5$ (5-day Biochemical Oxygen Demand, mg/L $O_2$) and Suspended Solids (mg of filterable solids per liter) can be easily evaded in some cases if the idiosyncrasies of these analytic techniques are known. In addition, charges to industry based on volumetric flow may not reflect the cost of treating a difficult waste.

The wastewaters that are most amenable to treatment by conventional biological sewage treatment are characterized by readily settleable solids, degradable and sorbable organics of not too high biochemical oxygen demand, such as a typical household waste with roughly 200 to 400 mg/L $BOD_5$. Biologically treatable wastes should have nutrient balance; deficiencies in nitrogen, phosphates, potassium or any of the 16 or so essential nutrients will limit biological treatment effectiveness. Normal municipal waste has an excess of nitrogenous and phosphate nutrients beyond what is necessary to metabolize the degradable carbonaceous load of the waste. Sometimes, therefore, the addition of carbonaceous BOD from industrial wastes, such as food processing or those containing altered hydrocarbons, brings the municipal wastewater into better nutrient balance. This situation is, however, rarely realized with most industrial waste discharges into municipal treatment plants.

## 2. CRITERIA

The industrial influent to a sewage treatment plant should resemble municipal waste in having a $BOD_5$ of 150 to 650 mg/L $O_2$. That is, it should be neither too dilute nor too concentrated in order not to alter the concentration of $BOD_5$ entering the plant to a value outside of this range. If the receiving municipal plant has a biological treatment unit process, the industrial wastewater should not contain refractory organics; although nonbiodegradable organic constituents do not fully register as BOD, they are serious environmental pollutants.

An industrial wastewater should not have a long-term biochemical oxygen demand ($BOD_{20}$ days) in excess of twice the $BOD_5$:

$$BOD_{20} < 300\text{-}1200 \text{ mg/L}$$

or the Total (Theoretical) Oxygen Demand (TOD) for carbonaceous material should not be significantly greater than the extrapolated ultimate BOD by a factor of 1.0 or 1.1:

$$TOD/BOD_{ultimate} < 1.1 \text{ (ideally 1.0)}$$

The presence of certain inhibitory or toxic constituents (like heavy metals, pesticides, certain chlorinated hydrocarbons) can make ordinarily biodegradable organics refractory. That is, these constituents alter the amenability of ordinary waste to biological treatment. Therefore, restraints on the discharge of certain industrial pollutants are necessary.

Table A is a list of pollutional constituents that need to be considered. It includes suggested acceptable concentrations or ranges of concentrations for each constituent listed. There are always extenuating circumstances in individual cases for being above or below this listed

concentration or range; standards are a guide, not a substitute for developed judgment.

It is nearly impossible to monitor for all possible inhibitors and toxics, especially since synergistic and antagonistic responses are complex. It is therefore, necessary to have a measure of toxicity of inhibition. Unfortunately, most of the toxicity tests now available take too long to perform for real-time evaluation of wastes. These toxicity tests are usually done on fish (two or more days) or by means of modified BOD ( 5 to 20 days) tests.

Most wastes contain, in addition to carbonaceous materials, nitrogenous pollutants such as proteins, alkaloids, peptides, ammonia, nitrates nitrites and/or cyanides. A ratio of the ultimate oxygen demand, expressed as TOD plus oxidizible nitrogen, divided by the ultimate BOD (Nitrogenous plus carbonaceous) is a more complete parameter for evaluating the total environmental insult to oxygen resources.

$$(TOD + TN)/BOD_u \lesssim 1.1$$

Since bacterial growth is always associated with organic degradation, the TOD and TN cannot equal the ultimate biochemical oxygen demand, there always being a residual of bacteria, organic nutrients, refractory materials and bacterial by-products.

Another criterion for evaluating industrial wastes is the refractory index (R.I.), a quantitative measure of refractory organics. The R.I. is defined as the ratio of ultimate BOD to ultimate TOD plus TN:

$$R.I. = BOD_u/(TOD + TN)$$

TABLE A

SUGGESTED LIMITS AND RANGES
FOR INDUSTRIAL WASTEWATER POLLUTANT INPUTS
TO MUNICIPAL SEWAGE TREATMENT PLANTS

| Constituent | Suggested Acceptable Limit and Range mg/L |
|---|---|
| Mercury | 0.0 - 0.05 |
| Manganese | 1.0 - 2.0 |
| Cadmium | 0.1 - 0.2 |
| Aluminum | 0.2 - 0.5 |
| Barium | 1.0 - 2.0 |
| Chromium $Cr^{VI}$ | 0.05- 0.1 |
| $Cr^{III}$ | 0.25- 0.5 |
| Copper | 0.2 - 0.5 |
| Lead | 0.05- 0.1 |
| Nickel | 1.0 - 2.0 |
| Silver | < 0.05 |
| Zinc | 0.5 - 0.05 |
| Total Metals | < 1.0 |
| Pesticides | * |
| Free and Combined Chlorine | 5 - 10 |
| Cyanides | 0.03- 0.1 |
| Fluorides | < 20 |
| Total Dissolved Solids | <3000 |

*Varies with specific pesticide.

Note - Metal concentration should be based on analysis of filtered waters. Many problem materials cannot be specified out of context of the industrial waste and type of treatment plant (e.g., blood from diseased persons and formaldehyde).

The interpretation of R.I. values is given in Table B:

TABLE B

INTERPRETATION OF REFRACTORY INDEX VALUES

| R.I. | Interpretation |
|---|---|
| 1.0 | Degradable |
| 0.2 - 0.80 | Stable Intermediates |
| 0 | Refractory |
| Negative* ( <0) | Inhibitory |

*Negative R.I. values provide only a qualitative indication of inhibition.

In the case of negative R.I. values, bioassays should be done as well as detailed analyses for special organic or inorganic materials that interfere with normal biodegradation.

The pH of industrial waste should be near neutral:

$$pH = 5.5 - 9.5$$

but, more important, it should be buffered in such a way that the treatment response at the sewage plant is not altered. Caustic alkalinity and mineral acidity should be decreased by neutralization processes.

Suspended solids offer buffering-like responses and impose a difficult loading and sludge management problem if applied to conventional treatment plants in large concentrations; therefore, there should be restrictions on industrial waste suspended solids input to conventional plants.

$$\text{Suspended Solids} \ll 350 \text{ mg/L}$$

Determination of settleable solids is both an easier test to perform and a more intuitively logical parameter to understand. It can be used to estimate precipitation loading for sludge management handling and is performed quickly.

There are many aesthetically offensive, obviously hazardous materials and gross indirect parameters that are easy to spot which indicate that restraints should be applied to the discharge of industrial wastewaters. These include:

Visible Oil Sheen
Floating Solids
Color (Unusual)
Horrendous Odor (Phenols, $H_2S$, $CN^-$, etc.)
Dispersing Agents
Materials that can cause clogging of pipelines
Foam
Gasoline, benzene, naphtha, fuel oil and other flammable or explosive materials
Viscous Solids
Aerosols and volatile materials

There are traditional restrictions on wastes:

| | |
|---|---|
| Phenols | ng range |
| Coliform Bacteria | varies |
| Pathogenic Microorganisms | -0- |
| Turbidity | < 200 JTU |
| Dissolved Oxygen | > 1.0 mg/L |
| Chlorine Residual | < 10 mg/L |
| Temperature | < 150°F |

3. NON-QUANTITATIVE FACTORS

This section recommends certain practices that can help the operation of conventional treatment plants.

a. Monitoring. It is pointless to monitor for pollutants that are known to be absent even if there is a standard test. On the other hand, rare but troublesome pollutants such as cadmium should be monitored if an industry has such a material in its inventory used in water-related processing.

b. Real-time measurements and controls. Ion selective probes such as nitrate detectors are real-time measurement techniques that, even considering some losses of precision, accuracy and sensitivity, can be more effective in controlling pollutantional output than slow time parameters such as BOD, toxicity and analyses for specific trace metals.

c. Pretreatment. If a waste does not meet the specifications, then pretreatment or partial treatment can be required before discharge into municipal treatment systems.

d. Power. In the case of power failure, provision should be made so that the burden of treatment is not shifted from the industry to the municipal plant.

e. Flow. In order to optimize the operation of the sewage treatment facility, flow should be designed to be continuous or supplied in such a way as to supplement the normal diurnal variations of the plant. Scheduling of industrial flow inputs can improve the operation of municipal plants by enabling it to approach steady state performance. Changes in flow or its characteristics should be reported by the industry and regulated by the controlling agency. This may require monitoring.

f. Corrosive materials. These should be excluded from municipal plants.

g. Odorous and explosive gases. Materials such as $H_2S$, HCN, $Cl_2$, $CH_4$ and volatile solvents should be removed from industrial discharges prior to municipal treatment plants.

h. Sequestered oils and metals. Because dispersing agents and emulsifiers have a detrimental effect on the performance of biological treatment, these should be removed by pretreatment.

i. Spills. Accidental or otherwise unavoidable spills should be reported by the industry to the controlling agencies for the municipal wastewater system.

j. Storm water runoff. Storm waters, especially the first flush of water after a heavy rain can be as dirty or dirtier than municipal waste. It must enter the municipal system in a nondisruptive manner and may require pretreatment or storage.

k. Industrial cooling water. The blowdown of cooling water often contains particularly difficult pollutants such as chromium, algicides and slime inhibitors and therefore should not enter municipal systems. These wastewaters should be internally cleaned in the industrial plant and recycled in the cooling system.

l. Radioactive materials. Radioactive materials with long half-lives should neither be discharged into municipal wastewater systems nor into any part of the ambient aquatic environment.

m. New and unknown wastes. Pilot plant tests can be used to evaluate unknown waste from new complex industrial processes.

n. Overall environmental impact and secondary pollution problems. The interrelation of industrial wastewaters, sludges, and air pollutants must be evaluated so that pollution problems are not shifted from one pocket of the environment to another. Dilution of pollutants is not the best long-term environmental protection practice for many industrial wastes, particularly since certain harmful refractory constituents can be reconcentrated biologically in the receiving environment.

### 4. Ultimate treatment

The ultimate treatment of wastewaters would consider them as reusable resources. In-plant treatment to reclaim chemicals and water is seen as the optimal goal. Since this goal is not always realizable, this section has set forth some of the restrictions on the discharge of industrial waste to municipal waste treatment plants.

## TECHNICAL REPORT DATA
*(Please read Instructions on the reverse before completing)*

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16. ABSTRACT

Refractory waterborne organics resist biodegradation, accumulate in the environment and can inhibit life forms. This research develops laboratory techniques for, and interpretations of, a Refractory Index (R.I.) to quantitatively assess the persistency of refractory organics and uses R.I. to evaluate some 38 industrial, natural and combined organics. R.I. values close to 1.0 characterize readily biodegradation; R.I. values near zero indicate refractory organics; negative R.I. values indicate inhibitors. The coefficient of variation for R.I. values is 13%. Since negative R.I. values are of qualitative significance only, a Biological Inhibition Value (B.I.V.) is developed and used to quantitatively assess those organics found to interfere with the biochemical tests used, long-term ultimate Warburg Respirometer determinations. In a few cases, confirmation of the R.I. interpretation was performed using a model activated sludge unit and by specific analysis. Correlations between oxygen demand tests ($BOD_5$, $BOD_u$, TOD) and organic parameters (TOC, TN) are presented. Suggestions for required pretreatments of industrial wastewaters before allowing discharge into municipal sewage treatment plants are included as an application of the Refractory Index criterion. The bibliography contains 49 references.

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| Activated sludge process<br>Chemical removal<br>Sewage disposal<br>Sludge digestion | Sewage treatment<br>Waste treatment | 13B<br>07A |

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