A PRELIMINARY EVALUATION OF THE EFFECT OF DISSOLVED OXYGEN ON NITRIFICATION AND OTHER BIOLOGICAL PROCESS PARAMETERS

By

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ABSTRACT:

This work examines the relationship between dissolved oxygen level and the nitrification process in municipal wastewaters. Carbon removal, chemical oxygen demand removal and sludge viability were also investigated. Parallel bench scale continuous activated sludge reactors operating at dissolved oxygen levels of approximately 2 mg/l and 8 mg/l were used.

The high dissolved oxygen level sludge produced significantly more nitrate nitrogen and exhibited significantly greater nitrification rates overall. The nitrification rate differential increased with temperature. The nitrification rate appeared to be more sensitive to temperature and sludge age at the higher dissolved oxygen level.

Significantly greater overall TOC reduction and TOC removal rates were observed at the high dissolved oxygen level although no one set of experimental conditions exhibited a significant difference at the 99% confidence level. No significant difference in effluent COD quality or removal rate was observed.

There was a significant difference at the 95% confidence level in unit ATP levels, indicating that the high dissolved oxygen level sludge may have been more viable.

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1.0 INTRODUCTION

1.1 GENERAL

The sixth decade of the twentieth century saw an upwelling of concern for the natural environment by the populace of the Western World. Protection of the air, land, and water became national imperatives, especially in Canada and the United States. When translated into the specific realm of municipal wastewater treatment, these concerns focused on the removal of nutrients and on the improvement of existing treatment technology.

Prior to the 1960's, conventional wastewater treatment had three primary objectives:

1. removal of suspended matter,
2. reduction of carbonaceous oxygen demand, and
3. elimination of pathogenic organisms.

To these three objectives now was added the removal of nitrogen and phosphorus. The need for the removal of these two nutrients has been based upon the following:

1. both nitrogen and phosphorus contribute to the eutrophication of rivers, lakes and streams,
2. ammonia nitrogen is toxic to both fish and human beings,
3. nitrate nitrogen is toxic to human beings, especially infants,

4. ammonia nitrogen exerts considerable oxygen demand on receiving waters,
5. ammonia nitrogen exerts considerable chlorine demand which affects the cost of water and wastewater disinfection by increasing both chlorine requirements and contact time, and
6. ammonia nitrogen causes corrosion of copper fittings.

Phosphorus removal has proven to be relatively easy and economical to accomplish. Many existing activated sludge plants now carry out phosphorus removal economically and most new treatment plants incorporate it.

Nitrogen removal, however, has not been as easy, nor as inexpensive to accomplish. Nitrogen occurs in many chemical forms in raw municipal sewage: in organic compounds in various valence states; as ammonia which is the predominant form; as nitrites and nitrates, although these are usually present in very small concentrations. This multiplicity of chemical forms makes efficient removal of nitrogen difficult. Three methods of removing nitrogen from municipal wastewater have received considerable investigation:

1. air stripping of ammonia,
2. ion-exchange removal of ammonia, and
3. biological nitrification-denitrification.

Of these, biological nitrification-denitrification has usually proven to provide the most economical and practical solution.

Biological nitrification-denitrification is a two step process. The first step, nitrification, involves the aerobic production of nitrate-nitrogen from ammonia-nitrogen by the autotrophic bacteria Nitrosomonas sp. and Nitrobacter sp. The second step, denitrification, involves the reduction of the nitrates to nitrogen gas. Denitrification is carried out anoxically by a wide variety of facultative heterotrophic microorganisms. In this research, investigation has centered on the first stage of this process, nitrification.

Union Carbide Corporation developed a new method of producing pure oxygen in the early 1960's. In seeking uses for this method, pressure swing adsorption, they decided to investigate the use of pure oxygen in municipal sewage treatment, a field first investigated by Okun (1948). The result has been the UNOX system.

The UNOX system has been a controversial subject since the first full scale results were reported by Albertsson et al (1970). The supporters of utilizing pure oxygen for aerating suspended growth activated sludge processes claim that the following major advantages accrue because of the use of pure oxygen:

1. a more active microbial mass,
2. enhanced settling characteristics, and

3. lower excess solids production compared to air activated sludge systems.

In addition, the volumetric utilization of the aeration tank will be higher as a higher MLSS concentration can be carried compared to air systems (Wilcox and Thomas, 1974).

Other researchers state that there is no basic microbiological difference between air and oxygen activated sludges. Any differences which appear to favor the oxygen system are due to physical manifestations of operational characteristics of oxygen aeration systems, such as lower mixing intensities and higher sludge ages.

The effect of dissolved oxygen level on nitrification is also controversial. Okun (1949), in his first experiments with the bioprecipitation process reported that higher oxygen levels enhanced nitrification. Lewandowski (1974), reported less nitrification at higher dissolved oxygen concentrations. Both Albertsson et al (1970) and Haug and McCarty (1971) report no differences in nitrification with changes in dissolved oxygen tension.

1.2 OBJECTIVES

In this work, parallel bench-scale suspended growth reactors were used to investigate the effects of dissolved oxygen upon a number of parameters in the activated sludge

system. The ten litre reactors, located in a temperature controlled room, were fed degritted raw sewage. Sludge age was used as the major operational parameter. A mixture of pure oxygen and argon was used as an aerating gas with additional mixing being provided by three three-bladed propellers in each reactor. Clarification was integral within each reactor.

The major objectives of this research were:

1. to determine if any difference in nitrifying ability can be attributed to differences in dissolved oxygen level,
2. to determine if any difference in removal of carbon (TOC) or oxygen demand (COD) can be attributed to the difference in oxygen tension, and
3. to determine if any difference between the viability of the activated sludge, as measured by mixed liquor volatile suspended solids or adenosine triphosphate is attributable to dissolved oxygen level.

Equipment employed and operational procedures are presented in Appendix A. Analytical procedures and statistical procedures are given in Appendix B and C respectively, while analytical results are presented in Appendix D.

2.0 LITERATURE REVIEW

2.1 NITRIFICATION

2.1.1 General

Webster's New Collegiate Dictionary defines nitrogen as a "colorless, tasteless, odorless gaseous element that constitutes 78 per cent of the atmosphere by volume and occurs as a constituent of all living tissues in combined form". Nitrogen has received considerable attention by chemists since its discovery, partially because of its preponderance in the biosphere and partially because of its complex chemistry.

Nitrogen has seven valance states (see Figure 1) and thus appears in a wide variety of chemical compounds which vary widely in form and physical and chemical attributes. This work is particularly concerned with three of the inorganic forms of nitrogen: ammonia, nitrite and nitrate, and the microbiological transformation of ammonia to nitrate and nitrite.

2.1.2 The Microbiology of Nitrification

Figure 2 illustrates the various processes mediated by microorganisms in the transformation of nitrogen and shows species usually associated with each reaction.

The aerobic transformation of ammonia nitrogen to nitrate nitrogen is termed nitrification and is carried

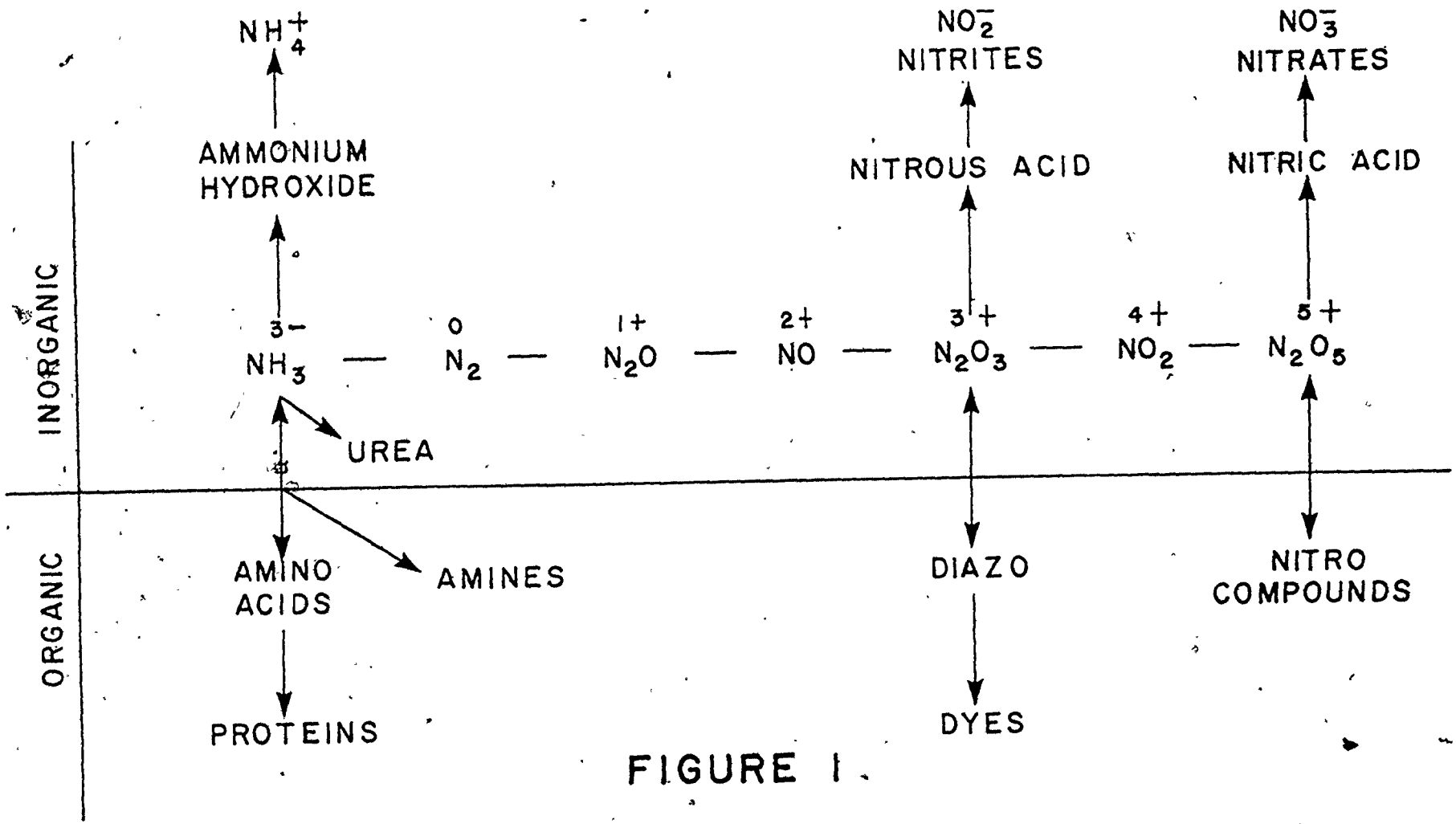


FIGURE 1.

THE PRESENCE OF NITROGEN IN ITS SEVEN VALENCE STATES

After WILSON (1975)

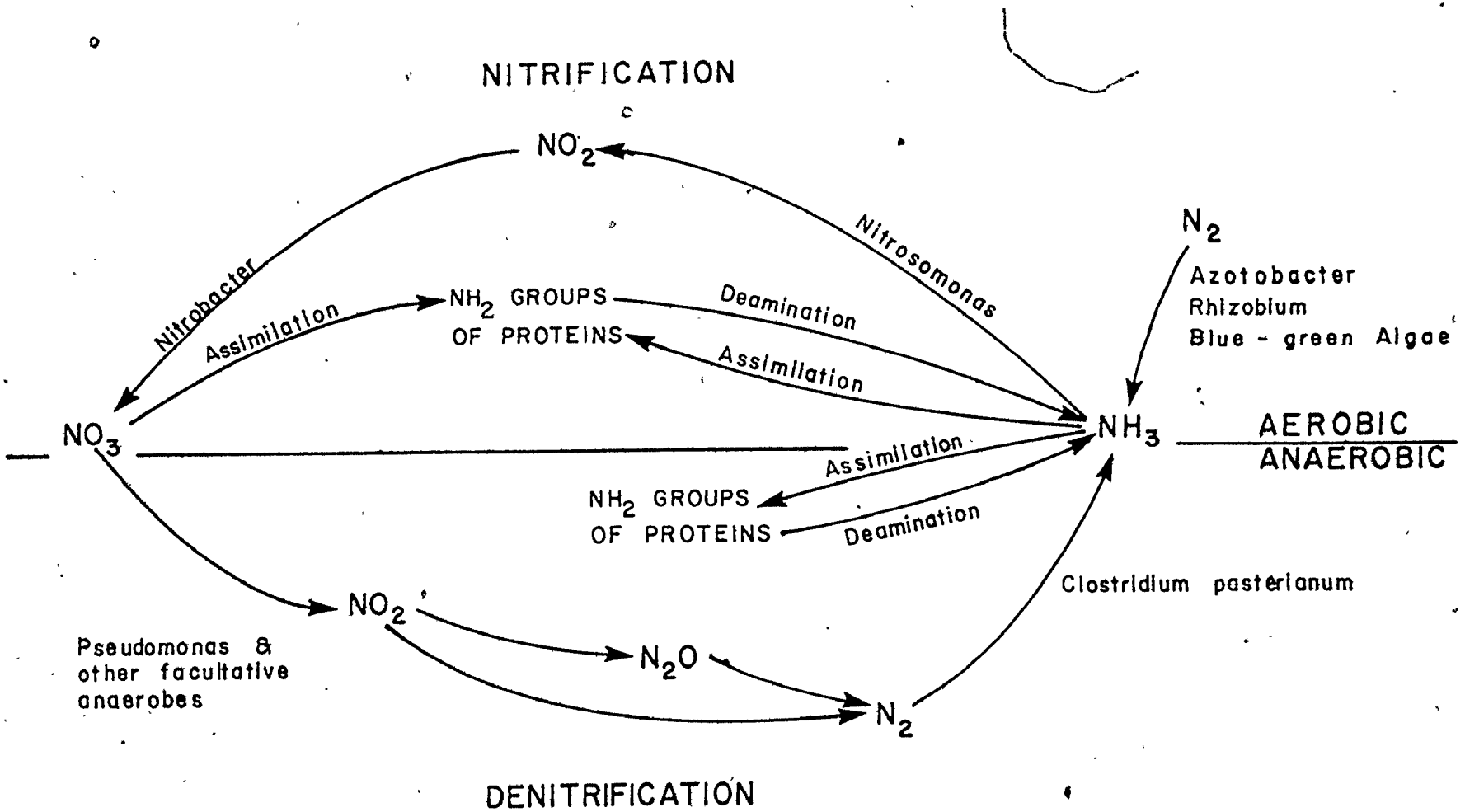


FIGURE 2

THE NITROGEN CYCLE

After Brock (1970)

out by a small group of organisms known as nitrifiers. Several genera have been identified as nitrifying bacteria, on the basis of morphology and the particular steps in the oxidation sequences they carry out (Table 1). It is likely that these genera are not all closely related, (Brock, 1970), but they have been traditionally classified into one group because of their unique autotrophic way of life.

Painter (1970) found that a small number of heterotrophic fungi oxidize ammonia to nitrite and that a smaller number produce nitrate. He also reported that a large number of heterotrophs, mainly soil fungi and bacteria, convert ammonia to nitrite or nitrite to nitrate. Haug and McCarty (1971) stated that Eylar and Schmidt (1959) found that Aspergillus flavus was able to form limited amounts of nitrates. Doxtoder and Roviera (1968) and Schmidt (1954) also reported nitrate production by this fungi. Fisher et al (1956) and Quastel and Scholefield (1949) reported that a limited number of heterotrophic organisms can nitrify but that none possessed the ability to produce the concentration of nitrate or nitrite produced by the Nitrobacteraceae.

Painter (1970) expressed doubt that Nitrosospira sp., Nitrosocystis sp. and Nitrosogloea sp. actually nitrify. Anthonisen et al (1976) stated that Breed et al (1966) identified seven genera of autotrophic nitrifying bac-

TABLE 1. NITRIFYING BACTERIA*

GENERA	GRAM STAIN	MORPHOLOGY
Ammonia Oxidizers		
Nitrosomonas sp	-	polarly flagellated rods
Nitrosococcus sp	+	non-motile coccus
Nitrosospiras sp	-	spiral shape
Nitrosocystis sp	-	polarly flagellated; cysts formed
Nitrosogloea sp	-	coccoid; aggregate into encapsulated clumps
Nitrite Oxidizers		
Nitrobacter sp	-	polarly flagellated rods
Nitrocystis sp	-	encapsulated rods aggregating into clumps

* After Brock (1970)

teria but that Nitrosomonas sp. and Nitrobacter sp. are responsible for most naturally occuring nitrification.

In the waste-treatment literature the process of Nitrification is commonly ascribed to these last two mentioned bacteria genera although no identification is usually carried out. This practice has been followed in this thesis.

2.1.3 Kinetics

A Michaelis-Menton or Monod kinetic model is frequently used by researchers attempting to describe the rate of bacterial growth and uptake of substrate. This model is usually presented in the form:

$$\frac{dF}{dt} = \frac{u_m XS}{K_s+S} \qquad (1)$$

where: $\frac{dF}{dt}$ is the rate of substrate uptake per unit volume with respect to time,

u_m is the maximum rate of substrate uptake per unit weight of microorganisms,

X is the concentration of microorganisms,

K_s is the half velocity coefficient, equal to the substrate concentration when $\frac{dF}{dt} = 1/2\ u_m$, and

S is the concentration of substrate.

Equation (1) reduces to a zero order model with respect to substrate if $S \gg K_s$ and to a first order model if $S \ll K_s$. Most authors have found the two nitrification

reactions to follow zero order kinetics in activated sludge systems (Table 2). Monod models based on their observations, have had K_s values in the order of 0.5 to 2.0 mg/l. In domestic sewage treatment where S (i.e. NH_4) concentrations are approximately 20 mg/l, $S \gg K_s$ and the Monod model reduces to a zero order expression.

Nitrification purportedly is carried out by two different organisms; Nitrosomonas sp., which oxidizes ammonia-nitrogen to nitrite-nitrogen; and Nitrobacter sp., which oxidizes nitrite-nitrogen to nitrate-nitrogen. The respective reactions are:

$$NH_4^+ + \frac{3}{2} O_2 \xrightarrow{\text{Nitrosomonas sp.}} NO_2^- + 2 H^+ + H_2O \quad (2)$$

$$NO_2^- + \frac{1}{2} O_2 \xrightarrow{\text{Nitrobacter sp.}} NO_3^- \quad (3)$$

The energy released in these reactions is used by the bacteria in cell maintenance and growth.

If bacterial cell composition is assumed to have the formula $C_5H_7NO_2$ the autotrophic assimilation reaction can be written as:

$$NH_4^+ + 2 H_2O + 5 CO_2 \longrightarrow H^+ + 5 O_2 + C_5H_7NO_2 \quad (4)$$

The overall equations for the formation of Nitrosomonas sp. and Nitrobacter sp. were given by Haug and McCarty (1971) as:

Nitrosomonas sp.

$$29 NH_4^+ + 37 O_2 + 5 CO_2 \longrightarrow$$
$$28 NO_2^- + 57 H^+ + 26 H_2O + C_5H_7NO_2 \quad (5)$$

TABLE 2

KINETIC MODELS

Author	System Description*
Zero Order	
Bishop, Heidman and Stamberg (1974)	Batch and continuous bench scale and continuous pilot scale on domestic wastes
Jenkins (1969)	Pilot and full scale on domestic waste
Poduska and Andrews (1974)	Continuous bench scale on synthetic waste
Sawyer et al (1973)	Pilot scale on domestic waste
Sutton, Murphy and Jank (1975)	Pilot scale on domestic waste
Wong-Chong and Loehr (1975)	Bench scale on synthetic waste (enriched culture of nitrifiers)
Wilson (1976)	Pilot scale on domestic waste (rotating biological reactor)
First Order	
Adams (1972)	Bench scale on industrial waste high in NH_3
Anthonisen et al (1976)	Bench and pilot scale on domestic and agricultural wastes
Stover and Kincannon (1975)	Bench scale on synthetic waste (rotating biological contactor)

TABLE 2 cont'd....

Monod	
Lijklema (1973)	Literature data in computer modelling

* Mixed culture, suspended growth reactor unless otherwise noted.

and

Nitrobacter sp.

$$96\ NO_2 + 43\ O_2 + 5\ CO_2 + NH_4^+ + 2\ H_2O \longrightarrow 96\ NO_3 + H^+ + C_5H_7NO_2 \quad (6)$$

Stankewich reported these equations as:

Nitrosomonas sp.

$$55\ NH_4^+\ 76\ O_2 + 5\ CO_2 \longrightarrow 54\ NO_2^- + 109\ H^+ + 52\ H_2O + C_5H_7NO_2 \quad (7)$$

Nitrobacter sp.

$$5\ CO_2 + 400\ NO_2^- + 195\ O_2 + NH_3 + 2\ H_2O \longrightarrow C_5H_7NO_2 + 400\ NO_3^- \quad (8)$$

Stankewich's equations (7 and 8) result in lower yield values than do Haug and McCarty's (4 and 5): 0.114 mg Nitrosomonas sp./mg NH_4^+ oxidized versus 0.216 mg/mg and 0.015 mg Nitrobacter sp./mg NH_4^+ oxidized versus 0.063 mg/mg but all these values are within the range reported in the literature (Table 3). The significant point to be noted is that the yield values for both organisms are much lower than those reported for heterotrophic organisms. The impact of these low yield values is reflected in the minimum sludge ages required to maintain nitrification.

Equations 1 and 2 show that 3.43 mg of oxygen are required to oxidize 1 mg of ammonia-nitrogen to nitrite-

TABLE 3

YIELD COEFFICIENTS FOR NITRIFYING BACTERIA*

Author	Yield Coefficient (mg cells/mg N oxidized)
Nitrosomonas sp.	
Baas-Becking and Parks (1927)	0.054
Skinner and Walker (1961)	0.06
Nelson (1931)	0.13
Loveless and Painter (1968)	0.04 - 0.1
Winogradsky (1949)	0.06
Downing, Painter and Knowles (1964)	0.05
Meyerhof (1916)	0.05
Hofman and Lees (1952)	0.068
Downing, Tomlinson and Truesdale (1964)	0.05
Lawrence and McCarty (1969)	0.29
Downing (1968)	0.05
Knowles, Downing and Barrett (1965)	0.05
Haug and McCarty (1971b)	0.29
Stankewich (1972)	0.114
Nitrobacter sp.	
Lees and Simpson (1957)	0.02
Boon and Laudelot (1962)	0.02
Gould and Lees (1960)	0.04 - 0.07
Baas-Becking and Parks (1927)	0.019
Lawrence and McCarty (1969)	0.084
Knowles, Downing and Barrett (1965)	0.02
Downing (1968)	0.02
Meyerhof (1916)	0.06
Haug and McCarty (1971b)	0.084
Stankewich (1972)	0.019

* after Painter (1970)

and Stankewich (1972)

nitrogen and 1.14 mg of oxygen is required to oxidize 1 mg of nitrite-nitrogen to nitrate-nitrogen for a total amount of oxygen required of 4.57 mg per mg of ammonia oxidized. However, some of the oxygen is acquired from the fixation of CO_2 which results in an overall oxygen demand of 4.33 mg per mg of ammonia oxidized. Wezernak and Gannon (1962) and Montgomery and Borne (1966) reported experimental values of 3.22 and 1.10 respectively. This high nitrogenous oxygen demand is one reason for removing nitrogen from wastewater.

2.1.4 Temperature Effects

The activity of both Nitrosomonas sp. and Nitrobacter sp. is very temperature sensitive and most authors examining biological nitrification have attempted to model the effects of temperature. Tables 4,5, and 6 list observed Monod half velocity constants, substrate utilization rates and maximum growth rates respectively.

Painter (1970) reported that little if any growth of Nitrosomonas sp. takes place below 5°C. The lower temperature limit of Nitrobacter sp. is reported to be 8°C by Nelson (1931) and 4°C by Deppe and Engel (1960).

Most authors report an Arrhenius type relationship between nitrification rate and temperature. Stankewich (1972) reported that many authors have utilized

TABLE 4

EFFECT OF TEMPERATURE ON MONOD HALF VELOCITY CONSTANT (K_s)

K_s	Temperature (°C)	Reference
Nitrosomonas		
1.0 mg/l	20	Lijklema (1973)
8.0 mg/l	29	Lijklema (1973)
10.0 mg/l	30	Hofman and Lees (1953)
3.5 mg/l	25	Ulken (1963)
1.0 mg/l	20	Loveless and Painter (1968)
1.0 mg/l		Haug and McCarty (1971)
Nitrobacter		
6.0 mg/l	30	Lees and Simpson (1957)
5.0 mg/l	28	Gould and Lees (1960)
8.4 mg/l	32	Laudelot and Van Tichelen (1960)
5.0 mg/l	25	Ulken (1963)

TABLE 5

EFFECT OF TEMPERATURE ON SUBSTRATE UTILIZATION RATE (k)

k mg-N/hour/gm MLVSS	Temperature (°C)	Reference
0.66-1.75	15.5	Bishop, Heidman and Stamberg (1974)
3.45-3.96	25.0	Ibid
4.58	27.0	Ibid
5.00	26.5	Ibid
0.5-6.0 (avg. 1-2)	15-20	Painter and Jones (1963)
0.6		Jenkins (1969)

TABLE 6

EFFECT OF TEMPERATURE ON GROWTH RATE (u)

u (day^{-1})	Temperature (°C)	System	Reference
Nitrosomonas sp.			
0.09-0.24	9-18	activated sludge - continuous	Jenkins (1969)
0.55	14.5	Thames estuary	Ibid
0.18-0.26	15-18	activated sludge - continuous	Ibid
0.7	15-20	activated sludge - fill and draw	Ibid
0.33	29	culture flasks	Ibid
0.25-0.35	30	continuous culture	Ibid
1.0-1.5	30	continuous culture	Ibid
0.33	20	activated sludge	Dept. of Scientific and Industrial Research (1964a)
0.7	20	Thames River	Knowles et al (1965)
0.46	30	mineral suspension	Lees (1952)
1.03	30	mineral suspension	Bomeke (1946)
1.19	30	mineral suspension	Engel (1930)
1.2-1.5	30	clear media - continuous culture	Skinner and Walker (1961)
2.2	30	clear media - flask culture	Ibid
1.5	30	clear media	Engel and Alexander (1958)
1.3	27	clear media	Loveless and Painter (1968)
Nitrobacter sp.			
0.14	20	activated sludge	Painter (1970)
1.0	20	Thames River	Ibid
2.2	32	Thames River	Ibid
1.39	32	pure culture	Boon and Laudelot (1962)

the following modified form of the Arrhenius relationship for a limited temperature range:

$$\hat{u}_T = \hat{u}_{15} e^{x(T-15)} \qquad (9)$$

where: $\hat{u}_T$ is the maximum growth rate at T°C,

$\hat{u}_{15}$ is the maximum growth rate at 15°C, and

x is the coefficient.

"X" for Nitrosomonas sp. was reported by Knowles, Downing and Barrett (1965) to be 0.095 and by Jenkins (1969) to be 0.116. The most commonly reported value was 0.12 (Downing and Knowles, 1966; Carlson, 1970; Downing, 1968; Eckenfelder, 1967; Downing and Hopwood, 1964; Downing, Jones and Hopwood, 1965). The value of "x" for Nitrobacter sp. is reported to be 0.057 by Knowles, Downing and Barrett (1965), 0.069 by Jenkins (1969) and 0.056 by Lawrence and McCarty (1969). The maximum growth rate of Nitrosomonas sp. at 15°C is usually taken to be 0.18 day^{-1} and of Nitrobacter sp., 0.30 day^{-1}, Stankewich (1972).

Other authors (Sawyer, 1973; Sutton et al, 1975; Sutton, Murphy and Jank, 1975; Wilson, 1976) have utilized the following form of the Arrhenius formula:

$$K = K^* \exp\left(-E/R \left[\frac{1}{T} - \frac{1}{T_o}\right]\right) \qquad (10)$$

where: K is the reaction rate constant (hr^{-1}),

K^* is equal to A exp $(-E/R\ T_o)$,

A is the frequency factor,

E is the activation energy (cal/g - mole),

R is the universal gas constant (cal/g - mole °K),

T is the temperature (°K), and

T_o is the median of the temperature range (°K).

This form is especially suitable for computer analysis of data since its logarithmic form is linear.

The half velocity coefficients are also known to be a function of temperature. Knowles, Downing and Barrett (1965) found the following models fit their data:

$$K_{s_{Nitrosomonas}} = 0.405e^{.118(T-15)} \quad (11)$$

$$K_{s_{Nitrobacter}} = 0.625e^{.146(T-15)} \quad (12)$$

Sludge age has a large effect on the nitrifying ability of a sludge because of the very low growth rate-yield values. Because of the marked temperature sensitivity of the nitrifying bacteria, many authors have attempted to define a relationship between sludge age and temperature (Adams, 1972; Barnard, 1975; Poduska and Andrews, 1974; Sutton et al, 1975; Sutton, Murphy and Jank, 1975). A minimum sludge age of four to five days at 25°C is usually reported. Sutton et al (1975) stated that a sludge age of at least 10 days is required at 5 °C. Reduced temperature sensitivity with longer sludge

ages was observed by Sutton, Murphy and Jank (1975). Wilson (1976) reported less temperature sensitivity with a fixed film reactor (bio-disc) than a suspended growth reactor.

2.1.5 pH Effects

Nitrification reactions produce two moles of hydrogen ions per mole of ammonia oxidized (Equations 2, and 3) and thus the pH can decrease when nitrification occurs. The magnitude of the decrease will depend upon the alkalinity of the wastewater. Since most domestic wastewaters have pH's in the range 7.0 to 8.5, bicarbonate alkalinity is consumed by the production of hydrogen ions according to the following equation:

$$H^+ + HCO_3^- \longrightarrow CO_2 + H_2O \qquad (13)$$

This would suggest that approximately 7 mg of bicarbonate alkalinity are required to neutralize the hydrogen ions produced in the oxidation of 1 mg of ammonia nitrogen.

Haug and McCarty (1971) showed that if all the CO_2 produced remained in solution, the alkalinity must be 10 times the amount of ammonia nitrogen to be oxidized to ensure that the pH would not drop below 6.0 and thus inhibit the reaction. They observed that nitrification ceased when the residual alkalinity decreased to approximately 10 mg/l as $CaCO_3$.

The accumulation of dissolved CO_2 in the final stages of UNOX system aeration tanks has been reported by Lewandowski (1974) to inhibit nitrification by lowering the pH. This phenomena was also reported by Stankewich (1972) but he found that after an acclimatization period of approximately one month, the nitrification rate was re-established at maximum levels. He found that changes in pH over short time periods drastically affected the nitrification rate.

Painter (1970) in his extensive literature review, reported that the optimum pH for growth of Nitrosomonas sp. and Nitrobacter sp. to be on the alkaline side of neutral but optima were reported in the range pH 6.0 to pH 9.0. Downing, Jones and Hopwood (1965) reported a sharp decline in the first order rate constant below pH 7.0 but little affect above pH 7.0.

Sawyer et al (1973) found operation of their pilot scale equipment at pH 8.4 provided optimum nitrification. Wong-Chong and Loehr (1974) reported that the maximum nitrite oxidation rate decreased exponentially with decreasing pH in the range 8.5 to 6.0. The reaction rate constant had an optimum in the range pH 7.0 to 7.5.

Prakasam et al (1974) used nitrifying oxidation ditch mixed liquor acclimatized at pH 6.1 and observed little difference in total oxygen uptake from pH 5.0 to pH 10.0. In this set of experiments, no oxygen uptake

was observed at pH 4.0 and the greatest total uptake was observed at pH 11.0. The higher uptake at pH 11.0 was attributed to the hydrolysis of some substrate materials making them more amenable to biological degradation. In another set of experiments with a similar culture acclimatized at pH 5.7, they found the highest total oxygen uptake occurred between pH 6.0 and 8.0. There was no significant difference in total uptake between the control culture and one at pH 4.0. The alkaline cultures growing at pH 10.0 and 11.0 showed a marked decrease in total oxygen uptake. They concluded that it was not advantageous to control pH if a system can achieve non-inhibitory pH levels by itself.

Prakasam et al (1974) cited a previous study by Prakasam and Loehr (1972) who found the degree of nitrification to be independent of pH.

2.1.6 Inhibition

Painter (1970) has reviewed the work of some 19 authors who have examined a wide range of substances to determine their effect on both Nitrosomonas sp. and Nitrobacter sp. The findings of these authors are shown in Table 7. In most cases the inhibiting concentration was determined but not the mechanism of the inhibition nor whether the general metabolic processes of the cell were disturbed or merely the primary oxidation reactions slowed or stopped.

TABLE 7

INHIBITORY AGENTS

Inhibiting Agent	Reference
Nitrosomonas sp.	
Chelating agents (eg. thiourea and allyl-thiourea)	Lees (1952)
2-chloro-6(trichloromethyl)pyridine	McBeath (1962)
Mannose	Jenson (1950)
Hydrazine	Hofman and Lees (1953)
Deionized water (passed through Amberlite IR4B)	Gunderson (1958)
Various metals (see text)	Meiklejohn (1954) Tomlinson et al (1966) Skinner and Walker (1961) Loveless and Painter (1968)
Nitrobacter sp.	
Cyanate and chlorate	Lees and Simpson (1957)
Cyanide, azide, various metal binding agents, p-chloramercuribenzoate, antimycin A, heptyl-4-hydroxyquinoline-N-oxide	Aleem and Nason (1963)
Nitrosomonas sp. and Nitrobacter sp.	
Lipid soluble compounds (eg. narcotics)	Meyerhof (1916, 1917)
Peptone	Boltjes (1935)
Light	Ulken (1963)
Ammonia and nitrite (See text)	Meyerhof (1916) Lewis (1959) Pokallus (1963) Boon and Laudelot (1962) Aleem (1959) Ulken (1963)

after Painter (1970)

Many metals have been examined to determine at what level they completely or substantially inhibit the respiration of Nitrosomonas sp. and Nitrobacter sp. Meiklejohn (1954) reported the following as the most toxic metals to Nitrosomonas sp.:

Ag	0.25 mg/l
Hg^{++}	2 mg/l
Ni	12 mg/l
Co	59 mg/l

Aluminum was toxic at 270 mg/l while 27,400 mg/l of barium was required to achieve complete inhibition. Skinner and Walker (1961) reported much more severe inhibitions to Nitrosomonas sp.:

Ni	0.25 mg/l
Cr	0.25 mg/l
Cu	0.5 mg/l

Jenkins (1969) reported inhibition of nitrification in a full scale activated sludge plant treating metal industrial wastes in Birmingham, England.

Painter (1970) cited the observations of several authors who had examined the toxicity of ammonia and nitrite to both Nitrosomonas sp. and Nitrobacter sp. and concluded that each organism is affected more by its own product than by its substrate. Nitrobacter sp. is reported to be quite sensitive to even relatively low concentrations of ammonia (Ulken, 1963).

Anthonisen et al (1976) reported that un-ionized ammonia (FA) and un-ionized nitrous acid (FNA) concentrations inhibited nitrification. They reported the following inhibitory concentrations:

Nitrosomonas sp.	10-150 mg/l	free ammonia
	0.22-2.8 mg/l	free nitrous acid
Nitrobacter sp.	0.1-1.0 mg/l	free ammonia
	0.22-2.8 mg/l	free nitrous acid

Both species were said to be able to acclimatize to FA and FNA and inhibition was not permanent. Temperature was said to be a factor as was pH. They presented the following equations for calculating FA and FNA:

$$\text{FA as } NH_3\text{(mg/l)} = \frac{17}{14} \times \frac{\text{total ammonia as N(mg/l)} \times 10^{pH}}{\frac{Kb}{Kw} + 10^{pH}} \quad (14)$$

where: Kb is the ionization constant of ammonia equilibrium equation,

Kw is the ionization constant of water, and

$$\frac{Kb}{Kw} = \exp(6344/[273 + °C])$$

$$\text{FNA as } HNO_2\text{(mg/l)} = \frac{46}{14} \times \frac{\text{total nitrite as N(mg/l)}}{Ka \times 10^{pH}} \quad (15)$$

where: Ka is the ionization constant of nitrous acid equilibrium equation and equal to $\exp(-2300/[273 + °C])$

Inhibition by FA and FNA is generally not a factor in domestic waste treatment. In this study, FA and FNA were found not to be in the range of inhibition with one exception, the FA value being 0.138 mg/l and therefore on

the borderline.

Bishop, Heidman and Stamberg (1974) reported that addition of methanol to their pilot scale aerobic-anaerobic reactor appeared to inhibit nitrification, although recent Canadian studies do not support this observation. Being unable to increase the available carbon in this manner during periods of dilute wastewater flow would restrict the overall nitrogen removal performance of aerobic-anaerobic single reactor process sequences.

2.1.7 Nitrogen Losses

Many researchers have reported apparent losses of total nitrogen in the activated sludge process. Jenkins (1933) reported losses of 13% to 14%. Wuhrman (1954) observed losses of from 24% to 32%, the losses increasing with decreasing loading. Truesdale *et al* (1961) experienced losses as high as 33.6% during a one year experiment on eight pilot scale filters. The Department of Scientific and Industrial Research (1962) reported losses of 14.4% in laboratory scale activated sludge units and losses of 8.5% in filters run at the same time. Wuhrman (1963) reports a 26% loss of total nitrogen in a system operating at a dissolved oxygen concentration of 6.7 mg/l while no loss of nitrogen occurred in a companion system run at 0.7 mg/l dissolved oxygen. He observed that nitrification took place only in the higher dissolved oxy-

gen level system. Painter (1970) cited an unpublished WPRL report showing nitrogen losses of 6% to 50% occurring on lab scale filters. The data was obtained from 20 daily tests over a three month period. Stamberg (1972) observed a 40% loss of nitrogen through the Washington, D.C., Blue Plains UNOX plant during the summer when nitrification was occurring but only approximately 10% losses during the winter when no nitrification occurred. Wong-Chong and Loehr (1975) reported a loss of total nitrogen during batch experiments of about 50 to 100 mg/l.

Painter (1970) reviewed some of the possible reasons for these apparent nitrogen losses. Analytical limitations such as the TKN test not being comprehensive especially for heterocyclic nitrogen compounds and the nitrite and nitrate tests picking up other compounds, were cited.

During storage, samples could undergo bacterial denitrification or hydrolysis of organic nitrogen to ammonia. Ammonium and nitrite ions are known to react to produce nitrogen gas. Acid inhibitors could cause nitrogen losses through chemical reaction while alkaline inhibitors may hydrolyse organic nitrogen or cause a loss of ammonia or volatile amines.

Aeration in the experimental reactor could cause stripping of ammonia or volatile amines. Jenkins (1933) reported no loss of ammonia vapor and suggested that the

evolution of nitrogen gas caused the observed loss. Wuhrman (1954) and Wong-Chong and Loehr (1975) also attribute their observed losses to bacterial denitrification and the resultant evolution of nitrogen gas.

Painter (1970) also stated that researchers rarely account for the nitrogen in the sludge produced. Based on the empirical formula $C_5H_7NO_2$, 100 pounds of dry sludge contains 12.4 pounds of nitrogen. Adams (1972) stated that non-degradable residual biomass will contain approximately 7% nitrogen by weight. Barnard (1975) stated that the nitrogen content of cells is 8% to 9% when carbon is abundant and increases to 12% when carbon is limiting. He also stated that the nitrogen content will drop to approximately 7% at long sludge ages. Recent Canadian studies have found 5% to 6% nitrogen in sludges.

Painter (1970) concluded that imbalances of nitrogen must be greater than 10% before they can be used as evidence for losses or gains by processes not measured directly.

2.2 DISSOLVED OXYGEN EFFECTS

2.2.1 General

Aerobic bacteria require a certain minimum (critical) level of dissolved oxygen to survive. Microbial respiratory activity will be inhibited at low dissolved oxygen levels. The level varies with each bacterial species. Pure culture dispersed growth experiments are usually used in determining critical dissolved oxygen levels. These have been fairly firmly established for a large number of bacterial genera and usually range from 0.01 mg/l to 0.10 mg/l (Harrison et al, 1969).

The effects of dissolved oxygen on the heterogeneous flocculant growth cultures which exist in activated sludge treatment plants have not been firmly established and have been the subject of some controversy since the late 1940's. Wuhrman (1963) stated that based on his evluation of the literature he would conclude that the critical D.O. in activated sludge flocs is 0.1 mg/l.

Others (Matson et al, 1972; Wuhrman, 1963; Pasveer, 1956) have reported or calculated much higher levels of bulk dissolved oxygen as being rate limiting in the activated sludge process. Englande and Eckenfelder (1973) reported that the critical liquid D.O. for activated sludges ranges from 0.2 to 0.5 mg/l.

Transport phenomena can often play a large role in the activated sludge process and has often been overlooked by researchers attempting to attribute varying degrees of treatment to different levels of oxygen tension. Schoberl and Engel (1964) reported that the limiting dissolved oxygen concentration they observed was directly proportional to cell concentration. Their reactors were probably mass transfer limited and not oxygen limited. Ball, Humenick and Speece (1972) attributed all the reported differences between air and oxygen activated sludges to differing physical environments in the aeration tank.

2.2.2 Transport Phenomena

Figure 3 illustrates the mechanisms and reactions involved in biological waste treatment. Of the nine "reactions" shown only two, R_5 and R_9, are not physical phenomena. R_5, the solubilization of substrate, and R_9, the metabolic processes of the microbe, can be severely limited by any of the seven transport reactions.

R_1, the transfer of oxygen from the bulk gas phase to the bulk liquid phase, is adequately covered by most text books on transport phenomena and waste treatment engineering. The rate of transfer is a function of the difference between saturation concentration and existing bulk liquid concentration. Pure oxygen aeration treat-

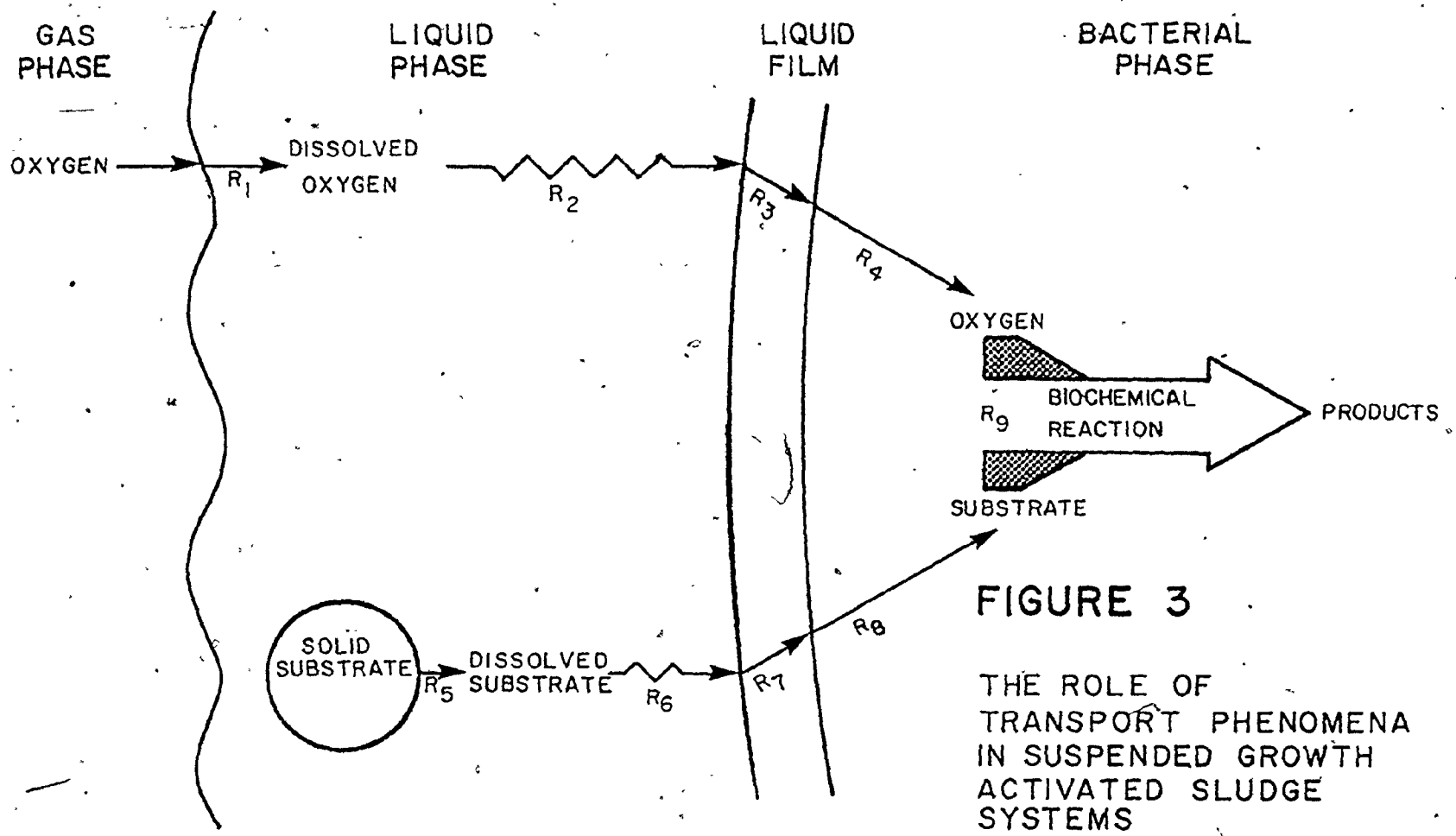


FIGURE 3

THE ROLE OF TRANSPORT PHENOMENA IN SUSPENDED GROWTH ACTIVATED SLUDGE SYSTEMS

AFTER SWILLEY ET AL (1964)

ment processes increase the rate of transfer by increasing this driving force from about 7 mg/l to 37 mg/l or by a factor of approximately five.

Rates R_2 and R_6, the transfer of oxygen and substrate from the liquid-gas boundary to the liquid-floc interface, are a function of mixing intensity. Kalinske (1971) stated that increased microturbulence produced the same effect on oxygen and nutrient uptake as increasing the dissolved oxygen concentration. It has been shown by many authors that increasing the degree of mixing increased the oxygen uptake rate (Finn, 1954; Schroepfer et al, 1955; Kehrberger et al, 1964; Norman and Busch, 1954; Wise et al, 1969; Murphy, 1971; Matson et al, 1972).

Pasveer (1956) stated that surface renewal at the liquid-floc interface is the rate limiting step in the activated sludge process. He proposed higher turbulence levels and increased dissolved oxygen levels to overcome this. Wise et al (1969) showed that for relatively quiescent systems ($0 \leq R_e < 2$) the presence of viable organisms had no noticeable effect on bubble collapse rates. They concluded that the effect of microorganisms in a mixed system was to diminish the quasi-static liquid surface film surrounding the gas bubble thus decreasing the gas-liquid interface film thickness and increasing the mass transfer rate. It would follow that mixed liquors with higher solids levels such as in pure oxygen systems should

exhibit higher mass transfer rates.

Finn (1954) reported that for a single cell, liquid film resistance was negligible. Mueller et al (1968) showed that for the specific type of flocculant culture they employed, liquid film diffusion, (R_3 and R_7 in Figure 3), was not a rate limiting phenomena.

Wuhrman (1963) derived formulas from mass balances on differential shells which predict the maximum dimension of bacterial flocs assuming that diffusion, R_4 and R_8, is the only mechanism whereby metabolites can be transferred through the floc. He arrived at a maximum aerobic floc size of 400 to 500 microns for bulk dissolved oxygen concentrations in the range of 1.5 to 2.5 mg/l. He also concluded that oxygen is not necessarily the rate limiting metabolite in the treatment of low strength domestic wastes as long as the bulk concentrations exceeded 1.5 mg/l to 2.5 mg/l. He stated that oxygen transfer could be rate limiting for high strength industrial wastes.

Matson, Characklis and Busch (1972), using almost exactly the same diffusivities and floc sizes, concluded that oxygen transfer is rate limiting because of the lack of sufficient mass concentration gradient in conventional activated sludge plants.

Mueller, Boyle and Lightfoot (1968) related a descriptive floc dimension to the limiting oxygen concentration utilizing anoxic core equations. They assumed that

the critical oxygen concentration at the cell surface was zero. Limiting bulk oxygen concentrations were found to be in the range 0.2 to 4.0 mg/l for flocs of the size usually found in activated sludge plants.

Englande and Eckenfelder (1973) concluded from both their own work and the work of others that oxygen diffusion into flocs is not a critical factor in the activated sludge systems.

2.2.3 Effect on Nitrification

Pure culture work has established that Nitrobacter sp. is more sensitive to low dissolved oxygen tensions than Nitrosomonas sp. (Painter, 1970). Growth of Nitrobacter sp. was observed to be inhibited at dissolved oxygen concentrations below 2 mg/l. No growth was observed at 0.2 mg/l at 32°C with K_s reported at 0.5 to 1.0 mg/l at 32°C and 0.25 mg/l at 18°C (Boon and Laudelot, 1962). Oxygen was not growth limiting above a concentration of 0.9 mg/l at 30°C for Nitrosomonas sp. with a corresponding K_s of 0.5 mg/l (Painter, 1970). Loveless and Painter (1968) reported a K_s of 0.3 mg/l at 20°C for Nitrosomonas sp. No ammonia oxidation was observed at an oxygen tension of 0.2 mg/l (Painter, 1970). Painter (1970) also reported that although research showed that a minimum concentration of dissolved oxygen was required for the growth of autotrophic nitrifiers, the absence of oxygen for pro-

longed periods was not lethal. This is confirmed by the work of researchers such as Prakasam et al (1974) who alternated periods of aeration with zero dissolved oxygen conditions in a single vessel to achieve nitrogen removal in suspended growth systems.

In activated sludge systems, the observed nitrification rate-limiting dissolved oxygen concentration varies widely. Jenkins (1969) found that nitrification occurred at D.O.'s of 0.5 mg/l and Painter and Jones (1963) achieved nitrification at oxygen tensions much below 0.5 mg/l. Downing and Hopwood (1964) suggested a minimum D.O. of 1 mg/l, while Haug and McCarty (1971) found the limiting D.O. to be in the range of 1 to 2 mg/l for their submerged filter. Barnard (1975) reported the rate limiting D.O. concentration to be 2 mg/l. Downing, Jones and Hopwood (1965) reported that the optimum D.O. for nitrification is 2 mg/l or slightly higher. Garret (1961) found nitrification to be independent of dissolved oxygen tensions greater than 3 mg/l. Wuhrman (1963) compared pilot scale plants operated at 1, 4 and 7 mg/l D.O. and found that considerably greater nitrification took place in the plants run at 4 and 7 mg/l D.O. than at 1 mg/l D.O. Okun (1948) reported that nitrification occurred in his high D.O. (11 to 25 mg/l) reactor but that none was observed in his low D.O. (0 to 5 mg/l) reactor. He attributed at least part of this observation

to denitrification occurring in the anaerobic upper part of the sludge blanket in his upflow unit which reduced all the nitrates formed in the main body of the reactor to nitrogen gas. The Ministry of Technology (1965) observed the same degree of nitrification at dissolved oxygen tensions of 2, 4 and 8 mg/l but found a slower rate at 2 mg/l D.O. than at 4 and 8 mg/l. Albertsson et al (1970) observed no difference in nitrifying ability between their side-by-side full scale units. Haug and McCarty (1971) found inhibition of nitrification if less than the stoichiometric amount of oxygen was supplied to their submerged filter.

The effect of high dissolved oxygen concentrations on nitrification is not clear from the literature. Okun and Lynn (1956) cited five researchers who observed nitrification inhibition at high D.O.'s. Fewson and Nicholas (1961) reported that high dissolved oxygen tensions inhibit nitrate production. Okun (1948) found no inhibition effects at dissolved oxygen concentrations up to 33 mg/l. Haug and McCarty (1971) found no inhibition at D.O.'s up to 60 mg/l.

Stankewich (1972) postulated a Monod-type relationship between growth rate and dissolved oxygen concentration:

$$u_{(DO)} = \frac{\hat{u}\,[DO]}{K_{O_2} + [DO]} \qquad (16)$$

where: K_{O_2} is the oxygen saturation constant and equals the dissolved oxygen concentration when the growth rate is one half of its theoretical maximum.

He stated that K_{O_2} is also temperature dependent and from his own data and literature data, derived the following relationship:

$$K_{O_2(T)} = K_{O_2(15°C)} \exp(0.069[T-15]) \qquad (17)$$

where: $K_{O_2(15°C)} = 0.21$ mg/l

Figure 4 illustrates this relationship for a single temperature.

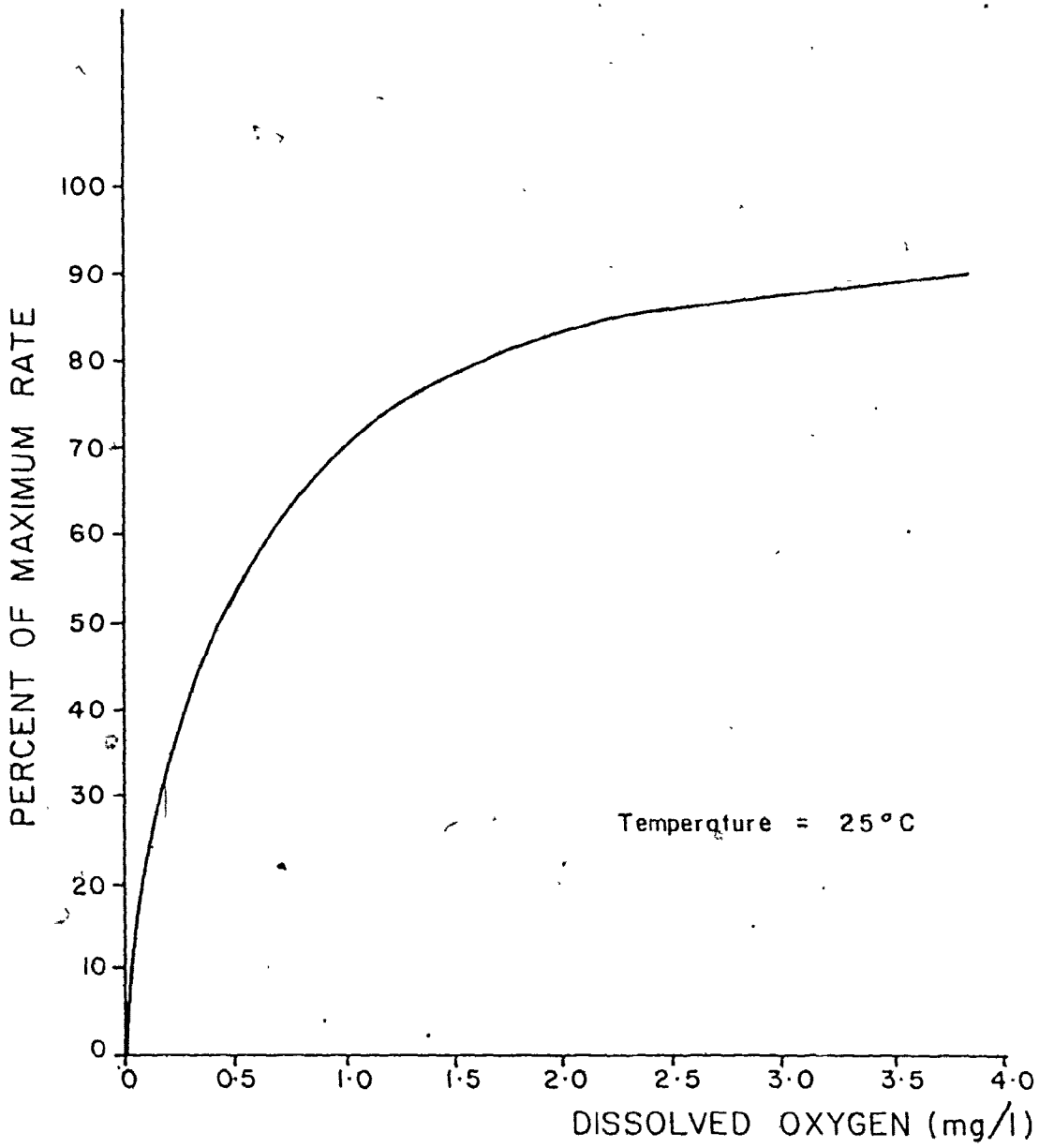


FIGURE 4

EFFECT OF DISSOLVED OXYGEN ON THE GROWTH RATE OF NITRIFIERS

After STANKEWICH (1972)

2.3 PURE OXYGEN

2.3.1 History

The idea of using high purity oxygen in the activated sludge sewage treatment process dates back to 1946 when Malcolm Pirnie wrote a memo suggesting its use and outlining a method which he thought would employ oxygen to advantage. This process, termed "Bio-precipitation", was investigated at the bench scale by Okun (1948 and 1949).

Okun used an upflow reactor similar to the one shown in Figure 5. The sewage was saturated with oxygen prior to its introduction at the bottom of the reactor and effluent was recycled to provide enough oxygen for the activated sludge. Recycle ratios of 3.2 to 23.2 were required. This was a major failing of the system.

Okun reported that he could consistently maintain greater concentrations of organisms in the reactor utilizing pure oxygen. He attributed this to the greater amount of oxygen available through the use of pure oxygen. He predicted possible savings of 75% of current aeration tank volumes.

Following Okun's work, little appeared in the literature until the mid-1950's, although Dorr-Oliver Ltd. did market small package plants utilizing the bio-precipitation process.

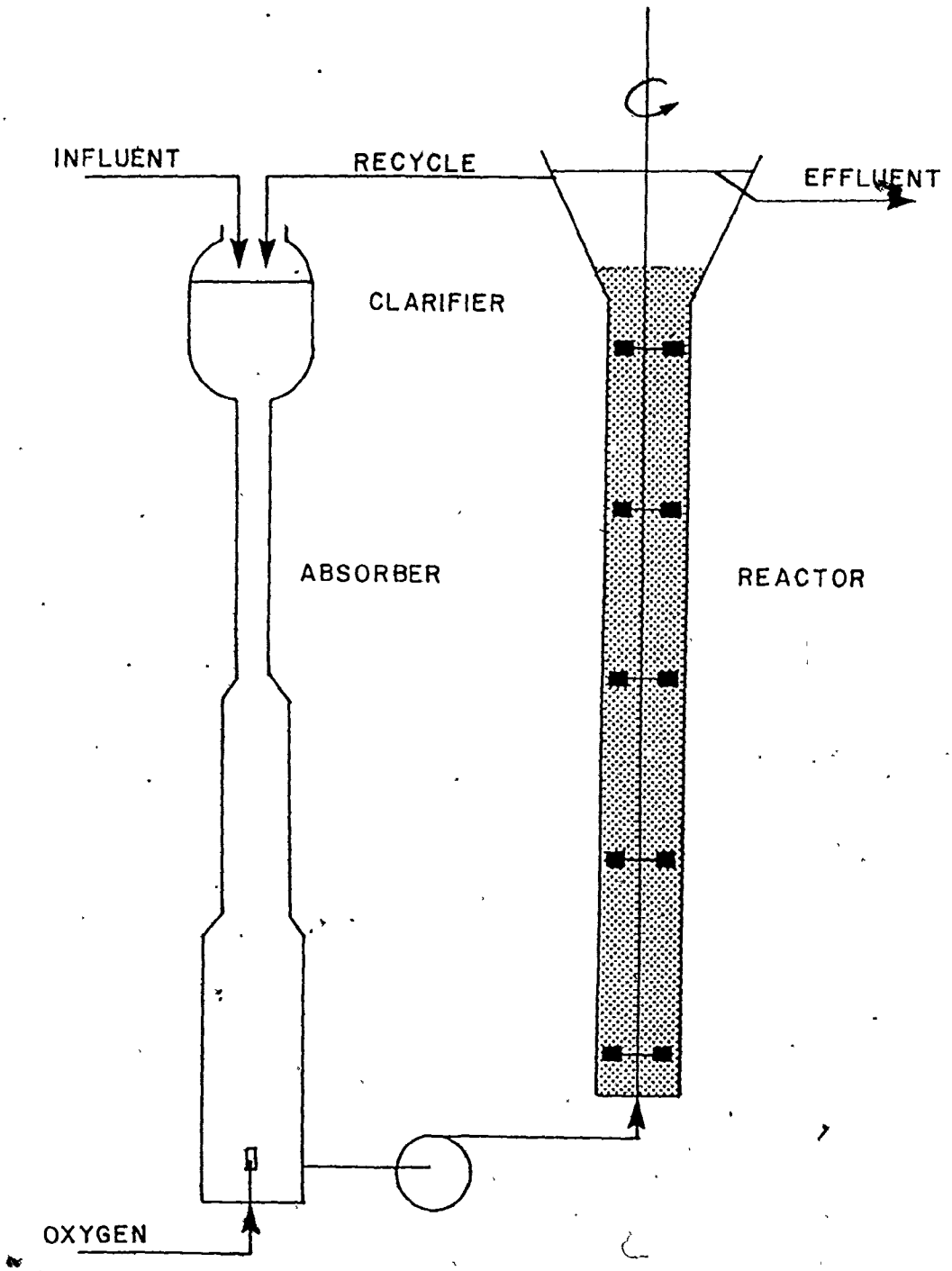


FIGURE 5

OKUN'S BIOPRECIPITATION UNIT

After Ball et al (1972)

Okun and Lynn (1956) reported on the results of batch tests using three purities of oxygen (100%, 60%, 21%). They observed better BOD_5 removal with increasing oxygen tension. They attributed this to the difference in the period of anaerobicity during settling rather than to any effects of higher oxygen tensions on the activity of the microorganisms. Okun (1957) in further studies drew much the same conclusions. Okun (1957) also stated that oxygen was rate-limiting in conventional activated sludge systems.

Budd and Lambeth (1957) reported on results obtained with Dorr-Oliver Ltd.'s package plant. They reduced Okun's aeration tank volume reduction prediction to 30% but claimed that the "bio-precipitation" configuration would result in 50% less total tank area than conventional activated sludge. The efficiency of oxygen transfer was 20% to 25%. The operating horsepower for the "bio-precipitation" process was said to be equivalent to that required by a conventional activated sludge unit.

Little work was done from the mid-50's to the mid-60's when Union Carbide Corporation began bench and pilot scale experiments into the use of pure oxygen for the U.S. Federal Water Quality Administration. The results of this work were so encouraging that the FWQA funded the conversion of one half of the Batavia, New York activated

sludge plant into the new oxygenation system, termed UNOX.

The UNOX system, shown schematically in Figure 6, consists of a series of completely mixed tanks. Pure oxygen flows cocurrently with the wastewater. The gas is drawn from the air space above each tank, compressed and introduced into the waste through a sparged turbine. The air space is kept under a pressure of 1 to 3 inches of water and pure oxygen is introduced into the head end of the system when the head space pressure drops below a given set point. The system therefore works in a manner similar to a respirometer. Waste gas, characteristically composed of 50% oxygen, is drawn off the downstream tank and released to the atmosphere in order to prevent build-up of CO_2.

The heart of the system, however, is the oxygen generation unit. The development of this process, Pressure Swing Adsorption (PSA), first lead Union Carbide to the waste treatment field in search of a market. PSA employs a series of 3 or more reactors filled with granular molecular sieve arranged in parallel, followed by a surge tank. The rate of oxygen production can be controlled with this process such that only as much oxygen is produced as is required.

The Batavia report prepared by Albertsson et al (1970), has led to a vast amount of research into the

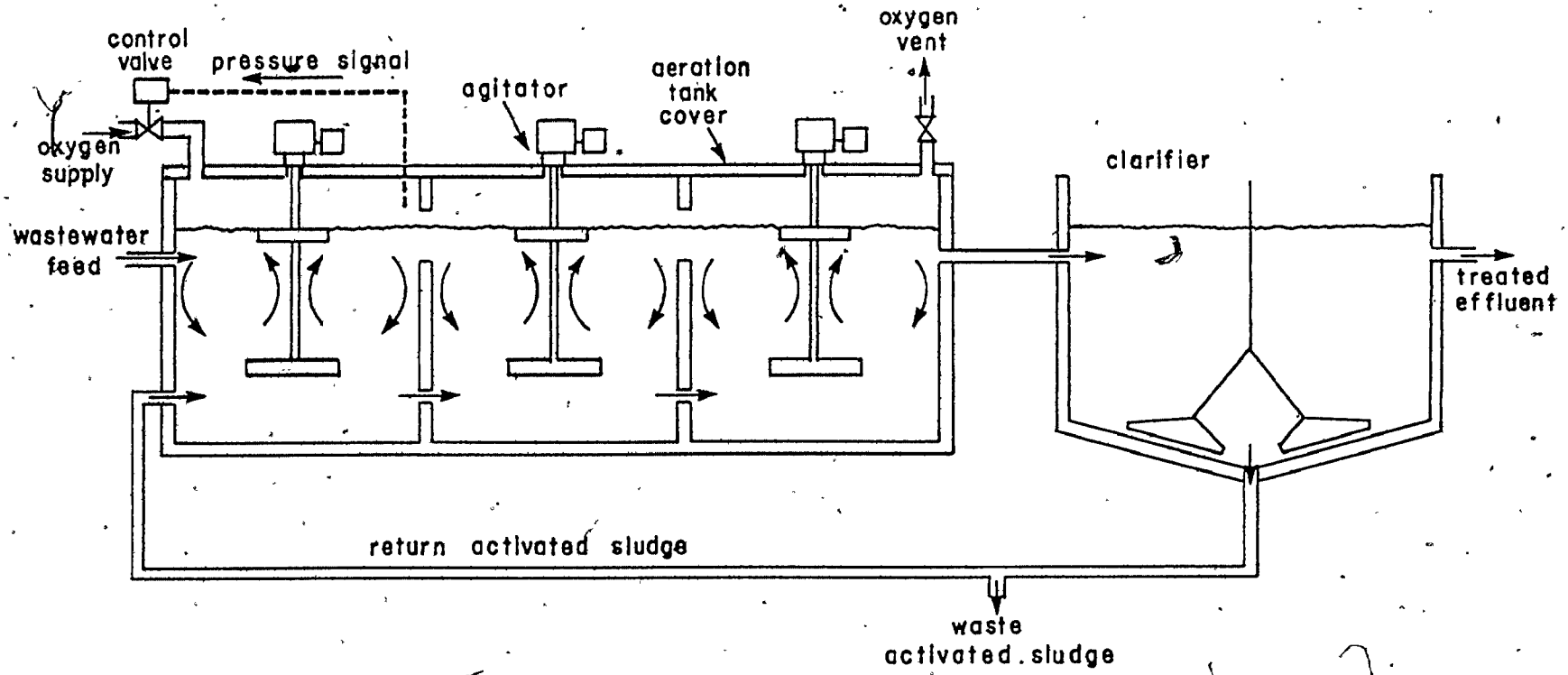


FIGURE 6

THE UNOX SYSTEM

after Wilcox and Thomas (1974)

field of oxygenation in the 1970's. Albertsson et al (1970) formed three major conclusions:

1. Oxygenated sludge was more easily settlable than aerated sludge,
2. oxygenated sludge had lower yields than aerated sludge, and
3. significant capital and operating cost savings could be made with the UNOX system.

The first two conclusions unleashed a storm of controversy which will be examined later in more depth but the large number of UNOX plants currently in the design, construction or operational phases attest to the veracity of their final conclusion.

The popularity of the UNOX system led other researchers into examining ways of utilizing existing aeration tanks as oxygenation tanks. Las Virgenes Municipal Water District (1971) proposed to cover their existing spiral roll tank and introduce oxygen via conventional spargers. The U.S. Environmental Protection Agency subsequently extended aid to this project (Stamberg, 1972). Lewis and Bays (1974) reported on the use of ceramic diffusers used to introduce oxygen into brewery waste. Problems with biological growth clogging the diffusers has suspended further research.

Various authors have reported on the results of pilot and full scale UNOX plants on wastes ranging from

domestic, (Gray et al, 1976; Nash et al, 1974 and 1977; Stamberg, 1972; Newton and Wilson, 1973), to kraft mill effluent, (Peterson, 1975), to lemon processing wastes (Tolaney, 1975), and to phenolic wastes (Englande and Eckenfelder, 1973).

2.3.2 Major Advantages

The use of pure oxygen as an aerating gas is claimed to offer three main advantages over conventional aeration systems:

1. more active sludge,
2. lower net yield, and
3. better settling sludge.

2.3.2.1 Sludge Activity

Okun and Lynn (1956), were among the first researchers to report increased BOD_5 removals with increasing dissolved oxygen tensions. They suggested the lower BOD_5 removal in air systems could be related to the sludge settling period when the sludge went anaerobic. They believed that this period of anaerobicity was less at the higher dissolved oxygen tensions. They were able to decrease the difference in BOD_5 removal by shortening the settling times in their batch fill and draw reactors. They suggested that the higher D.O. levels prevented anoxic conditions from occurring in areas of

high cell concentration, such as flocs. They observed no difference in oxygen utilization at the different dissolved oxygen tensions they used. From their results they concluded that the dissolved oxygen level does not influence the activity of the microorganisms.

Okun (1957), reported that higher dissolved oxygen levels gave better BOD_5 removals after two hours in a series of batch fill-and-draw experiments. He attributed this, as he had previously (Okun and Lynn, 1956), to shorter periods of zero dissolved oxygen during the settling period at the higher dissolved oxygen levels. To examine further the effects of anaerobic conditions, Okun let the contents of his batch reactors settle for periods varying from 10 to 120 minutes. With the oxygen aerated systems, no difference in BOD_5 removal efficiency with settling period was noted. However with the air aerated systems much poorer efficiencies were noted at the longer settling times, even though the period of zero measurable D.O. was only 10 to 15 minutes longer for the air systems than for the oxygen systems. He hypothesized that the oxygen was somehow bound-up in the floc structure or in the cytoplasm of the bacteria themselves. He observed no differences in oxygen utilization rates with D.O. level and again concluded that there was no difference in the activity of the microorganisms themselves.

Rickard and Gaudy (1968) found no correlation between dissolved oxygen level and oxygen uptake rates although they observed increasing COD and glucose removal efficiencies with increasing dissolved oxygen tension. They used bench scale continuous flow activated sludge units fed with synthetic waste. Tests were performed at a constant temperature of 30°C and mixing levels were kept constant by providing a constant flow (1 litre/min) of aerating gas.

Ball and Humenick (1972), in evaluating the data of Albertsson et al (1970), found no difference in unit substrate removal rates with respect to dissolved oxygen level.

Sherrard and Schroeder (1972) found no difference in BOD_5 removal efficiency with dissolved oxygen level for the same sludge age.

Stein et al (1972) reported that the oxygen uptake was greater for their air systems than for their oxygen systems. They were investigating aerobic digestion at bench scale in both continuous and batch reactors. No dissolved oxygen levels were mentioned.

Ball, Humenick and Speece (1972), using bench scale apparatus and a synthetic feed, found no significant difference in substrate removal kinetics with D.O. level when compared at the same solids concentrations, sludge ages and mixing levels. They also found no differ-

ences in any of a series of sludge characterization parameters: dehydrogenase activity, extracellular carbohydrates, cellular bound water. No differences could be found by microscopic examination. They concluded that the use of high dissolved oxygen levels did not cause any significant fundamental changes to the organisms.

Using an activated sludge culture and a glucose-yeast extract feed, Ball and Humenick (1973) observed no difference in substrate removal kinetics between their air and pure oxygen units.

Englande and Eckenfelder (1973) observed no differences in substrate removal rate, oxygen utilization or dehydrogenase activity level with dissolved oxygen level with their activated sludge culture grown on a phenolic waste.

Drnevich and Gay (1973) suggested that there are microbiological differences between air activated and oxygen activated sludges. They stated that oxygen activated sludges contained a greater amount of higher life forms such as rotifers and ciliata and that therefore kinetic differences existed.

McCormick (1974) also noted an increased population of rotifers and ciliata with increased dissolved oxygen tension.

2.3.2.2 Yield

One of the major conclusions of Albertsson et al (1970) was that the process sequence utilizing tonnage oxygen produced less excess solids than the parallel air system. Their correlations provided yields of 1.38 lbs. of excess volatile solids produced per pound of BOD_5 removed for the air system versus a figure of 1.05 for the oxygen system. Decay coefficients were reported at 0.17 day^{-1} for air and 0.27 day^{-1} for oxygen. These figures are significantly higher than those normally reported in the literature. Ball, Humenick and Speece (1972) attribute this to the effect of high influent solids levels coupled with the lack of primary sedimentation at the Batavia installation.

Drnevich and Gay (1973), reported statistically significant differences in sludge production between two systems operated at dissolved oxygen levels of 0.5 mg/l and 5.0 mg/l respectively. The net mass yield rate expressed as pounds of VSS formed per pound of COD removed was 29.3 per cent lower at the higher D.O. level. The reasons for this difference in yield were said to be periodic oxygen limitation periods in the air system plus more auto-oxidation occurring in the oxygen system, especially at night, because of the high MLVSS concentrations and therefore longer periods of metabolite "starvation". Lewandowski, (1974) stated that the UNOX system produces

less sludge than air activated sludge systems because the effect of endogenous respiration was greater with the higher MLSS concentrations carried in UNOX systems and because the sludge activity was higher with higher D.O. concentration.

Drnevich and Stuck (1975) utilized the data of Ball, Humenick and Speece (1972) to show a reduction of the endogenous decay rate, k_d, with D.O. below a dissolved oxygen level of 3 mg/l. They also cited the work of a number of other authors who reported lower sludge production in high D.O. systems (Hegemenn, 1973; Wuhrman, 1964; Water Pollution Research Laboratory, England, 1973).

Nash (1974 and 1977) in a New York City full scale side-by-side comparison of a modified aeration system and UNOX system, reported up to 36% less sludge produced by the UNOX system during summer conditions and up to 34% less sludge produced during winter conditions when filamentous growth was rampant in both systems. Stamberg (1972) reported on four U.S.E.P.A. large scale tests on pure oxygen systems and found that lower yields were found with the systems than are usually reported for air activated sludge systems. Wilcox and Thomas (1974), in a U.S.E.P.A. Technology Transfer Seminar publication, claimed the UNOX system produced less excess solids for the same loading as an air system because of a highly aerobic floc and because the food-to-mass ratio decreased

through the plant therefore achieving higher degrees of stabilization in the latter stages of the system.

Jewell and Mackenzie (1973) reported yields up to 29% less with high D.O. levels in side-by-side bench scale suspended growth reactors utilizing a glucose feed mixture. The low level reactors ran at D.O.'s of 2 to 6 mg/l and the high level reactors were run at D.O.'s of 12-22 mg/l. At comparable sludge ages the high oxygen tension sludges consistently produced less sludge. They hypothesized that the reason for the lower yields is that more of the floc is aerobic. That part of the floc which is aerobic, (compared to an air system floc), may not be able to get other metabolites because the concentration gradient is not great enough to drive them to the center of the floc. These microorganisms would undergo aerobic endogenous respiration which would lower the net yield.

Okun and Lynn (1956) reported little difference in sludge production between three reactors run at dissolved oxygen levels of 25.25 mg/l, 13.95 mg/l and 6.50 mg/l.

Ball and Humenick (1972) reviewed the data on the Batavia study of Albertsson et al (1970) and concluded that there was no difference in solids production between the air and oxygen systems for equivalent sludge ages. They attributed the low solids production levels

of the UNOX system to the long sludge ages maintained. Sherrard and Schroeder (1972) concurred, noting that their experiments showed decreasing yields with increasing sludge ages.

Poon and Wang (1972) ran parallel batch reactors using synthetic feed at 2 to 9 mg/l and 15 mg/l D.O. and observed greater average yields at the high D.O. level except at the highest F/M ratio, (3.0), at which they conducted their experiments. However there is a large overlap in the reported yields and one cannot tell from the data presented that whether any statistically significant differences were observed. They also reported that the yields in the high dissolved oxygen system decreased with increasing loading while the yields in the low D.O. system increased with increasing loading.

Rickard and Gaudy (1968) found no correlation between D.O. levels and cell mass yield. Stein et al (1972) also found no significant differences in yield between air and oxygen system, although they did not state the D.O. levels at which they ran their bench scale experiments.

Experiments run with a synthetic glucose-yeast extract feed and activated sludge cultures by Ball and Humenick (1973) showed no significant differences in cell production and decay rates between air and oxygen systems.

Englande and Eckenfelder (1973) concluded that D.O. had no effect on the yield of an activated sludge treating a phenolic industrial waste.

No significant difference in sludge yield was found by Ball, Humenick and Speece (1972) when their bench scale, synthetic feed systems were compared at the same solids concentrations, sludge age and mixing levels. They stated in their literature review that the Albertsson et al (1970) data do not give true yield values and that the results are biased because of the lack of primary sedimentation in the process sequence.

2.3.2.3 Settling

Okun and Lynn (1956) were probably the first to report that activated sludge settling was affected by dissolved oxygen levels. They found slightly, but consistently better sludge settling with increasing D.O. from 6.5 to 25.25 mg/l

Albertsson et al (1970) stated that the UNOX system produced a highly flocculant and readily settlable sludge which required no thickening prior to further processing for disposal. Approximately 3% to 4% recycle solids were obtained when the clarifier operation was closely controlled.

Adams (1972) reported that dissolved oxygen tensions in the range of 6.0-10.0 mg/l produced denser

sludges than conventional activated sludge systems. More of the floc was said to be aerobic and therefore no gaseous anaerobic by-products were given off to decrease the density of the flocs.

Wilcox and Thomas (1974) claimed that the UNOX system produces a highly aerobic floc with enhanced settling characteristics. Lewandowski (1974) also reported that the UNOX system produces a better settling and more easily dewaterable sludge than conventional activated sludge systems.

Peterson (1975) reported on a pilot plant study using kraft mill effluent where a UNOX system was run in parallel with an air activated sludge unit. He stated that the UNOX system produced a better settling sludge although no comparative data is given.

Ball, Humenick and Speece (1972) found no significant differences in sludge initial settling velocities with dissolved oxygen level when they were compared at the same solids concentrations, sludge ages and mixing levels. They claimed that the Sludge Volume Index is not a perfectly valid way of comparing sludge settlabilities since the higher the original MLSS concentration, the lower the S.V.I. will be. They attribute this to lower initial settling velocity and a greater weight of overburden.

No significant difference in sludge settlability

was observed by Ball and Humenick (1973) on their activated sludge culture feed on a glucose-yeast extract mixture. They suggested that the differences noted by other experimenters could be due to the lower mixing intensities, (and therefore larger flocs), in the pure oxygen systems as compared to conventional air systems.

Englande and Eckenfelder (1973) concluded that neither dissolved oxygen level or turbulence intensity had any significant effect on system performance or scale-up considerations, including sludge settlability.

2.3.3 Other Advantages

Okun (1949) reported large masses of filamentous organisms in his low D.O. reactor, while none were present in his high D.O. reactor. Adams (1972) stated that high dissolved oxygen levels substantially eliminate filamentous growth. Adams (1972) implied that filamentous growth problems in conventional plants could be substantially reduced by increasing turbulence levels thereby producing smaller flocs and reducing the locations of low D.O. tension within the sludge. His statement was supported by the work of Nash *et al* (1974 and 1977), who reported on a side-by-side comparison of an extended aeration plant and a UNOX plant at Newton Creek in New York City. The plants were seriously hampered during winter operations by filamentous organisms but the UNOX

plant gave consistently better performance in terms of BOD_5 removal and sludge yield.

Ball, Humenick and Speece (1972), reported that their search of the literature produced the following list of causes for filamentous takeover:

1. low oxygen tension,
2. high oxygen tension (one case only),
3. low pH,
4. high carbohydrate level,
5. low mixing levels,
6. high CO_2 concentrations, and
7. certain F/M ratios.

Lewandowski (1974), reported that UNOX systems tend to depress the pH of the waste because of high CO_2 concentrations, especially in the last compartments of the aeration basin.

One of the advantages claimed for pure oxygen systems is lower mixing intensities than conventional air activated sludge plants.

It can therefore be stated that pure oxygen systems are more likely to support filamentous growth if reasons 2., 3., 5., and 6 of Ball, Humenick and Speece (1972) are considered. The conditions listed as 1., 4., and 7. are more likely to occur in conventional air systems. Until more experience is gained in this aspect of pure oxygen systems, the issue of filamentous growth

will probably remain a contentious, if minor, issue.

Adams (1972) listed the ability to withstand shock loadings as an attribute of pure oxygen systems. This is a reasonable claim because of the higher MLSS carried by oxygen systems.

Perhaps the greatest advantage of pure oxygen systems is the reduction in aeration tank volume. Budd and Lambeth (1957) suggested that the "bio-precipitation" process required 30% less aeration tank volume than conventional activated sludge systems. Albertsson et al (1970) also concluded that the UNOX system afforded reductions in aeration tank volume although no percentage figure was given. The Las Virgenes proposal (1971) suggests that plant capacity can be upgraded by a factor of 2.4. The reason for this reduction in volume is the ability of the pure oxygen systems to carry high concentrations of organisms in the aeration tank. This allows the maintenance of proper F/M ratios while reducing tank volume.

Ball and Humenick (1973), concluded that the choice between a pure oxygen system and a conventional air activated sludge system is purely economic. They did, however, list four advantages of a pure oxygen system:

1. ability to meet high oxygen demands at the head end of the plant,
2. odor control because of the covered tanks,

3. significant amounts of dissolved oxygen in the effluent, and
4. efficient oxygen transfer at low mixing velocities.

2.4 SUMMARY

2.4.1 Nitrification

Nitrification, the oxidation of ammonia to nitrate, is most probably carried out by Nitrosomonas sp. and Nitrobacter sp. in the activated sludge process. These bacteria have lower growth rates and lower yield values than the heterotrophs generally found in activated sludge. Therefore longer sludge ages are required to maintain ammonia oxidation than to maintain carbon oxidation. Growth of both species is a strong function of temperature. They have wide pH optima. Nitrosomonas sp. and Nitrobacter sp. are inhibited by a wide variety of substances including metals, free ammonia and free nitrous acid. Nitrogen losses of ten per cent and greater are common in systems where nitrification is occurring.

2.4.2 Dissolved Oxygen Effects

Systems which may appear to be oxygen limiting may, in fact, be mass transfer limiting. The literature is not unanimous on the critical dissolved oxygen level required for nitrification in the activated sludge process. The effect of the high dissolved oxygen level is also in dispute.

2.4.3 Pure Oxygen

The three major advantages claimed for the use of high dissolved oxygen levels: greater microbial activity; lower yield; greater settlability, have not been proven conclusively. Physical characteristics of pure oxygen systems such as longer sludge ages and lower mixing intensities have been identified as factors contributing to experimental observations. Pure oxygen systems do have the ability to carry higher MLSS concentrations and therefore require less aeration tank volume than air systems and may have greater ability to prevent filamentous growth and withstand shock loadings.

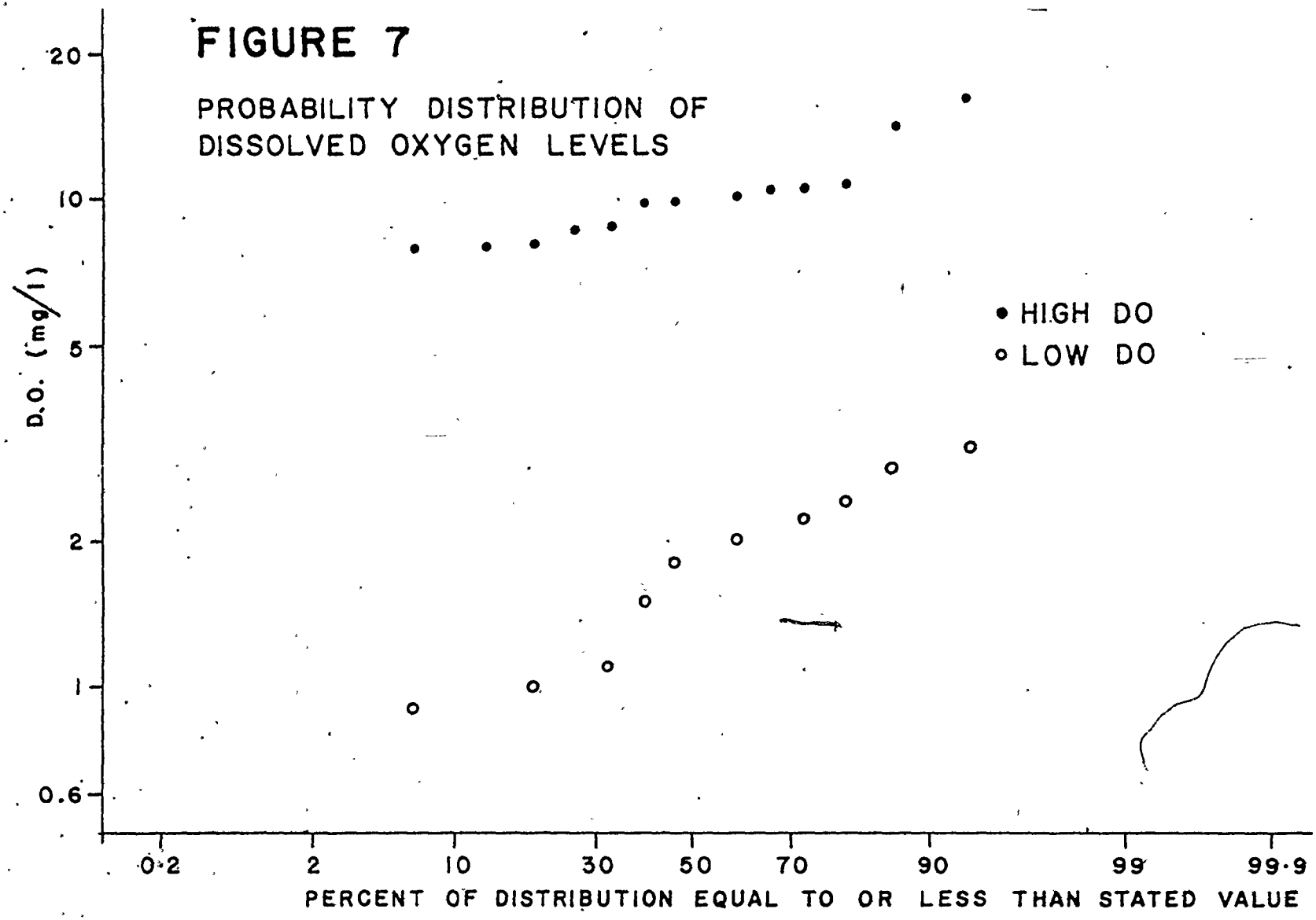
3.0 RESULTS AND DISCUSSION

3.1 INTRODUCTION

It was felt that the levels of the various operational parameters: dissolved oxygen level, temperature, and sludge age, should be equivalent to levels found in practice. Therefore two oxygen levels were chosen, 2 mg/l and 10 mg/l, these being values normally found in air activated sludge systems and oxygen activated sludge systems respectively. Figure 7 illustrates the dissolved oxygen levels recorded during the experiment. The temperature extremes, (5 C and 20 C), are commonly experienced sewage temperatures in Canada with the midrange temperature, (12.5 C), chosen as the mid-point for experimental design purposes. Sludge ages were chosen such that in a complete 3^2 experimental design nitrification was expected to cease at the low temperature-low sludge age condition, up to expected complete nitrification at the high temperature-high sludge age condition.

FIGURE 7

PROBABILITY DISTRIBUTION OF
DISSOLVED OXYGEN LEVELS



3.2 EQUIPMENT AND OPERATIONAL PROCEDURES

3.2.1 Reactor Design

At the outset of the experimental period, it was decided to employ reactors similar to those of Okun (1948) as modified by McCormick (1974). Because of difficulties encountered by McCormick (1974) in achieving good clarification, various combinations of internal baffle and clarifier arrangements were tested on a prototype. A suitable configuration was found and the two identical reactors were built. Neither of these reactors achieved satisfactory solids separation and considerable time was spent in achieving good clarification.

Each reactor was built of 6 inch diameter plexiglas with a total volume of 9.80 litres. The volume of the internal clarifier was 0.50 litres with the reaction zone volume being 9.30 litres. The wall of the clarifier and the baffle were constructed of high density polyethylene, because it was found that biological growth would not adhere to this substance to the same extent as plexiglas.

Mixing was provided by a Canlab heavy duty variable speed laboratory stirrer, model RZR1. Each reactor had its own stirrer. The high torque arm of the stirrer

provided speeds from 45 to 220 rpm. A speed of 100 rpm was found to provide a constant MLSS throughout the depth of the reactor and was used throughout the experiment. The speed was set by utilizing a strobe light and was checked weekly. Each stirrer consisted of a stainless steel rod with three four-bladed propellers. One of the propellers was positioned approximately three inches from the reactor bottom; one was just below the top of the baffle and one was approximately three inches below the top water level in the reactor. The high mixing intensity, characteristic of bench scale reactors, produced small flocs. These small flocs have minor D.O. gradients compared to flocs in full scale reactors and therefore would be more aerobic.

3.2.2 Reactor System Operation

The feed was saturated with oxygen prior to its introduction to the reactor. This saturation was achieved in a counter-current absorber similar to that used by McCormick (1974). Additional oxygen was introduced into the reactor in the eye of the bottom propeller mixer.

Early experimentation demonstrated that adding only pure oxygen into the reactor did not provide enough control over the dissolved oxygen concentration to achieve the two distinct levels required for the work. It was found that the introduction of argon into the aerating gas allowed much closer control of the oxygen tension. Dissolved oxygen levels were checked six to eight times per day.

The two reactor systems were installed in a temperature controlled room in the Wastewater Group laboratory in the Engineering Building on the McMaster University campus. The temperature was maintained at the set point $\pm$ 1°C over most of the experimental period. Icing of the coils was a constant problem at 5°C because of the high humidity in the room. Occasional building power outages; circuit breaker replacement; a compressor failure and one instance of severe coolant leakage all served to compound any other problems which happened to occur.

Raw waste was fed to the saturator-reactor systems by a Cole-Parmer variable speed peristaltic pump. Considerable difficulty in maintaining a constant feed rate was encountered. Switching from a separate pump for each system to a single pump with a "twin head" lessened the variations in flow but did not eliminate them. A low pump rpm was necessary to attain the required flow rate through the large diameter Tygon tubing used. Therefore small changes in pump speed would produce significant differences in flow rate. Flow rates varied from 14 to 27 ml/min over the experimental period despite frequent checks.

It was initially planned that a synthetic feed would be used to remove one variable from the experiment. It was felt that a mixture of yeast extract, glucose and

inorganic nutrients would be suitable. Considerable experimentation showed that the mixtures produced a rapid growth of filamentous organisms with subsequent loss of clarification ability. In some cases, the entire reactor contents would go "over the weir" within a period of 6 hours.

To overcome this difficulty, raw degritted domestic sewage was used as feed. For the first three experimental runs (February 14 to March 18, 1975) the sewage was obtained daily from the City of Burlington Skyway Sewage Treatment Plant at the Canada Centre for Inland Waters Technology Development Building. The logistics of utilizing this feed were almost unmanageable from the first and over the period March 18 to March 20, 1975 a change was made to raw degritted domestic sewage from the Town of Dundas Pollution Control Centre. Analyses of Total Organic Carbon, Chemical Oxygen Demand, Total Kjeldahl Nitrogen and Nitrate plus Nitrite Nitrogen indicated no significant differences existed in these parameters between the two sewage feeds.

The sewage was transported to the laboratory in five gallon plastic containers. It was screened through two or three layers of cheese cloth to remove the larger solids and poured into a two hundred litre plastic container in the temperature controlled reactor room. From this container it was pumped into the reactor systems.

All the effluent from each reactor was collected in a plastic container for each 24 hour period. The container was vigorously stirred for two or three minutes prior to taking a total composite sample. Although biological growth was always present on the bottom and sides of the container, it is felt that no significant growth occured in the container because of the low B.O.D. of the effluent.

Tygon tubing (R 3603 formulation) was used exclusively for carrying both liquids and gases. All tubing carrying liquids was cleaned every week by immersion for 20 to 30 minutes in denatured alcohol. It was then rinsed with tap water and air dried at room temperature.

3.2.3 Daily Procedure

The daily procedure was to obtain the raw feed during the period 1 PM to 2 PM. Once the sewage had been filtered through the cheese cloth, the feed pumps were turned off and samples of both the old feed and the new feed were taken and filtered. During this time the solids in the clarifiers had settled out into the main body of the reactor. Samples were then taken from each reactor and the composited effluent and suspended solids measurements carried out. While the filters were being dried, the old feed was drained off and the new feed

poured into the 200 litre container. Once the suspended solids measurements were complete, the required wasting was performed from each reactor by syphoning off the required volume of mixed liquor and filtering it through Whatmann No. 5 filters. The filtrate was then returned to the reactors, (not into the clarifiers), and the feed pumps were restarted. During this filtration process samples were taken of the composited effluent from each reactor. The remaining effluent was drained away and the containers were rinsed with tap water. The effluent samples were then filtered and preserved for later analysis as time permitted. The total shut down time varied from 30 to 60 minutes and depended in large part on the amount of solids which had to be wasted from the reactors.

3.2.4 Sampling Day Procedures

"Sampling days" were carried out for each set of experimental conditions to obtain "instantaneous" values of parameters being measured. These values were used in establishing substrate utilization rates and gave an indication of the variability of the parameters measured in the daily composite sampling.

Two sampling days were carried out for each set of experimental conditions. Forty eight hours elapsed between the start of one sampling day and the start of the next. This time period allowed a complete change

of active cells in the mixed liquor and thus independent experiments at the set conditions were carried out.

On sampling days the following procedure was used:

1. the flow rate from each reactor was determined,
2. effluent samples were collected from the reactor overflow,
3. influent samples were obtained from immediately upstream of the saturators,
4. the D.O. and pH in each reactor was measured,
5. Samples were filtered if necessary and proserved for future analysis,
6. dissolved nitrogen gas analysis was carried out on each saturator effluent and each reactor effluent,
7. raw feed was obtained,
8. the oxygen uptake experiment was carried out,
9. during the oxygen uptake experiment the raw sewage was coarse filtered, and the old feed changed for the new feed, and
10. immediately following the end of the oxygen uptake experiment the remainder of the daily procedure described above was carried out.

Between the two sampling days the ATP analyses were carried out and the M.P.N. nitrifier inoculations were done in addition to the normal daily procedure.

M.P.N. nitrifier inoculations were done prior to daily sludge wasting.

3.3, NITROGEN BALANCE

A nitrogen balance was calculated for each reactor on sampling days. The following equation was used in determining these balances:

$$\text{Accumulation} = \text{Total Nitrogen In} - \text{Total Nitrogen Out} \quad (18)$$

Total nitrogen was taken to be the sum of Total Kjeldahl Nitrogen in unfiltered samples plus nitrite-nitrogen plus nitrate-nitrogen plus nitrogen gas. The unfiltered TKN analyses account for soluble organic nitrogen compounds including ammonia as well as most nitrogen components in the non-soluble materials entering and leaving the reactors. Nitrogen gas was not found in the influent as it left the saturators immediately prior to entering the reactors or in the reactor effluents.

The mass balances are shown in Table 8.

All 14 of the mass balances on the low dissolved oxygen level reactor were within ± 10% of balancing, with the range being a gain of 8.4% to a loss of 6.5%. This is within the range of experimental error suggested by Painter (1970). There was an average gain over the 14 experimental runs of 1.06% or 0.60 mg/l total nitrogen.

The mass balances on the high dissolved oxygen level reactor exhibited a wider range of values than those of the low D.O. reactor, from a gain of 10% to a loss of 15.8% although only 3 runs exhibit gains or losses greater than 10%. There was an average percentage loss of 1.59% although an average gain of 0.24 mg/l total nitrogen was calculated. This can be accounted for by noting that the large percentage losses occurred when the influent total nitrogen concentration was low. There does not appear to be any reason to suspect denitrification as a cause of the observed losses.

In addition to the error sources discussed previously in the literature review section, the Technicon Auto-analyzer was subject to sampling errors when abstracting the unfiltered TKN samples from the sample cups.

TABLE 8

MASS BALANCES

DATE AND PARAMETER	INFLUENT (mg-N/l)	HIGH D.O. REACTOR EFFLUENT (mg-N/l)	DIFF (out-in) (mg-N/l)	DIFF %	LOW D.O. REACTOR EFFLUENT (mg-N/l)	DIFF (out-in) (mg-N/l)	% DIFF ($\frac{\text{out-in}}{\text{in}}$ x 100)
Feb. 18							
TKN-UF	33.7	23.8			21.9		
NO_3+NO_2	0.2	13.5			10.5		
Total-N	33.9	37.3	+3.4	+10.0	32.4	-1.5	-4.4
Feb. 20							
TKN-UF	21.9	9.4			0.2		
NO_3+NO_2	2.3	15.3			15.5		
Total-N	24.2	24.7	+0.5	+2.1	24.7	+0.5	+2.1
Mar. 1							
TKN-UF	25.8	22.8			23.5		
NO_3+NO_2	4.2	5.8			8.6		
Total-N	30.0	28.6	-1.4	-4.7	32.1	+2.1	+7.0

TABLE 8 cont'd...

DATE AND PARAMETER	INFLUENT (mg-N/l)	HIGH D.O. REACTOR EFFLUENT (mg-N/l)	HIGH D.O. REACTOR DIFF (out-in) (mg-N/l)	HIGH D.O. REACTOR DIFF %	LOW D.O. REACTOR EFFLUENT (out-in) (mg-N/l)	LOW D.O. REACTOR DIFF (out-in) (mg-N/l)	LOW D.O. REACTOR % DIFF $(\frac{\text{out-in}}{\text{in}} \times 100)$
Mar. 3							
TKN-UF	20.5	16.8			16.3		
NO_3+NO_2	2.5	7.7			7.7		
Total-N	23.0	24.5	+1.5	+6.5	24.0	+1.0	+4.3
Mar. 16							
TKN-UF	20.0	10.7			13.8		
NO_3+NO_2	trace	7.9			6.3		
Total-N	20.0	18.6	-1.4	-7.0	20.1	+0.1	+0.5
Mar. 18							
TKN-UF	26.4	18.0			20.6		
NO_3+NO_2	trace	8.8			6.8		
Total-N	26.4	26.8	+0.4	+1.5	27.4	+1.0	+3.8

TABLE 8 cont'd...

DATE AND PARAMETER	INFLUENT (mg-N/l)	HIGH D.O. REACTOR EFFLUENT (mg-N/l)	HIGH D.O. REACTOR DIFF (out-in) (mg-N/l)	HIGH D.O. REACTOR DIFF %	LOW D.O. REACTOR EFFLUENT (out-in) (mg-N/l)	LOW D.O. REACTOR DIFF (out-in) (mg-N/l)	LOW D.O. REACTOR % DIFF ($\frac{\text{out-in}}{\text{in}}$ x 100)
Mar. 25							
TKN-UF	44.9	3.5			22.3		
NO_3+NO_2	2.7	44.9			29.3		
Total-N	47.6	48.4	+0.8	+1.7	51.6	+4.0	+8.4
Mar. 27							
TKN-UF	45.6	22.1			26.5		
NO_3+NO_2	3.2	26.8			22.5		
Total-N	48.8	48.9	+o.1	+0.2	49.0	+0.2	+0.4
Apr. 6							
TKN-UF	49.2	1.9			29.7		
NO_3+NO_2	2.3	53.1			22.1		
Total-N	51.5	55.0	+3.5	+6.8	51.8	+0.3	+0.6

TABLE 8 cont'd...

DATE AND PARAMETER	INFLUENT (mg-N/l)	HIGH D.O. REACTOR EFFLUENT (mg-N/l)	HIGH D.O. REACTOR DIFF (out-in) (mg-N/l)	HIGH D.O. REACTOR DIFF %	LOW D.O. REACTOR EFFLUENT (out-in) (mg-N/l)	LOW D.O. REACTOR DIFF (out-in) (mg-N/l)	LOW D.O. REACTOR % DIFF ($\frac{\text{out-in}}{\text{in}} \times 100$)
Apr. 8							
TKN-UF	36.5	7.1			14.6		
NO_3+NO_2	8.3	41.9			32.4		
Total-N	44.8	49.0	+4.2	+9.4	47.0	+2.2	+4.9
Apr. 13							
TKN-UF	17.3	1.3			4.0		
NO_3+NO_2	1.1	14.2			14.2		
Total-N	18.4	15.5	-2.9	-15.8	18.2	-0.2	-1.1
Apr. 15							
TKN-UF	13.8	1.8			2.0		
NO_3+NO_2	trace	10.3			10.9		
Total-N	13.8	12.1	-1.9	-13.8	12.9	-0.9	-6.5

TABLE 8 cont'd....

DATE AND PARAMETER	INFLUENT (mg-N/l)	HIGH D.O. REACTOR EFFLUENT (mg-N/l)	HIGH D.O. REACTOR DIFF (out-in) (mg-N/l)	HIGH D.O. REACTOR DIFF %	LOW D.O. REACTOR EFFLUENT (out-in) (mg-N/l)	LOW D.O. REACTOR DIFF (out-in) (mg-N/l)	LOW D.O. REACTOR % DIFF ($\frac{\text{out-in}}{\text{in}}$ x 100)
Apr. 20							
TKN-UF	14.3	6.2			4.2		
NO_3+NO_2	1.6	8.7			10.8		
Total-N	15.9	14.9	-1.0	-6.3	15.0	-0.9	-5.7
Apr. 22							
TKN-UF	18.2	10.5			13.4		
NO_3+NO_2	1.4	6.6			6.3		
Total-N	19.6	17.1	-2.5	-12.8	19.7	+0.1	+0.5

A "t" test at the 99% confidence level reveals that no difference exists in the calculated balances between the high and low level dissolved oxygen reactors.

3.4 INHIBITION

The data taken on rate days was sufficient to calculate the concentrations of free ammonia (FA) and free nitrous acid (FNA), both shown to be inhibitory to the nitrification process. The formulae developed by Anthonisen et al (1976) and presented previously in the literature review were used. The results are presented in Table 9.

A review of the results shows that FNA was never at inhibitory concentrations. Inhibitory concentrations of FA occurred only once, on February 18 in the high DO reactor. The concentration, 0.138 mg/l, is considered by Anthonisen et al (1976) to be on the lower boundary of the inhibition level. None of the other analyses carried out during this work implied that the nitrification process on February 18 was inhibited and it is concluded that no inhibition by FA or FNA occurred during this work.

3.5 NITRATE PRODUCTION

3.5.1 General

Daily 24 hour composited effluent nitrate plus

TABLE 9

CALCULATED* VALUES OF FREE AMMONIA (F.A.) AND FREE NITROUS ACID (F.N.A.)

DATE	TEMP (°C)	HIGH D.O. pH	TOTAL NH_3 (mg/l-N) (effluent)	F.A. (mg/l-NH_3)	TOTAL NO_2 (mg/l-N) (effluent)	F.N.A. (mg/l-HNO_2)	LOW D.O. pH	TOTAL NH_3 (mg/l-N) (effluent)	F.A. (mg/l-NH_3)	TOTAL NO_2 (mg/l-N) (effluent)	F.N.A. (mg/l-HNO
Feb. 18	12.5	7.35	22.8	0.138	0.6	0.0003	7.05	20.0	0.061	0.5	0.000
Feb. 20	12.5	6.75	2.9	0.004	2.2	0.0041	6.75	3.9	0.005	1.5	0.002
Mar. 1	5	7.25	13.0	0.034	0.4	0.0003	7.5	15.2	0.071	1.8	0.000
Mar. 11	12.5	6.5	13.8	0.012	4.9	0.00161	7.0	15.7	0.043	4.5	0.004
Mar. 16	12.5	6.75	8.5	0.013	0.8	0.0015	7.1	12.0	0.041	1.4	0.001
Mar. 18	12.5	6.85	17.9	0.034	3.2	0.0047	7.05	20.5	0.062	2.8	0.002
Mar. 25	20	6.2	2.7	0.002	0.5	0.0027	6.7	19.7	0.047	1.1	0.001
Mar. 27	20	6.8	21.1	0.064	0.3	0.0004	6.85	23.0	0.078	0.5	0.000
Apr. 6	12.5	5.95	0.6	0.0001	0.5	0.0058	7.0	26.8	0.073	0.5	0.000
Apr. 8	12.5	6.1	trace	-	0.7	0.0058	6.8	11.3	0.019	0.4	0.000
Apr. 13	12.5	6.6	0.1	0.0001	0.2	0.0005	6.7	0.4	0.0005	0.3	0.000
Apr. 15	12.5	6.6	0.2	0.0002	0.3	0.0008	6.75	trace	-	0.7	0.001
Apr. 20	5	6.3	3.5	0.001	0.1	0.0006	6.95	2.3	0.003	0.3	0.000
Apr. 22	5	6.9	6.7	0.008	0.1	0.0002	7.3	6.5	0.019	0.2	0.000

*After Anthonisen et al (1976)

nitrite analyses were used to determine the concentration of nitrate plus nitrite produced during each 24 hour period in each reactor.

A "t" test at the 95% confidence level indicated that there was no significant difference between the total flow through the high D.O. level and low D.O. level reactors (Appendix C) and therefore concentration difference rather than total weight of nitrate plus nitrite produced was used.

Only those results from March 10 to the end of the experimental period were used. The concentrations of nitrate plus nitrite prior to this date were determined by use of an Orion nitrate probe. Starting on March 10 samples were taken for analysis by the Technicon Autoanalyzer and compared to the probe results. After a period of two weeks it became obvious that a low correlation existed between the two sets of results and the use of the probe was discontinued.

3.5.2 Hypothesis Testing

Hypothesis tests were used to determine if there was any significant difference in nitrate production between high and low level dissolved oxygen reactors at each set of pseudo-steady-state experimental conditions.

The tests used the paired "t" statistic as follows:

$$H_o : x_H - x_L = 0 \quad \text{or} \quad D = 0 \tag{19}$$

$$H_1 : x_H - x_L > 0 \quad \text{or} \quad D > 0 \tag{20}$$

where: x_H is the average daily concentration of $NO_3 + NO_2$ produced in high D.O. level reactor, and

x_L is the average daily concentration of $NO_3 + NO_2$ produced in low D.O. level reactor.

$$t = \bar{D}/S_{\bar{D}} \tag{21}$$

where: $\bar{D}$ is $\frac{1}{n} \sum_{i=1}^{n} D_i$ (22)

D_i is $x_{H_i} - x_{L_i}$, (23)

n is the number of paired data points,

$S_{\bar{D}}$ is $\sqrt{\frac{\sum D_i^2 - \frac{1}{n}(\sum D_i)^2}{n(n-1)}}$, and (24)

t has n-1 degrees of freedom.

For the pooled data:

$$t = \frac{\bar{D}}{S_{\bar{D}_p} \times \frac{1}{\sqrt{n}}} \tag{25}$$

where: $\bar{D}$ is $\frac{1}{n} \sum D_i$, (26)

D_i is $x_{H_i} - x_{L_i}$, (27)

n is the total number of paired data points,

$$S_{\bar{D}_p} \text{ is } \sqrt{\frac{(n_3 - 1)S_{\bar{D}_3}^2 + \ldots + (n_7 - 1)S_{\bar{D}_7}^2}{n_3 + n_4 + n_5 + n_6 + n_7 - 5}} \quad (28)$$

t has 20 degrees of freedom.

The results of these "t" tests are summarized in the following table together with the conclusion at the 95% confidence level:

TABLE 10

SUMMARY OF HYPOTHESIS TESTS

DAILY NITRATE PRODUCTION

Run	Sludge age (days)	Temp (°C)	t	df	Conclusion @ 95% C.L.
Pool			17.079	22	Reject H_o
3	4	12.5	5.399	6	Reject H_o
4	8	20.0	5.850	3	Reject H_o
5	12	12.5	3.211	6	Reject H_o
6	8	12.5	-.614	2	Accept H_o
7	8	5.0	.591	5	Accept H_o

The pooled "t" value indicates that the higher dissolved oxygen level sludge produced significantly more nitrates plus nitrites during the experiment. This was true during 3 of the 5 separate runs. Temperature may have inhibited nitrate production during Run 7 to such an extent as to mask the dissolved oxygen effect. The lack of steady state data during Run 6 tends to reduce the

validity of the computed "t" value.

3.5.3 Temperature and Sludge Age Effects

Variances were calculated from the data for each run, first without differentiation between results at the two dissolved oxygen levels and then with results from each dissolved oxygen level separated. A series of "F" tests were then carried out. The results of these "F" tests are shown in Table 11. Those results that are significant at the 95% and 99% confidence limits are indicated.

Significant differences at the 95% confidence level (C.L.) occurred between most undifferentiated runs and it was therefore felt that only significant differences at the 99% confidence level could be utilized in this analysis.

There were significant (99% C.L.) differences between the mid-point run, (Run 6), and Runs 3 and 5, i.e. at differing sludge ages , but a common temperature indicating that sludge age has some effect on the ability of a sludge to convert TKN to NO_3-N. The lack of (99% confidence limit) difference between Runs 3 and 5 could be due to the relatively wide scatter in the data.

There were significant (99% C.L.) differences between Run 4 and Runs 6 and 7, i.e. at differing temperatures, but a common sludge age indicating that temp-

TABLE 11

SUMMARY OF "F" TESTS

NITRATE CONVERSION

(a) Undifferentiated Data

Run	3	4	5	6	7
3	-	5.634	2.835*	0.046**	0.247*
4		-	0.503	0.008**	0.044**
5			-	0.016**	0.087**
6				-	5.327*
7					-

(b) High D.O. Level Data

Run	3	4	5	6	7
3	-	2.312	3.547	0.250	1.001
4		-	1.534	0.108	0.433
5			-	0.070*	0.282
6				-	4.010
7					-

(c) Low D.O. Level Data

Run	3	4	5	6	7
3	-	1.004	3.439	0.017*	0.324
4		-	3.424	0.017*	0.323
5			-	0.005	0.094*
6				-	18.828
7					-

* Significant difference at 95% confidence level.

** Significant difference at 99% confidence level.

erature has some effect on the ability of a sludge to convert TKN to NO_3-N. The lack of difference (99% C.L.) between the lower temperature Runs 6 and 7 could indicate an Arrhenius-type temperature dependency.

The other significant (99% C.L.) difference occurs between Runs 5 and 7, i.e. between the low temperature run and the high sludge age run. In view of the evidence in the literature and in this work regarding temperature and sludge age effects, it appears reasonable to expect such a difference to occur.

There is a lack of significant differences between runs when the data from each dissolved oxygen level is considered separately. This together with the differences exhibited by the undifferentiated data supports the conclusion reached in the analysis of the hypothesis tests that dissolved oxygen tension affects the ability of a sludge to convert organic nitrogen to nitrate nitrogen.

3.6 NITRIFICATION RATE

3.6.1 Determination of Reaction Order

For the purpose of this work the rate of nitrification was taken to be the rate of formation of nitrite plus nitrate nitrogen. Those data where the concentration of nitrite plus nitrate nitrogen formed exceeded

the concentration of unfiltered TKN in the influent (i.e., where the apparent conversion was greater than one) were excluded from the analyses.

There is no significant difference at the 95% confidence level between either the MLSS or MLVSS levels in the two reactors at any temperature or sludge age or during the overall experiment. It was felt that expressing rates as unit rates served no purpose in this work and because of the lack of significant difference in solids levels, unit rates were not used.

In order to determine the order of the reaction it was first necessary to eliminate temperature effects. This was accomplished by using dimensionless rates. The dimensionless rates were calculated by dividing each value at a particular temperature by the average rate at that temperature. Similar transformations were carried out on the influent TKN concentrations and the effluent nitrite plus nitrate nitrogen concentrations.

From Levenspiel (1972) the following expression can be derived for the nitrification rate in a single completely mixed reactor:

$$\text{Nitrification Rate} = \frac{C - C_o}{t} \qquad (29)$$

where: C_o is the influent concentration of nitrite plus nitrate nitrogen,

C is the effluent concentration of nitrite plus nitrate nitrogen, and

t is the theoretical detention time. Both rates determined from the 24 composited samples and the "instantaneous" samples were used in the analysis of rates. Both the rates and the dimensionless rates are shown in Table 12.

In order to determine if the reaction rate could be approximated by a first order model, the dimensionless rates were plotted versus the dimensionless effluent unfiltered TKN concentration in Figures 8,9,10 and 11. No significant correlation was found to exist at the high dissolved oxygen level. At the low dissolved oxygen level the daily rates were significantly negatively correlated at the 99% confidence level and the instantaneous rates were significantly negatively correlated at the 95% confidence level. The correlations at the low D.O. level, although statistically significant, are weak, and this together with their negativity lead to the conclusion that a first order model does not fit the data.

A zero order model was tested by plotting dimensionless rates versus dimensionless influent unfiltered TKN concentration as shown in Figures 12,13,14 and 15. The only statistically significant correlation occurs at the high dissolved oxygen level daily rates. This correlation coefficient (0.501) was quite weak and it was felt that the data as a whole supported the conclusion that a zero order kinetic model was adequate to describe the data.

TABLE 12

NITRIFICATION RATES

DAILY NITRIFICATION RATES

RUN	TEMP (°C)	HIGH D.O. RATE	HIGH D.O. DMLS RATE	LOW D.O. RATE	LOW D.O. DMLS RATE
7	5	.738	1.127	.631	1.000
		.598	.913	.661	1.048
		.847	1.293	.702	1.113
		.524	.800	.518	.821
		.704	1.075	.628	.996
		.518	.791	.645	1.022
	Avg.	.655	1.000	.631	1.000
3	12.5	1.839	1.221	.914	.980
		1.444	.958	.412	.442
		1.497	.994	1.296	1.390
		1.637	1.086	.912	.978
		1.160	.770	.879	.943
		1.565	1.039	.980	1.051
		1.543	1.024	.666	.714
5	12.5	1.041	.691	.396	.425
		1.569	1.041	1.131	1.213
		1.454	.965	1.437	1.541
		1.218	.808	1.250	1.341
		1.518	1.007	.812	.871
		1.869	1.240	.510	.547

TABLE 12 cont'd...

RUN	TEMP	HIGH D.O. RATE	HIGH D.O. DMLS RATE	LOW D. O. RATE	LOW D. O. DMLS RATE
		1.416	.940	.320	.343
		2.653	1.761	.903	.969
		2.357	1.564	1.976	2.119
6	12.5	.937	.622	1.034	1.109
		.961	.638	.823	.883
		.950	.631	1.063	1.140
	Avg.	1.507	1.000	.932	1.000
4	20	5.325	1.190	2.406	.995
		4.190	.936	2.509	1.038
		3.910	.874	2.338	.967
	Avg.	4.475	1.000	2.418	1.000

INSTANTANEOUS RATES

RUN	TEMP	DATE	RATE HIGH RATE	RATE HIGH DMLS	RATE LOW RATE	RATE LOW DMLS
2	5	Mar 1	0.157	.294	0.458	.690
	5	Mar 3	.562	1.051	.569	.857
7	5	Apr 20	.782	1.463	1.014	1.527
	5	Apr 22	.637	1.192	.615	.926
	Avg.		.535	1.000	.664	1.000
1	12.5	Feb 18	1.855	1.057	1.336	.906
	12.5	Feb 20	2.117	1.206	2.020	1.370
3	12.5	Mar 16	1.112	.633	.887	.602
	12.5	Mar 18	1.428	.813	.999	.678

TABLE 12 cont'd....

RUN	TEMP	DATE	RATE HIGH RATE	RATE HIGH DMLS	RATE LOW RATE	RATE LOW DMLS
5	12.5	Apr 8	3.086	1.758	2.213	1.501
6	12.5	Apr 13	1.524	.868	1.564	1.061
		Apr 15	1.167	.665	1.301	.882
	Avg.		1.756	1.000	1.474	1.000
4	20	Mar 25	4.521	1.283	2.736	1.139
	20	Mar 27	2.529	.717	2.068	.861
	Avg.		3.525	1.000	2.402	1.000

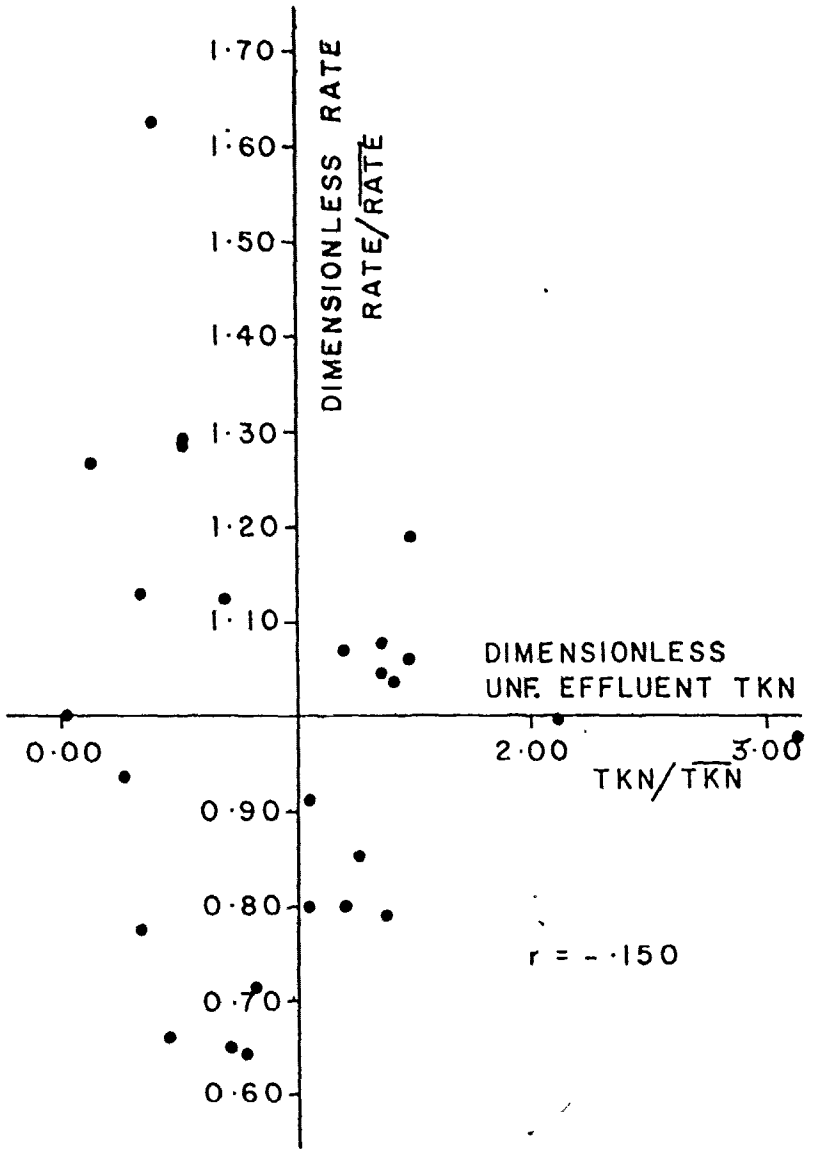


FIGURE 8

DAILY NITRIFICATION RATE
VERSUS
EFFLUENT CONCENTRATION
HIGH DISSOLVED OXYGEN LEVEL

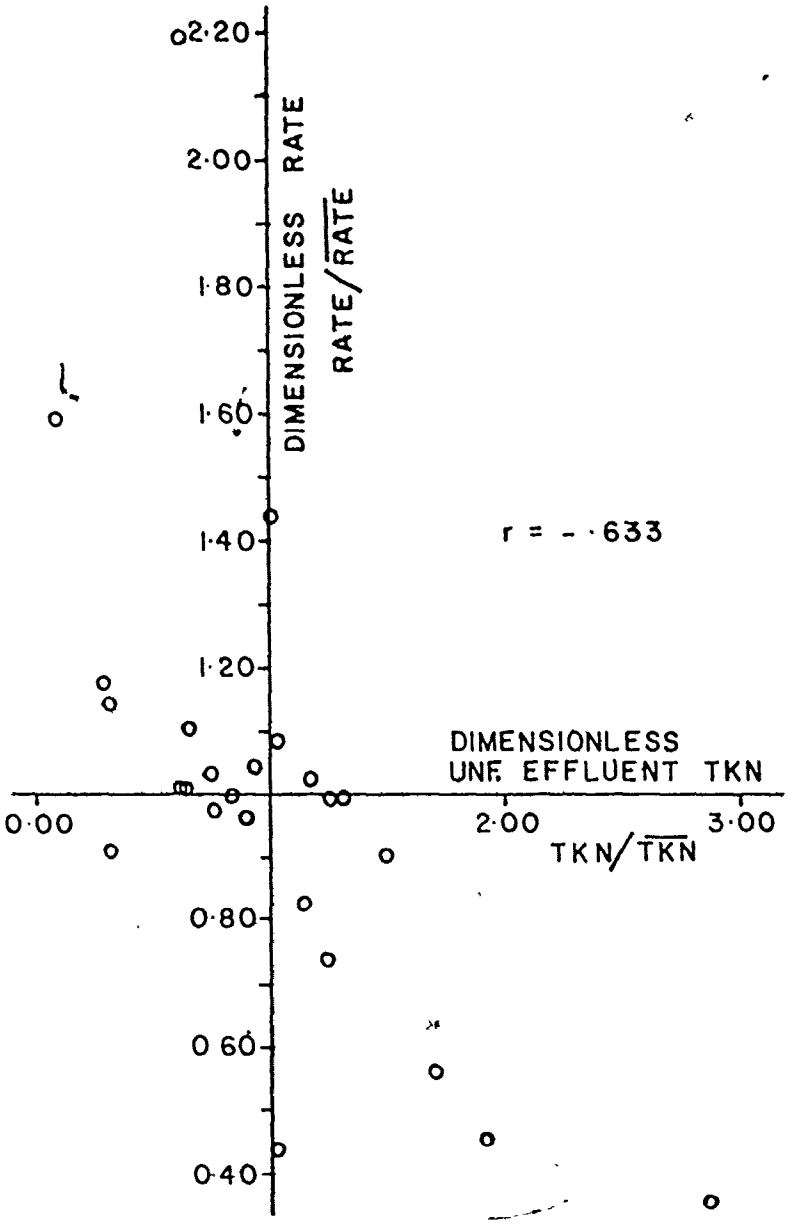


FIGURE 9

DAILY NITRIFICATION RATE
VERSUS
EFFLUENT CONCENTRATION
LOW DISSOLVED OXYGEN LEVEL

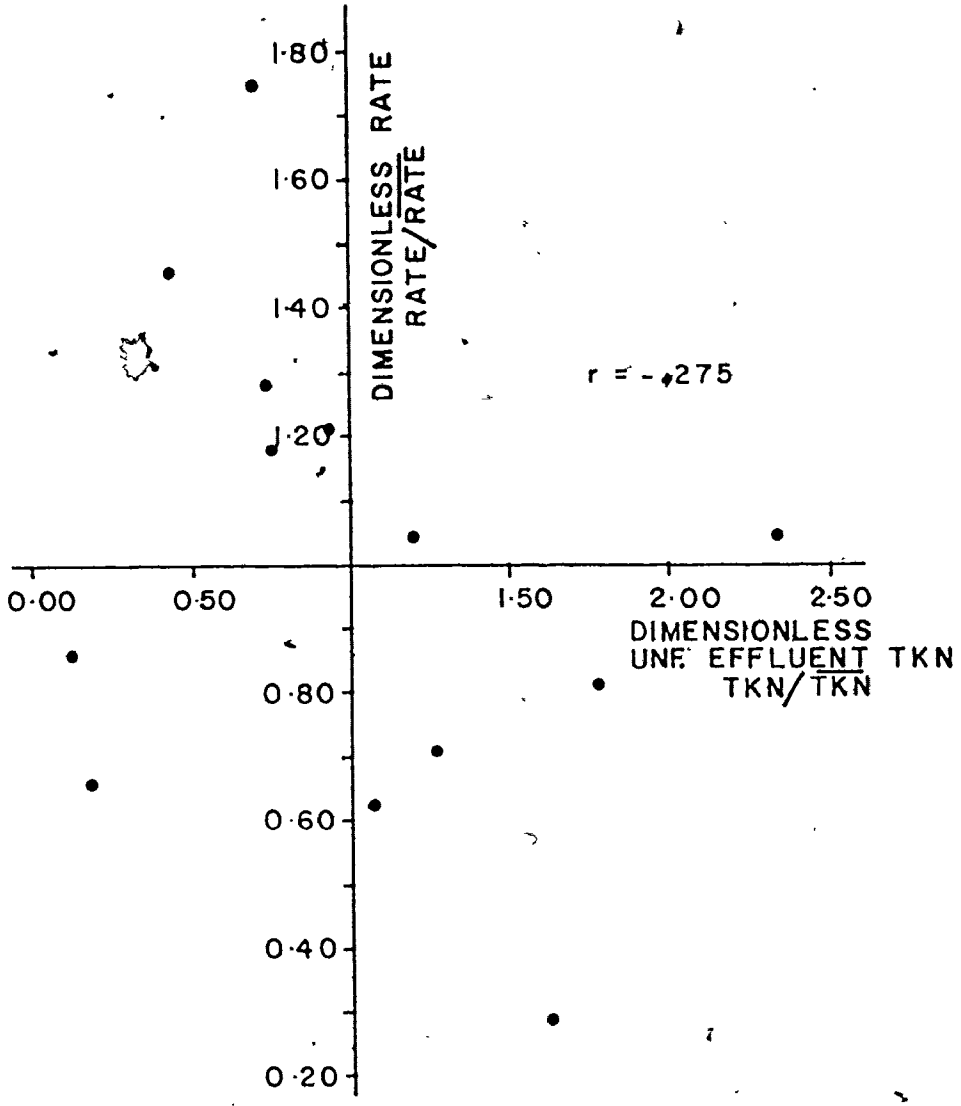


FIGURE 10

"INSTANTANEOUS"
NITRIFICATION RATE
VERSUS
EFFLUENT CONCENTRATION
HIGH DISSOLVED OXYGEN

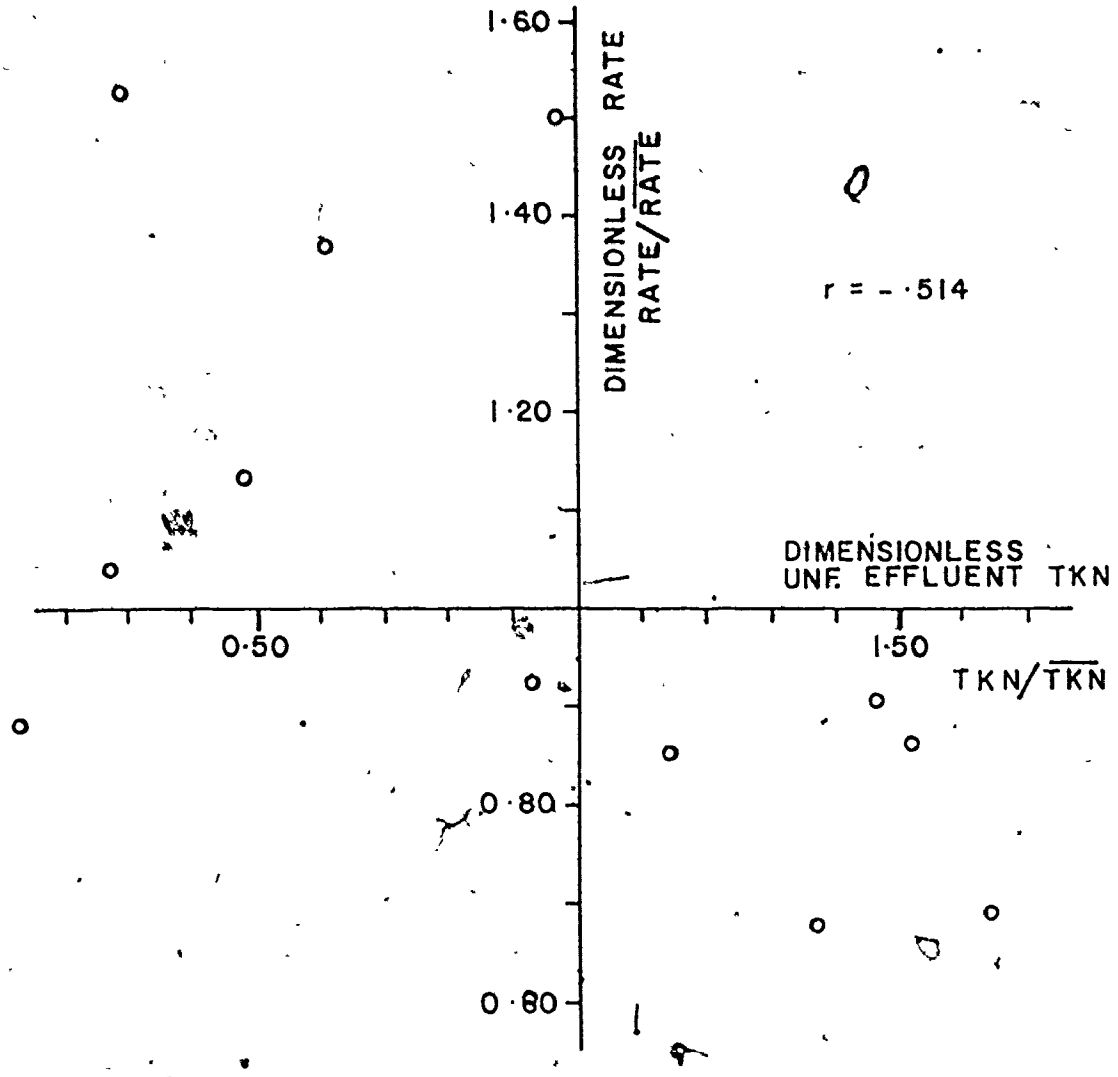


FIGURE 11

"INSTANTANEOUS"
NITRIFICATION RATE
VERSUS
EFFLUENT CONCENTRATION
LOW DISSOLVED OXYGEN

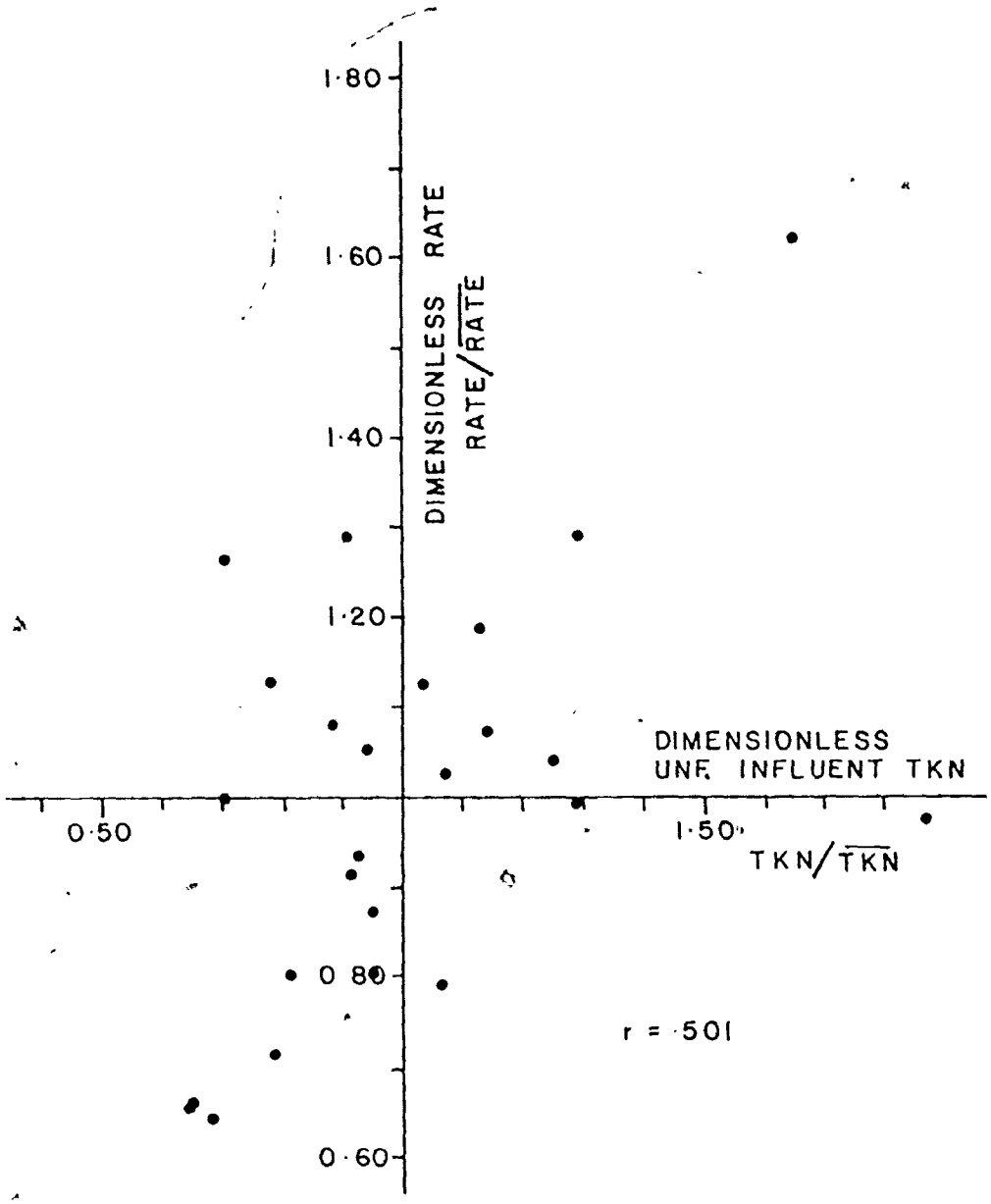


FIGURE 12

DAILY NITRIFICATION RATE
VERSUS
INFLUENT CONCENTRATION
HIGH DISSOLVED OXYGEN LEVEL

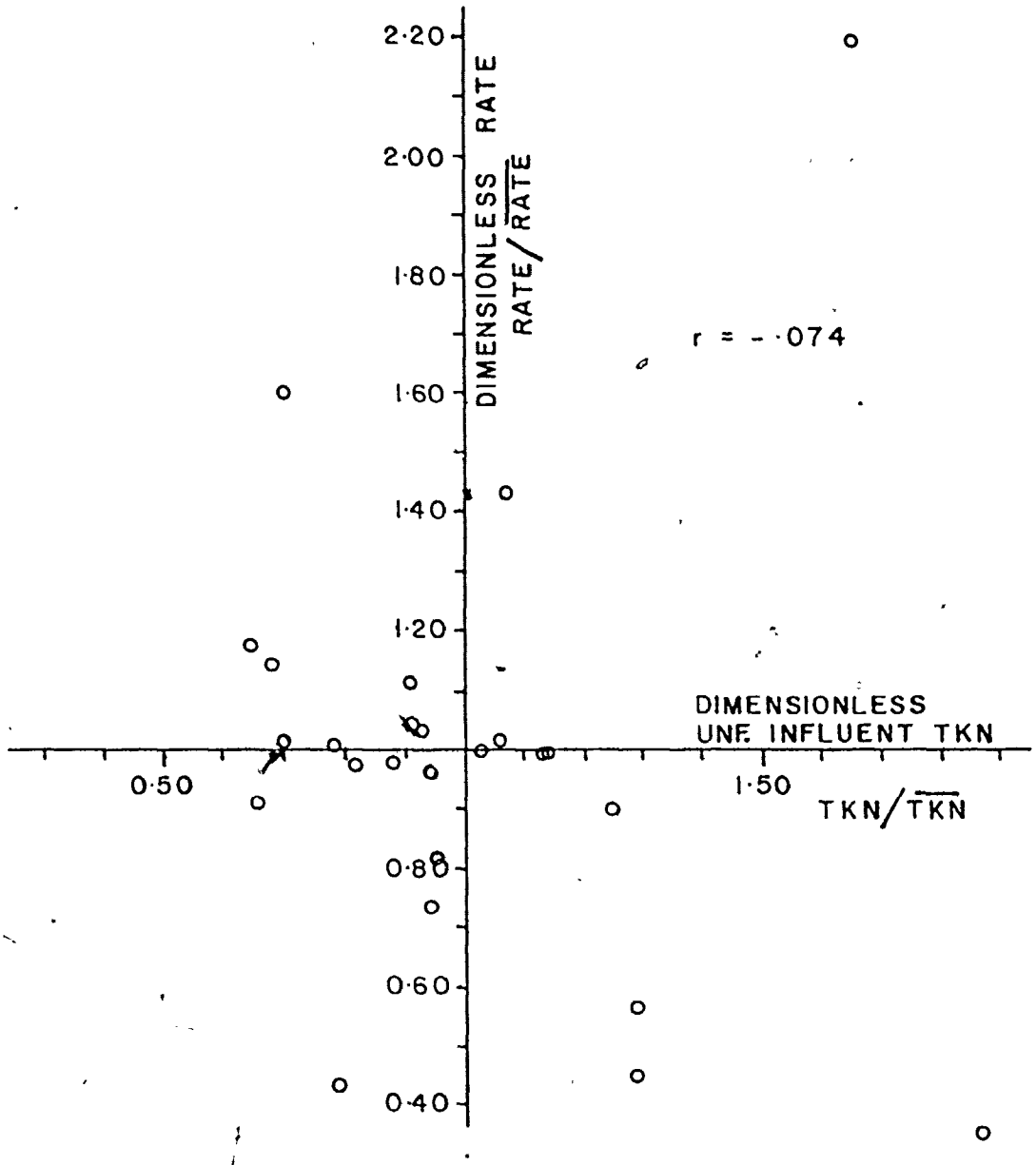


FIGURE 13

DAILY NITRIFICATION RATE
VERSUS
INFLUENT CONCENTRATION
LOW DISSOLVED OXYGEN LEVEL

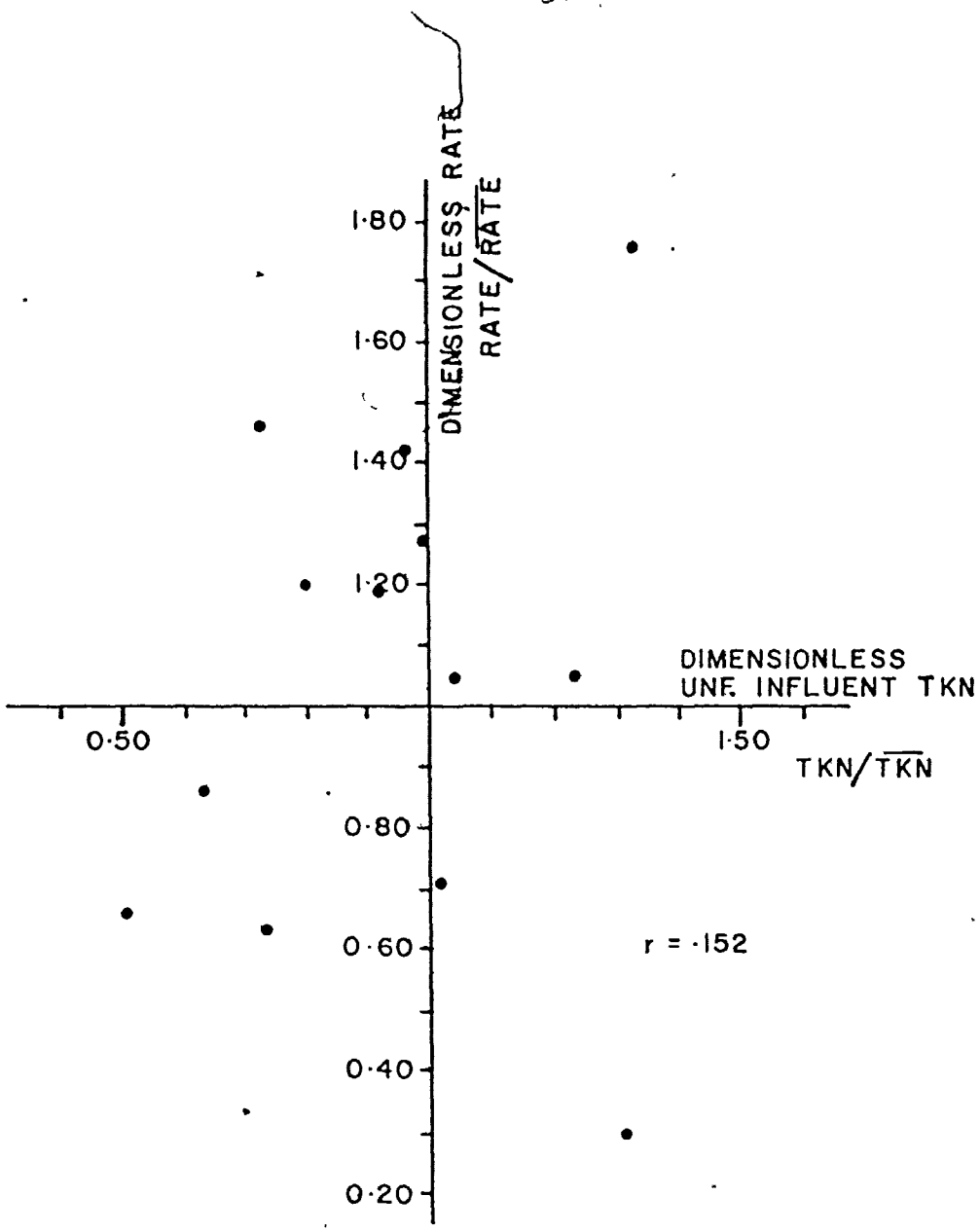


FIGURE 14

"INSTANTANEOUS"
NITRIFICATION RATE
VERSUS
INFLUENT CONCENTRATION
HIGH DISSOLVED OXYGEN LEVEL

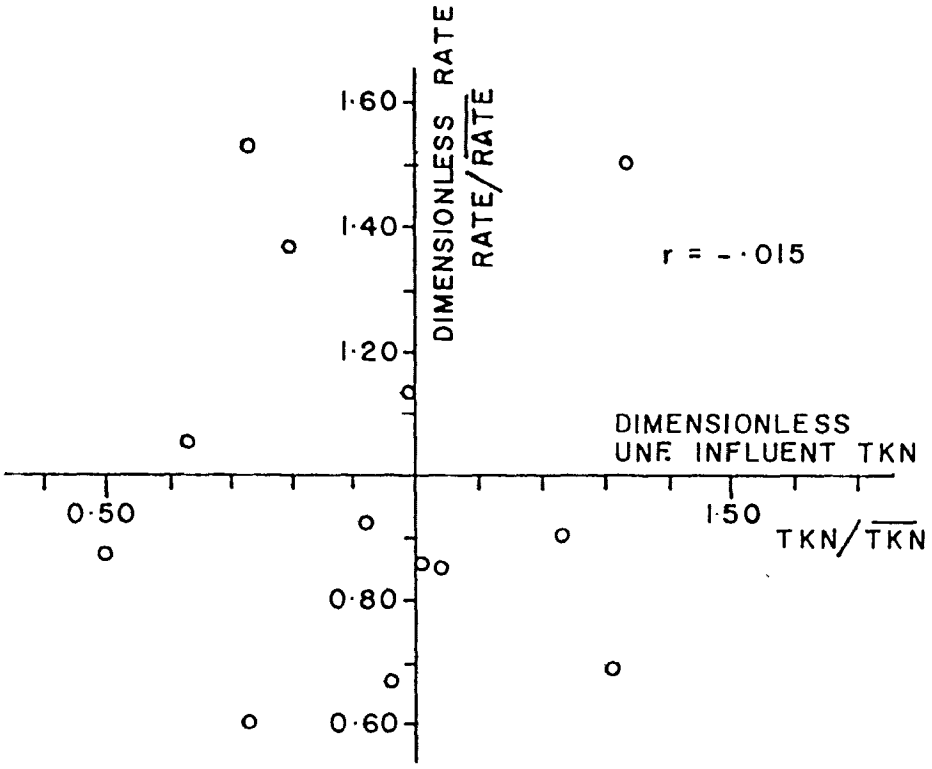


FIGURE 15

"INSTANTANEOUS"
NITRIFICATION RATE
VERSUS
INFLUENT CONCENTRATION
LOW DISSOLVED OXYGEN LEVEL

3.6.2 Hypothesis Testing

Table 13 below shows the results of hypothesis testing on daily nitrification rates using the paired "t" statistic as outlined in Section 3.4.2 and showing the conclusion at the 99% confidence level. The results are similar to those of the hypothesis tests on the total nitrate production. The pooled data indicates that overall, the high dissolved oxygen sludge nitrified at a faster rate than the low D.O. sludge. Temperature inhibition may have masked this effect during the 5°C run (Run 7) and lack of steady-state data during Run 6 reduces the validity of this analysis. During Runs 3,4, and 5 the oxygen level effect was clearly evident.

TABLE 13

SUMMARY OF HYPOTHESIS TESTS

DAILY NITRIFICATION RATE

Run	Sludge Age (days)	Temp (°C)	t	df	Conclusion
Pool			17.380	27	Reject H_o
3	4	12.5	5.440	6	Reject H_o
4	8	20.0	4.763	2	Reject H_o
5	12	12.5	3.540	8	Reject H_o
6	8	12.5	-.296	2	Accept H_o
7	8	5.0	.561	5	Accept H_o

3.6.3 Temperature and Sludge Age Effects By Analysis of Variances

The procedure set out in Section 3.4.3 was used to analyse the data for temperature and sludge effects. The results of the "F" tests are shown in Table 14.

Analysis of the results of the undifferentiated data indicate that both temperature and sludge age affect the nitrification rate and that there appears to be a strong interaction between the two parameters. The high temperature run (Run 4) is significantly different at the 99% confidence level from both Runs 6 and 7 at the same sludge age but differing temperatures. Because Runs 6 and 7 were not significantly different, an Arrhenius-type temperature-nitrification rate dependency was indicated. The midpoint run (Run 6) was significantly different from both Runs 3 and 5 at the same temperature but differing sludge ages. Runs 3 and 5 (high and low sludge ages), are significantly different at the 90% confidence level but not at the 95% confidence level. All the "perimeter" runs are significantly different at either the 95% or 99% confidence level indicating that a strong sludge age-temperature interaction exists.

When only the results at the high dissolved oxygen level are considered, the results are similar to the undifferentiated data. Runs 3 and 7, and 4 and 5 are not significantly different indicating that the temperature

TABLE 14

SUMMARY OF "F" TESTS

NITRIFICATION RATE

(a) Undifferentiated Data

Run	3	4	5	6	7
3	-	8.726*	2.330	.041**	.056**
4		-	.267*	.005**	.006**
5			-	.018**	.024**
6				-	1.358
7					-

(b) High D.O. Level Data

Run	3	4	5	6	7
3	-	13.283**	6.570*	.003**	.404
4		-	.495	.0003**	.030**
5			-	.001**	.061**
6				-	118.247**
7					-

(c) Low D.O. Level Data

Run	3	4	5	6	7
3	-	.099	3.891	.230	.051**
4		-	39.250*	2.315	.510
5			-	.059	.013**
6				-	.220
7					-

* Significant difference at 95% confidence level.

** Significant difference at 99% confidence level.

and sludge age effects cancel each other out, i.e. that they are of approximately equal magnitude.

The "F" test results on the low D.O. data differ considerably from those of both the high dissolved oxygen level and the undifferentiated data. These results indicate that the temperature-sludge age interaction is relatively strong but that neither parameter by itself exerts much influence. The lack of a statistical difference between Runs 3 and 4 may be due to the low number of degrees of freedom for Run 4.

It is concluded that decreased dissolved oxygen tension inhibits the sensitivity of an activated sludge systems nitrification rate to both temperature and sludge age.

3.6.4 Polynomial Model

To assist in determining whether differences in nitrification rates were due to differences in dissolved oxygen level, an empirical polynomial model was constructed, based on the experimental design:

$$y = B_o + B_1 x_1 + B_2 x_2 + B_{11} x_1^2 + B_{22} x_2^2 + B_3 x_3 + B_{13} x_1 x_3 + B_{23} x_2 x_3$$

where: y is the nitrification rate,

x_1 is the temperature,

x_2 is the sludge age, and

x_3 is the dissolved oxygen level.

The average daily nitrification rates for each

run were used to evaluate the parameters. The mean square of the residual was used as an estimate of the variance and the 95% confidence intervals of the parameters calculated. The results are shown below in Table 15.

TABLE 15

NITRIFICATION RATE POLYNOMIAL MODEL

Parameter	Value	95% confidence Limits Lower	Upper
$\hat{B}_o$	0.961	.578	1.344
$\hat{B}_1$	1.402	1.131	1.673
$\hat{B}_2$	0.064	-.207	.335
$\hat{B}_{11}$	1.084	.614	1.554
$\hat{B}_{22}$	0.299	-.171	.769
$\hat{B}_3$	0.342	.171	.513
$\hat{B}_{13}$	.508	.237	.778
$\hat{B}_{23}$	.012	-.259	.283

Because of the lack of daily data on Runs 1 and 2, no lack of fit test could be carried out.

The 95% confidence limits indicate that the sludge age parameters, $\hat{B}_2$, $\hat{B}_{22}$ and $\hat{B}_{23}$ do not differ significantly from zero. This suggests that sludge age was not a major factor over the range of sludge age used. The work of other authors has shown that major losses of nitrifying ability occur below sludge ages of 4 to 5 days. The effect of sludge age could be expected to be more pronounced at lower temperatures according to the literature.

The values of the other parameters indicate that

temperature has a greater effect than dissolved oxygen level on the nitrification rate. The model indicates that the higher dissolved oxygen system nitrification rate was approximately 0.7 mg/hr/l higher than the rate in the low dissolved oxygen system.

An extra sum of squares evaluation was used to determine whether excluding the dissolved oxygen level terms, would have a significant effect on the fit of the polynomial model. An "F" test conducted at the 95% confidence level on the mean square of the residuals of the two models showed that exclusion of the dissolved oxygen term had no significant effect on the fit of the model.

Although the extra sum of squares evaluation shows no significant differences between models with and without the D.O. term, the predicted difference of 0.7 mg/hr/l is greater than the average daily and average "instantaneous" rates under low temperature conditions (Run 7). Had the full experimental design been carried out, the usefulness of the polynomial model as a predictive tool would probably be enhanced. With the data available the model predicts that temperature has the greatest effect on nitrification rates, dissolved oxygen tension lesser effects and sludge age no effect.

3.6.5 Temperature Dependency

3.6.5.1 General

It has been shown earlier in this work that temperature has a large effect on the rate of nitrification.

The Arrhenius temperature-rate model has been shown by many authors to adequately describe observed nitrification rates and was therefore used in this work.

To eliminate any possible sludge age effects, only those rates from runs having an 8 day sludge age were used. Rates were determined from 24 hour composite samples except for Runs 1 and 2 where the rate is the average of the two rate day values.

3.6.5.2 Arrhenius Model

The Arrhenius rate model is quite difficult to fit in the form usually presented in chemical engineering texts because of the interaction between the frequency factor, and the activation energy:

$$K = Ae^{-E/RT} \qquad (31)$$

where: K is the rate constant,

A is the frequency factor, and

E is the activation energy.

A form of this equation has been developed which minimizes this interaction:

$$K = K^* e^{-E/R\left(\frac{1}{T} - \frac{1}{T_o}\right)} \qquad (32)$$

where: K^* is Ae^{-E/RT_o}, and

T_o is the median temperature in °K.

A logarithmic transformation was used to produce the following simplified equation:

$$\ln K = -\frac{E}{R}\left(\frac{1}{T} - \frac{1}{T_o}\right) + \ln K^* \qquad (33)$$

This equation was used to produce "best fit" lines for both the high and low dissolved oxygen results. These lines are shown in Figure 16. "F" tests at the 95% confidence level showed no lack of fit of either of the lines fitted to the high and low dissolved oxygen level data. However, there was no significant difference at the 95% confidence level between the activation energies at each oxygen tension.

Therefore one "best fit" line was produced as shown in Figure 17. This single line for both sets of data exhibited a lack of fit at the 95% confidence level but no lack of fit at the 99% confidence level. The 95% confidence limits for the activation energy of the single line contains the activation energies of both the high and the low D.O. "best fit" lines. This value of E is almost identical to that reported by Downing and Hopwood (1964).

It may be possible that at lower temperatures the effects of dissolved oxygen are hidden by the inhibiting effect of those low temperatures.

The Q_{10} from 10°C to 20°C was found to be 3.53,

FIGURE 16

NITRIFICATION RATE TEMPERATURE DEPENDENCY

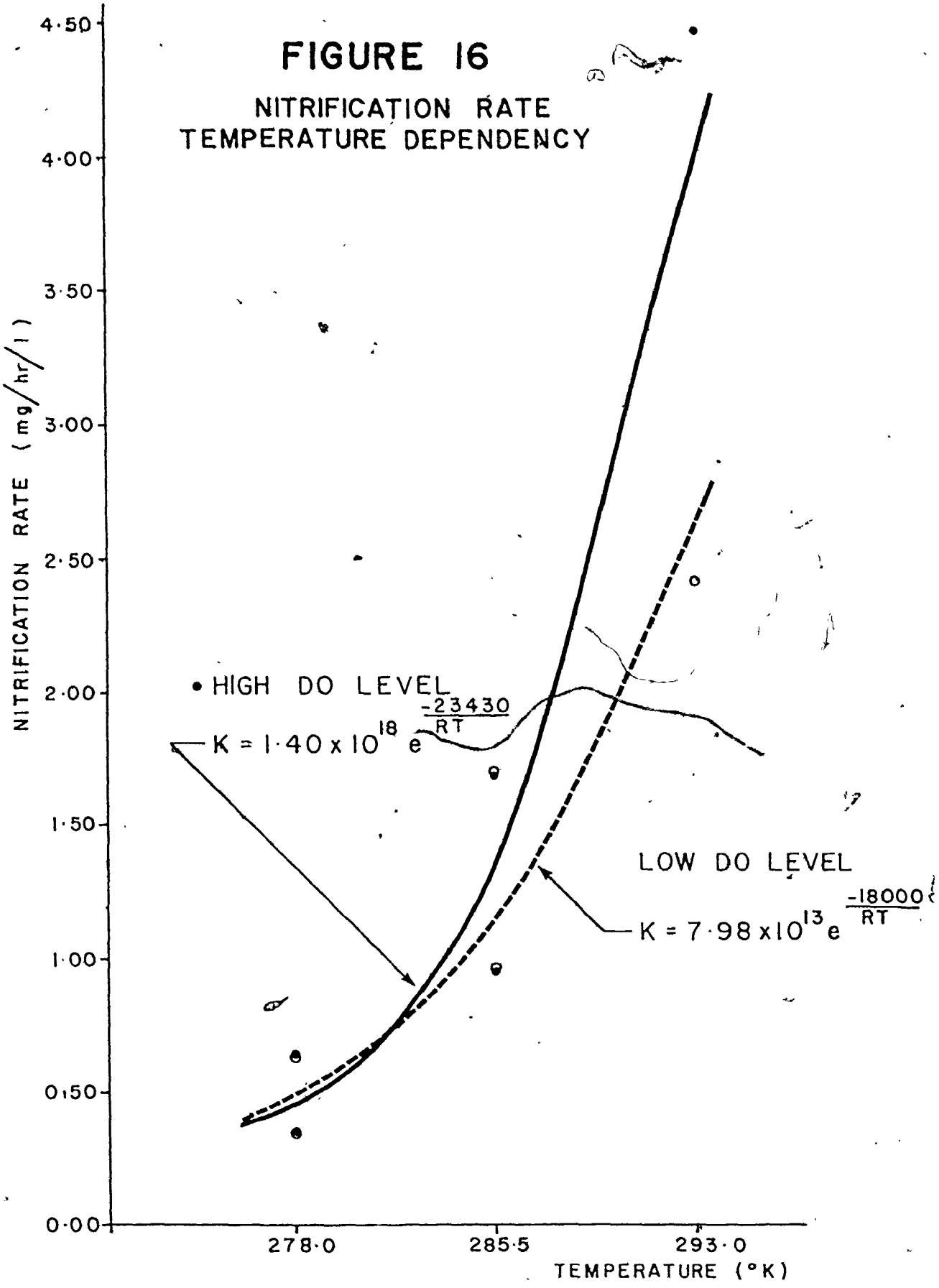
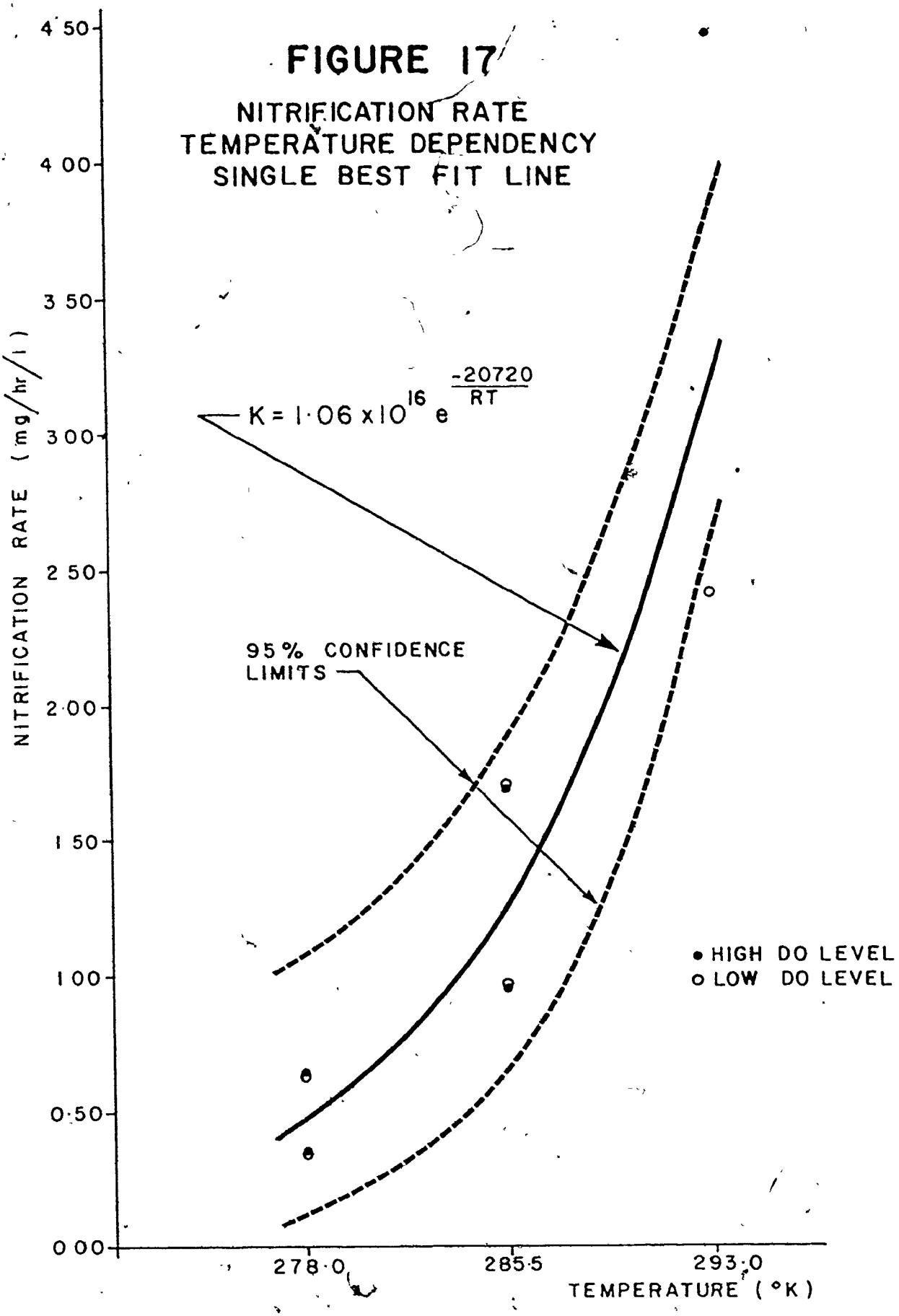


FIGURE 17

NITRIFICATION RATE TEMPERATURE DEPENDENCY SINGLE BEST FIT LINE



based on the single "best fit" line, 4.17 for the high D.O. and 2.99 for the low D.O. "best fit" line. The single "best fit" Q_{10} is close to that of 3.42 reported by Wilson (1975) at a sludge age of 7 days in a two stage activated sludge process. The 40% difference in Q_{10} nitrification values between the high D.O. and the low D.O. sludges, again suggests that low temperatures may hide any D.O. effects.

3.7 MOST PROBABLE NUMBER OF NITRIFIERS

3.7.1 General

A multiple tube technique (See Appendix B) was used to determine the most probable number of nitrifiers (MPNN) in each reactor during each run. Spot tests were done on each tube at the end of the incubation period to test for the presence of ammonia, nitrite and nitrite plus nitrate. All tubes showed positive reactions for both ammonia and nitrite and thus the results of the nitrite plus nitrate test were used in determining the MPNN. The detailed results are shown in Appendix D. Table 16 summarizes the results.

TABLE 16

SUMMARY OF MPNN RESULTS

Run	D.O. Level	
	High	Low
1	2,300,000/ml	430,000/ml
2	56,000	1,700,000
3	330,000	230,000
4	350,000	1,300,000
5	350,000	1,300,000
6	13,000,000	1,100,000
7	2,300,000	64,000

3.7.2 Hypothesis Tests

A hypothesis test was conducted on the paired data shown in Table 16. The results are shown below.

H_o: $D = 0$

H_1: $D > 0$

where: $D_i = MPNN_{H_i} - MPNN_{L_i}$

$t = 1.012$ $\quad df = 6$

$t_{crit} = 1.943$ at the 95% confidence level.

This test shows that there is no significant difference between the MPNN at the two oxygen levels.

3.7.3 Correlation With Nitrate Production and Nitrification Rate

The MPNN and $\log_{10}$ MPNN were plotted versus nit-

rate converted and nitrification rate. See Figures 18, 19, 20, and 21. All relationships showed a low level of correlation. None of the correlations were found to be significant at the 95% level of significance.

One reason for the low correlations found may be that the range of MPNN was limited, from 0.05 x 10^6/ml to 2.3 x 10^6/ml, except for the high D.O. level MPNN during Run 6.

3.8 TOC REDUCTION

3.8.1 Hypothesis Testing

TOC analyses were run daily on the 24 hour composite of the effluent from each reactor. This paired data from each run was subjected to hypothesis testing to determine whether any differences existed in the daily TOC reduction between the high and low dissolved oxygen level reactors. The results of these tests are shown in Table 17.

The hypothesis tests show that, with the exceptions of Runs 3 and 7, the high dissolved oxygen level sludge reduced more TOC than the low dissolved oxygen sludge. This reduction is statistically significant at the 99% confidence level when all the data is pooled. Pooling of the data from Runs 1 and 6 also indicates a significant difference at the 99% confidence level. How-

FIGURE 18

NITRATE CONVERTED VERSUS MPNN

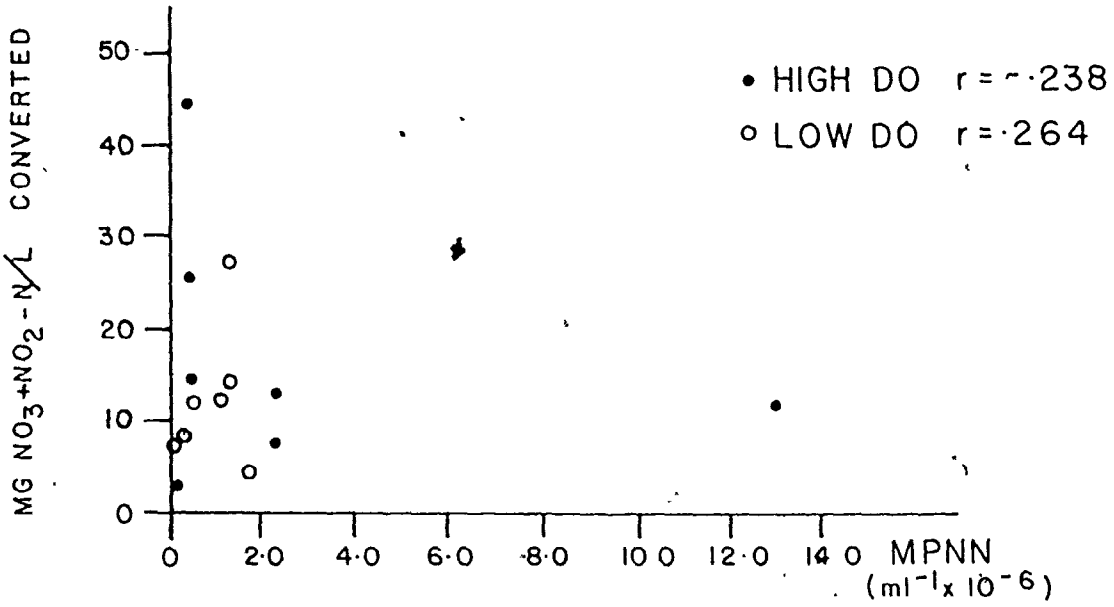


FIGURE 19

NITRIFICATION RATE VERSUS MPNN

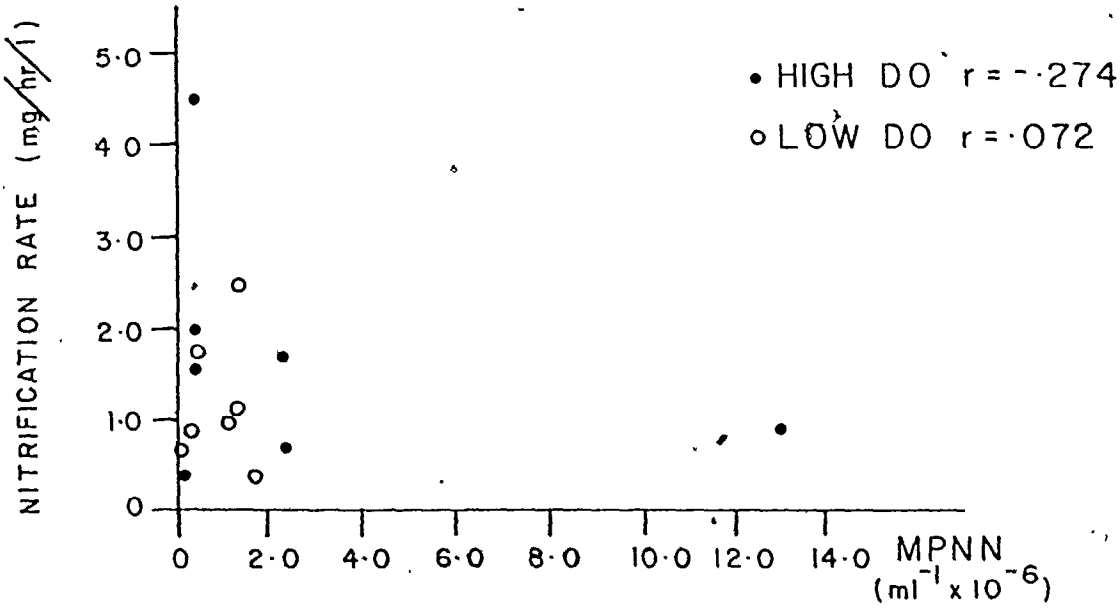


FIGURE 20

NITRATE CONVERTED
VERSUS
LOG MPNN

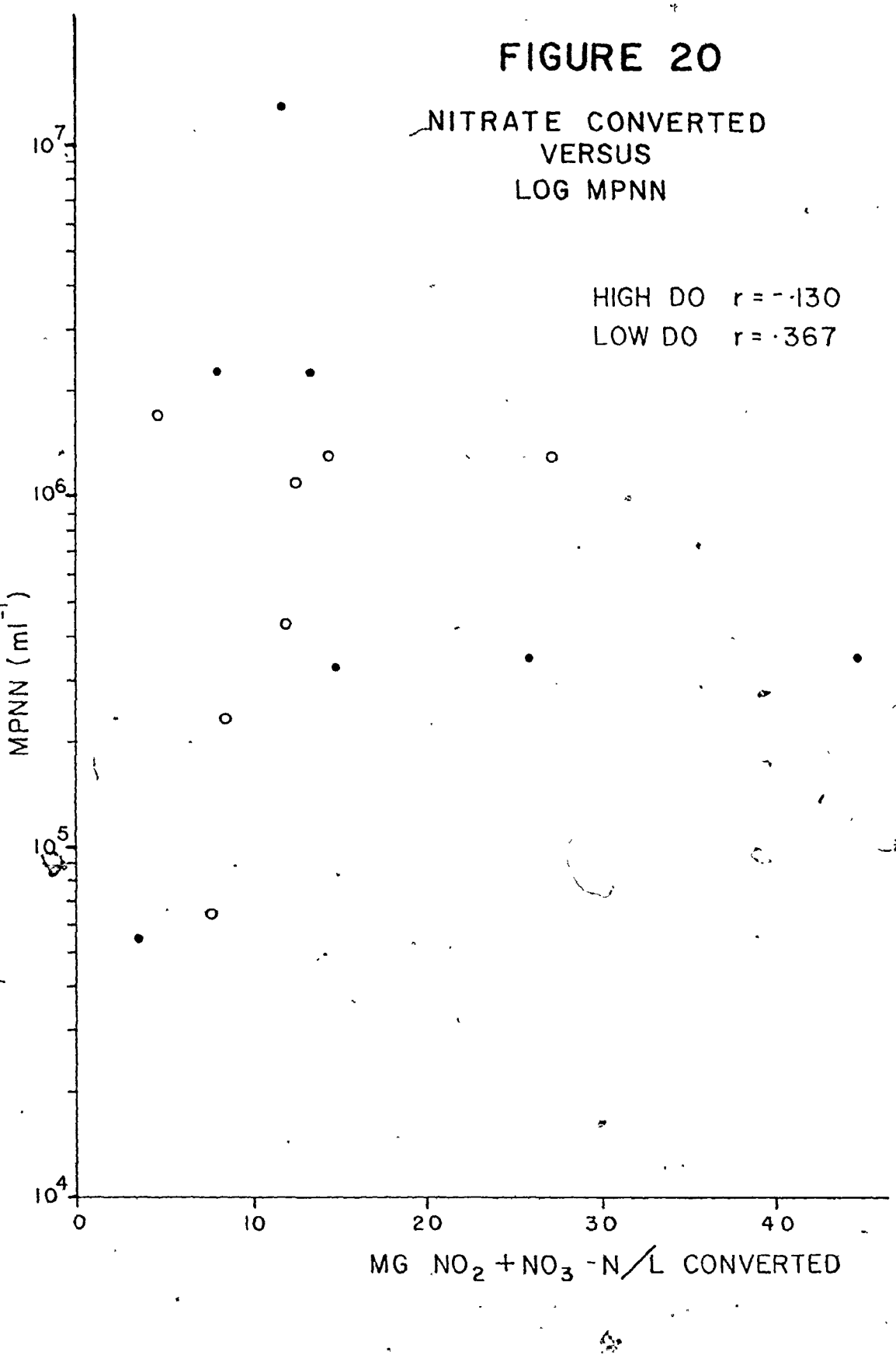


FIGURE 21

NITRIFICATION RATE
VERSUS
LOG MPNN

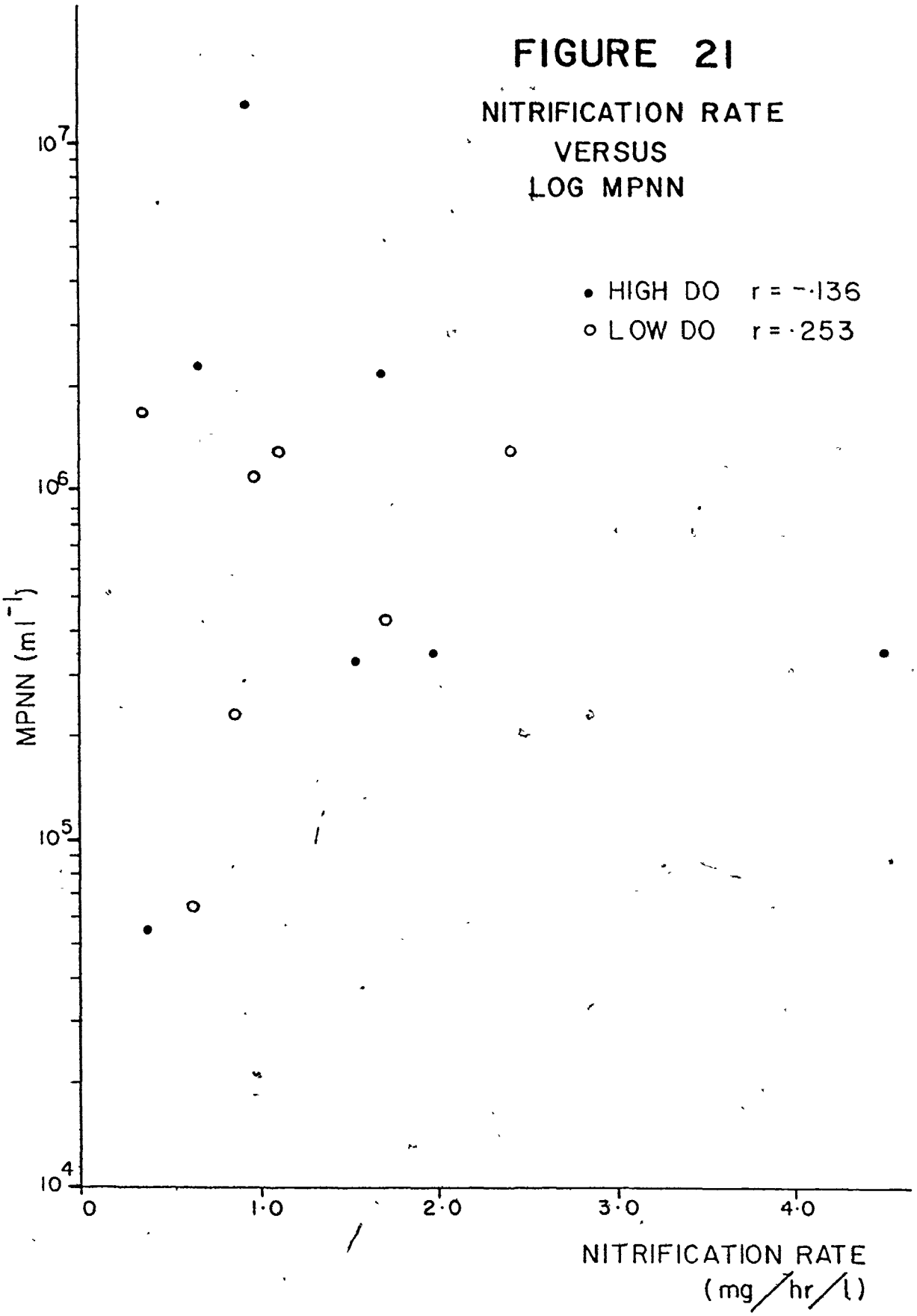


TABLE 17

SUMMARY OF HYPOTHESIS TESTS

DAILY TOC REDUCTION

Run	t	df	t_{crit} = 0.95	= 0.99
Pool	7.507	45	1.645	2.326
1	.889	6	1.943	3.143
2	.270	4	2.132	3.747
3	-.064	10	1.812	2.764
4	2.158	6	1.943	3.143
5	2.651	9	1.833	2.821
6	1.380	6	1.943	3.143
7	-2.484	4	2.132	3.747

ever, no single run exhibits significant differences at the 99% confidence level and only during Runs 4 and 5 was the TOC reduction significantly greater by the high D.O. sludge at the 95% confidence level. The data from Run 7 indicates that the low dissolved oxygen reactor reduced TOC to a significantly greater degree than the high D.O. reactor.

It was concluded that over the long term, an acttivated sludge reactor operating at a dissolved oxygen tension in the range 6 to 10 mg/l, will reduce more TOC than a reactor operating at a more normal dissolved oxygen tension of 0.5 to 2.0 mg/l.

3.8.2 Polynomial Model

A polynomial model similar to the one previously described for nitrification rate was used in evaluating the effect of dissolved oxygen, temperature and sludge age on the effluent TOC quality. See Appendix C. The model showed no lack of fit at the 95% confidence level. Table 18 lists the values of the parameters and their respective 95% confidence intervals. It indicates that the temperature, dissolved oxygen and dissolved oxygen interaction parameters do not differ significantly from zero and that sludge age is the largest factor in TOC reduction.

An "F" test on the extra residual sum of squares of the model created by dropping the dissolved oxygen

TABLE 18

DAILY TOC REDUCTION POLYNOMIAL MODEL

Parameter	Value	95% Confidence Limits Lower	Upper
$\hat{B}_o$	18.48	16.75	20.21
$\hat{B}_1$ (temp.)	1.30	-0.19	2.79
$\hat{B}_2$ (sludge age)	11.46	9.73	13.19
$\hat{B}_{11}$ (temp.)	0.84	-1.44	3.12
$\hat{B}_{22}$ (sludge age)	9.70	7.26	12.14
$\hat{B}_3$ (D.O.)	0.89	-0.05	1.83
$\hat{B}_{13}$ (temp-D.O.)	0.91	-0.53	2.35
$\hat{B}_{23}$ (sludge age-D.O.)	1.42	-0.31	3.15

terms showed no significant difference in the residuals at the 95% confidence level. There was no lack of fit of the model at the 95% confidence level without the dissolved oxygen term.

The model is in agreement with the results of the hypothesis tests. The high sludge age data (Run 5) indicates a significant difference in TOC reduction. One run at the middle sludge age and the pooled data of the two centre-point runs show significant differences while the low sludge age data shows no difference in TOC reduction performance.

3.8.3 Correlation With Process Parameters

Adenosine triphosphate (ATP) analyses and oxygen uptake rate determinations were carried out on each reactor during each run. The results of the ATP analyses were plotted against daily TOC reduction in Figure 22. The correlation coefficients between effluent TOC levels, and other process indicators, and ATP concentrations, MLVSS levels and oxygen uptake rates are listed in Table 19.

Daily TOC reduction was significantly correlated at the 99% confidence level with ATP concentration at the low dissolved oxygen level and with the pooled data. The limited range of TOC reduction evidenced by grouping of data around 20 mg/l, reduces the value of the comparison with ATP concentrations.

FIGURE 22

ATP VERSUS TOC REDUCTION

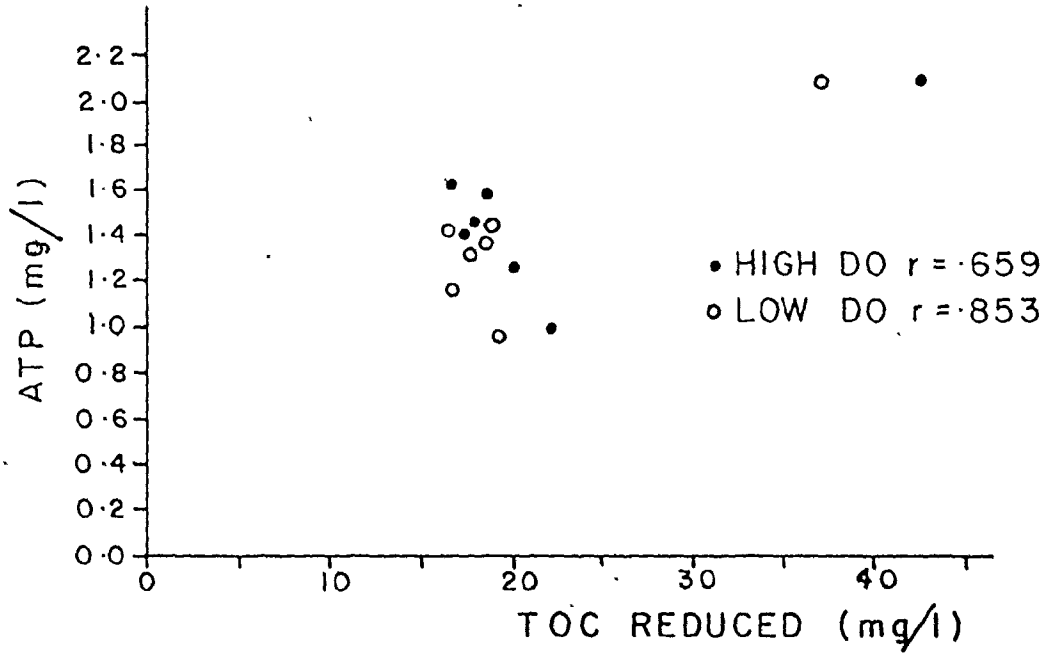


FIGURE 23

ATP VERSUS TOC REMOVAL RATE

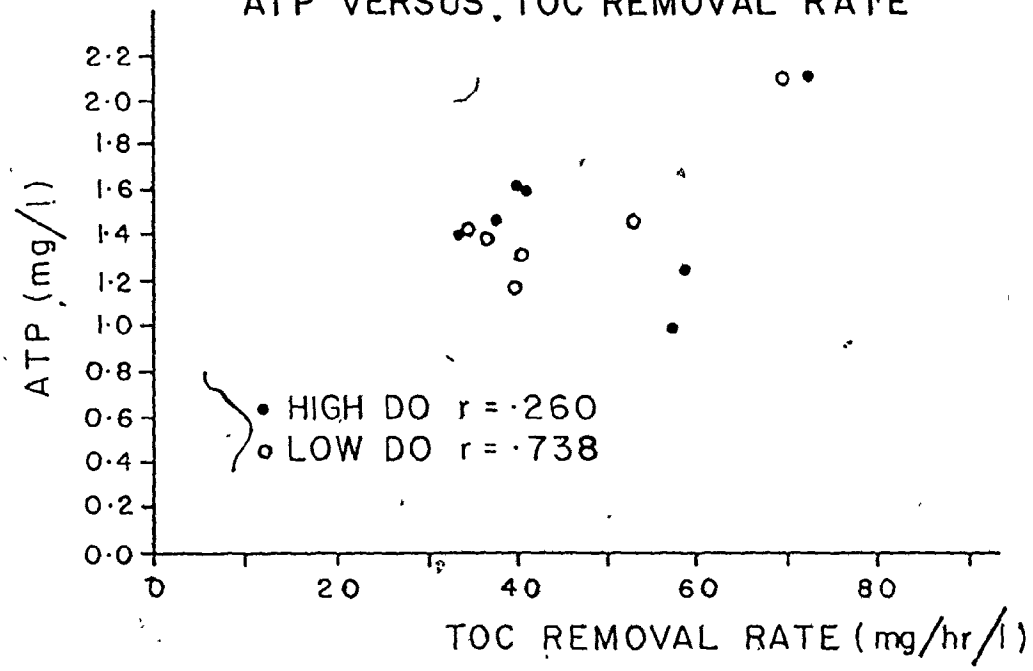


TABLE 19

ATP, MLVSS AND OXYGEN UPTAKE RATE CORRELATIONS

WITH PROCESS INDICATORS

Parameter	ATP	ATP/VSS	MLVSS	Overall D.O. Uptake	Unit D.O. Uptake
High D.O. Level					
TOC Reduction	.659	-.304	.757 (97.5%)	-.222	-.329
TOC Rate	.260	-.409	.598	.615	.468
Effluent COD	.337	.378	-.131	-.455	-.456
COD Rate	-.097	-.080	.082	.665	.037
Low D.O. Level					
TOC Reduction	.853 (99%)	-.182	.683 (95%)	-.574	-.528
TOC Rate	.738 (95%)	-.219	.661	-.042	-.281
Effluent COD	-.066	.310	-.177	-.335	-.130
COD Rate	.263	-.377	.390	-.630	-.699
Pooled					
TOC Reduction	.743 (99%)	-.236	.701 (99%)	.386	.045
TOC Rate	.487 (95%)	-.305	.607 (99%)	.489 (95%)	.147
Effluent COD	.166	.354	-.151	-.542	-.434
COD Rate	.025	-.001	.188	-.005	-.283

3.9 TOC REMOVAL RATE

3.9.1 Hypothesis Testing

TOC removal rates were calculated for each reactor from the data obtained daily on the raw influent and the composited effluent as follows:

$$\text{Removal Rate} = \frac{C_o - C}{t} \qquad (34)$$

where: C_o is the influent TOC,

C is the effluent TOC, and

t is the theoretical detention time based on 24 hour total flow.

The paired data were used in conducting hypothesis tests similar to those previously described. The results of this testing are given in Table 20.

The hypothesis test results are similar to those of the daily TOC reduction, i.e., the pooled data indicates a definite increase in TOC removal rates at higher dissolved oxygen levels but no one run shows a significant difference at the 99% confidence level. Runs 4 and 7 indicate significant differences in the removal rates at the 95% confidence level, run 4 showing increased rates at the high D.O. level and run 7 showing decreased rates at the high D.O. level. It was concluded that, over the long term, greater TOC removal rates would occur at dissolved

TABLE 20

SUMMARY OF HYPOTHESIS TESTS

TOC REMOVAL RATE

Run	t	df	t_{crit} = 0.95	t_{crit} = 0.99
Pool	4.178	40	1.645	2.326
1	0.898	6	1.943	3.143
2	0.082	4	2.132	3.747
3	0.066	9	1.833	2.821
4	2.547	3	2.353	4.541
5	0.979	8	1.860	2.896
6	1.699	6	1.943	3.143
7	-2.435	4	2.132	3.747

oxygen tensions in the range of 6 to 10 mg/l than at dissolved oxygen tensions in the range of 0.5 to 2.0 mg/l.

3.9.2 Polynomial Model

A polynomial model was constructed similar to that used to assist in the evaluation of the nitrification rate. There is a wide scatter in the data. Calculation of the confidence intervals on the individual parameters shows that none of the parameters differs significantly from zero at the 95% confidence level, indicating no correlation within the data.

3.9.3 Correlation With Process Parameters

The TOC removal rate at the low dissolved oxygen level was significantly correlated at the 95% level of significance with ATP concentration (Figure 23). The pooled TOC removal rates were significantly correlated at the 99% significance level with MLVSS concentration and at the 95% significance level with ATP concentration and overall dissolved oxygen uptake rate. In contrast Nutt (1974) found that the TOC removal rate was significantly correlated at the 99% significance level with ATP concentration, but was not significantly correlated with either MLVSS concentration or oxygen uptake rate.

3.10 COD EFFLUENT QUALITY AND REMOVAL RATE

3.10.1 Hypothesis Testing

Effluent COD was determined only during rate days and therefore only two data points per run are available. Therefore all the data were tested as one set rather than testing the data from each run separately.

The hypothesis tests indicates that there is no significant difference at the 95% level of confidence between COD effluent quality or COD removal rate at the two dissolved oxygen levels.

3.10.2 Polynomial Model

An analysis of the polynomial model for COD effluent quality indicates that there is no lack of fit in the model. However there is a large amount of scatter in the data as evidenced by a large residual mean square (89.21). Consequently none of the parameters are significantly different from zero at the 95% confidence level. This indicates temperature, sludge age, and dissolved oxygen level are not important parameters for predicting COD effluent quality.

The polynomial model for COD removal rate also exhibited no lack of fit. However because of the scatter in the data, only the $\hat{B}_o$ parameter was significantly different from zero at the 95% confidence level. This indi-

cated that an average should fit the data just as well as the polynomial. An "F" test at the 95% confidence level between the variance of the observed rates and the variance of the predicted rates showed no significant difference. Therefore it is concluded that the COD removal rate does not vary significantly within the range of conditions studied.

3.10.3 Correlation With Process Parameters

Effluent COD quality was not significantly correlated at the 95% confidence level with any of the process parameters measured, (i.e. ATP, MLVSS and oxygen uptake).

COD removal rate was not significantly correlated with any of the process parameters at the high dissolved oxygen level. At the low D.O. level the unit oxygen uptake was significantly, (albeit negatively), correlated at the 95% confidence level but not at the 97.5% confidence level.

It is concluded that none of the process parameters is a reliable indicator of the ability of a sludge to remove COD within the range of conditions studied in this work.

3.11 OXYGEN UPTAKE RATE

3.11.1 General

The oxygen uptake rate was determined from data gathered on each rate day. Linear regression was used to determine the rate as mg-D.O./l/min. These rates were also converted to mg-D.O./mg-MLVSS/day. The data is presented in Appendix D. Table 21 lists the calculated rates.

3.11.2 Hypothesis Testing

Oxygen uptake rates are often used as an indicator of process performance and it was thought that if a more active microbial mass existed at higher dissolved oxygen levels it may be reflected in higher uptake rates.

The hypothesis tests outlined previously in this work were carried out on the calculated rates shown in Table 21. The hypothesis tests showed that there was no significant difference at the 95% confidence level between the rates at the two D.O. levels for both the overall rates (mg-D.O./l/min) and the "unit" rates (mg-D.O./mg-MLVSS/day):

straight rates $t = -1.45$

unit rates $t = -1.78$

$t(95\%) = 1.78$

The negative "t" values indicate that the rates

TABLE 21

OXYGEN UPTAKE RATES

DATE	D.O. LEVEL	D.O. UPTAKE RATE	D.O. UPTAKE UNIT RATE
Feb. 18	H	.198	.263
	L	.117	.148
Feb. 20	H	.261	.284
	L	.136	.160
Mar. 1	H	.077	.121
	L	.090	.128
Mar. 3	H	.085	.140
	L	.084	.132
Mar. 16	H	.060	.169
	L	.182	.624
Mar. 18	H	.111	.291
	L	.118	.347
Mar. 25	H	-	-
	L	.334	.641
Mar. 27	H	.133	.351
	L	.377	.835
Apr. 6	H	.170	.195
	L	.258	.290
Apr. 8	H	.144	.171
	L	.346	.477
Apr. 13	H	.082	.144
	L	.062	.095
Apr. 15	H	.111	.188
	L	.112	.154

TABLE 21 cont'd....

DATE	D.O. LEVEL	D.O. UPTAKE RATE	D.O. UPTAKE UNIT RATE
Apr. 20	H	.138	.225
	L	.188	.320
Apr. 22	H	.082	.124
	L	.120	.232

at the lower D.O. level tend to be higher than those at the higher D.O. level.

3.12 ADENOSINE TRIPHOSPHATE

3.12.1 General

The results of the adenosine triphosphate (ATP) analyses are listed below in Table 22 in two forms: the first is the actual concentration of ATP measured; and the second is in the form usually found in the literature.

TABLE 22

ATP RESULTS

Run	ATP Concentration			
	mg/l		ug/(mg VSS)	
	High D.O.	Low D.O.	High D.O.	Low D.O.
1	1.25	1.45	1.30	1.27
2	1.60	1.32	1.75	1.50
3	1.62	1.17	3.60*	2.85
4	1.00	0.97	1.53	1.46
5	2.12	2.09	1.56	1.52
6	1.48	1.42	1.48	1.55
7	1.40	1.38	1.99	1.38

* Assumes MLVSS = 0.8 x MLSS

The concentrations as ug/mg VSS are in the range generally reported in the literature although most are

somewhat lower than the average of 2.0 to 2.1 ug/mg VSS reported by Patterson et al (1970).

3.12.2 Hypothesis Testing

Hypothesis tests similar to those described previously were carried out on both the ATP concentrations and the unit concentrations. The results showed that at the 95% confidence level no significant differences existed between the ATP concentrations observed at the high and the low dissolved oxygen levels.

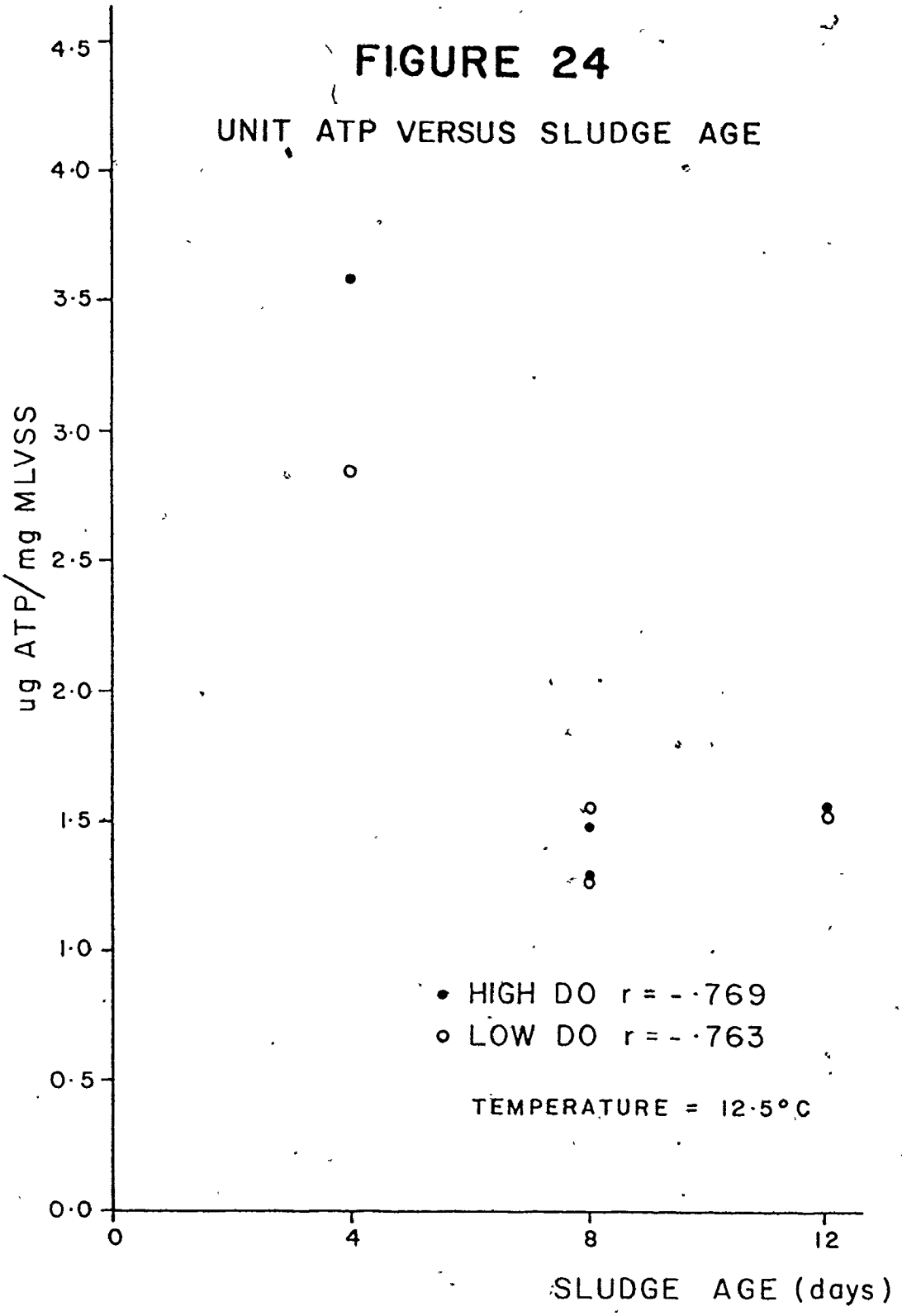
However, the unit ATP concentrations were significantly different at the 95% confidence level although they were not significantly different at the 99% confidence level. This suggests that in the high D.O. sludge, a greater percentage of the microorganisms were viable than in the low D.O. sludge. This difference in viability does not appear to be large as in a period of approximately one week for an average run, no significant difference was usually detected in the TOC effluent quality. But as shown in Table 19 this difference in viability could be significant over a long period of time.

3.12.3 Sludge Age Effects

Figure 24 illustrates the correlation between ATP unit concentration and sludge age. This correlation is negative, indicating that the viable fraction of the MLSS

FIGURE 24

UNIT ATP VERSUS SLUDGE AGE



drops with increasing sludge age. This is in keeping with conventional descriptions of the activated sludge process as the longer sludge ages represent a culture moving towards the death phase.

4.0 CONCLUSIONS

1. Over the temperature range of 5°C to 20°C the high dissolved oxygen level sludge produced significantly more nitrite plus nitrate nitrogen during the course of the experiment. An increase in sludge age in the range of 4 to 12 days also increased nitrate production.

2. Within the temperature range of 5°C to 20°C the rate of nitrification was significantly greater overall at the high dissolved oxygen level. At 5°C virtually no difference was observed but a significant difference was apparent at 20°C.

3. The majority of the nitrogen mass balances were within 10% which is within the range of experimental error indicating no loss of nitrogen; there was no significant difference in the balances between the two dissolved oxygen levels.

4. No significant difference in effluent COD quality or COD removal rate was observed between low and high dissolved oxygen over the range of sludge age and temperature investigated. No process parameter measured was a reliable indicator of COD effluent quality or removal rate.

5. Significantly greater overall TOC reduction and TOC removal rates were obtained at the high dissolved oxygen level although no one set of conditions showed a significant difference at the 99% confidence level.

6. Overall ATP concentration was significantly different at the 95% confidence level indicating that the high dissolved oxygen sludge may have been more viable.

7. The most probable number of nitrifiers was not significantly correlated with either the nitrification rate or total nitrate converted.

8. No significant difference in overall or unit oxygen uptake rates was observed between the two dissolved oxygen levels over the range of sludge ages and temperatures investigated.

5.0 RECOMMENDATIONS

The following is recommended for further study:

1. A more extensive comparison of the relationship between nitrification rate and dissolved oxygen level, with the object of substantiating that oxygen tension affects nitrification rates and determining the mechanism for this interaction. Work should be concentrated at temperatures above 10°C and at sludge ages in excess of 8 days.

ABBREVIATIONS AND SYMBOLS

ATP	:	adenosinetriphosphate
BOD_5	:	5 day biochemical oxygen demand
°C	:	degrees celcius
COD	:	chemical oxygen demand
D.O.	:	dissolved oxygen
hr	:	hour
l	:	litre
mg/l	:	milligrams per litre
MLSS	:	mixed liquor suspended solids
MLVSS	:	mixed liquor volatile suspended solids
S.A.	:	sludge age
TKN	:	total Kjeldahl nitrogen
TKN-F	:	filtered (soluble) total Kjeldahl nitrogen
TKN-UF	:	unfiltered total Kjeldahl hitrogen
TOC	:	total organic carbon
ug/mg	:	microgram per milligram

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APPENDIX A

REACTOR MIXING, WASTEWATER CHARACTERIZATION AND EXPERIMENTAL DESIGN

REACTOR MIXING CHARACTERIZATION

The reactor configuration is shown in Figure 25.

Tracer studies were carried out on each reactor to ascertain whether in fact they were completely mixed. Dilute-out curves were determined for the minimum and the maximum flow rates measured during the study.

Each reactor was filled with tap water containing glutamic acid at approximately 1,000 mg/l TOC. Tap water was pumped into the reactors and samples of the effluent taken at various time intervals.

A mass balance on the reactor yields the following relationship between concentration and detention time:

$$\text{Change} = \text{In-Out} \pm \text{Reaction} - \text{Sinks} + \text{Sources} \quad (40)$$

where: In, Reaction, Sinks and Sources are zero

$$\text{then } \frac{dm}{dt} = \text{out} = \frac{-d}{dt}(VC) = QC \quad (41)$$

$$-V\frac{dC}{dt} = QC \quad (42)$$

$$\text{or } \ln(C/C_o) = \frac{Q}{V}t = Dt \quad (43)$$

where: D is the dilute-out rate (hours^{-1}),

C_o is the initial TOC (mg/l),

C is the TOC at any time (mg/l),

Q is the flow rate in influent (litres/hour)

and,

V is the reactor volume (litres).

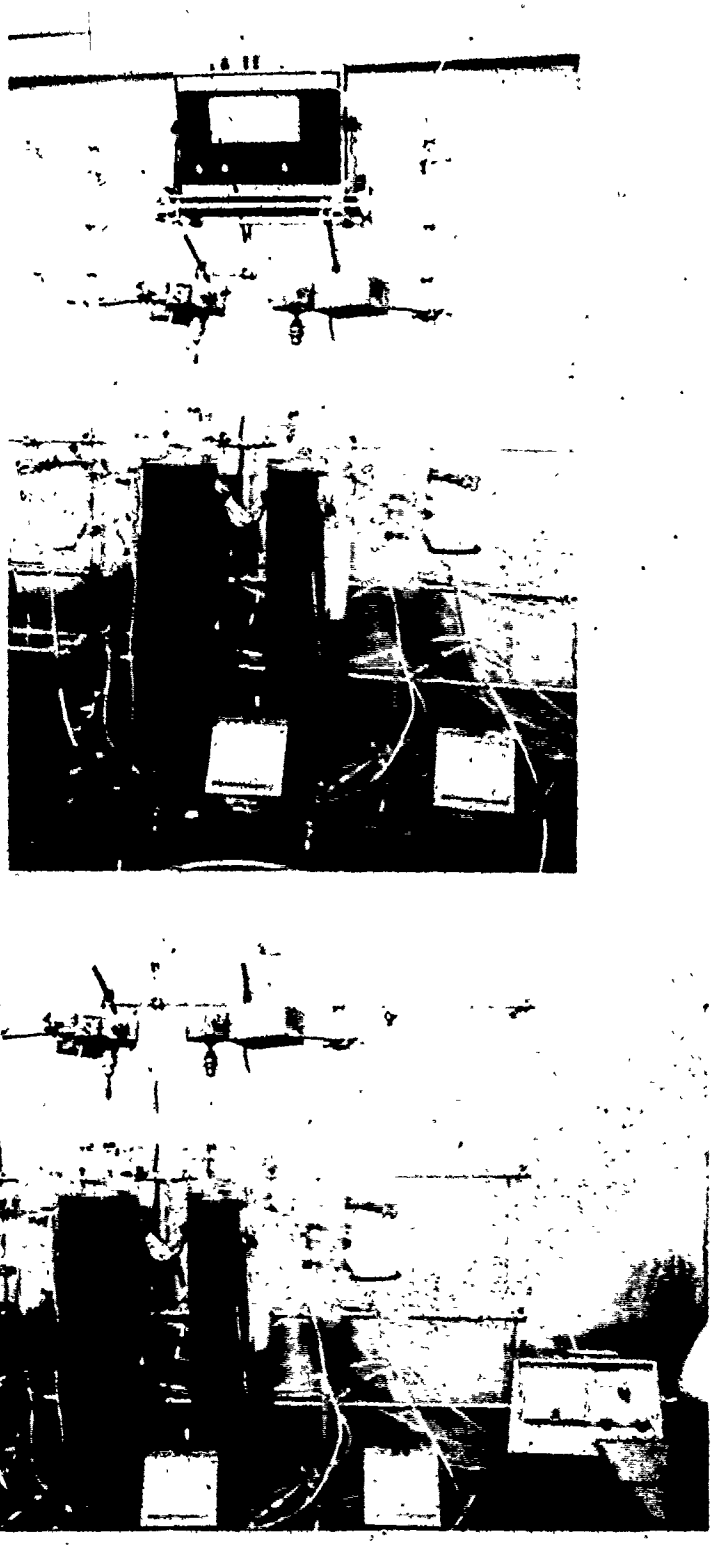


FIGURE 25

EXPERIMENTAL APPARATUS

The results, shown on Figures 26 and 27, indicate that the detention times calculated from the dilute-out curves are within about 3% of the theoretical space time at the lowest flow rates and within approximately 7.5% at the highest flow rates. It was concluded that the reactors could be considered completely mixed for all flow rates used in this work.

The pumping rate increased approximately 3 ml/minute during each tracer study. The average of the initial and final pumping rates was used in calculating the theoretical space time, i.e., it was assumed that the rate of increase in the pumping rate was linear. This is a source of experimental error in addition to sampling error and analytical error.

WASTEWATER CHARACTERISTICS

The raw sewage feed was analyzed daily for Chemical Oxygen Demand, Total Organic Carbon, Total Kjeldahl Nitrogen (filtered and unfiltered samples) and nitrate plus nitrite. The results of these analyses are shown in the form of probability plots in Figures 28, 29, 30, and 31.

The waste contained Kjeldahl nitrogen and nitrate plus nitrite nitrogen in quantities considered normal for domestic sewage by most authors. However, it contained much less organic carbon and oxygen demanding substances than are usually reported for domestic sewage.

FIGURE 26

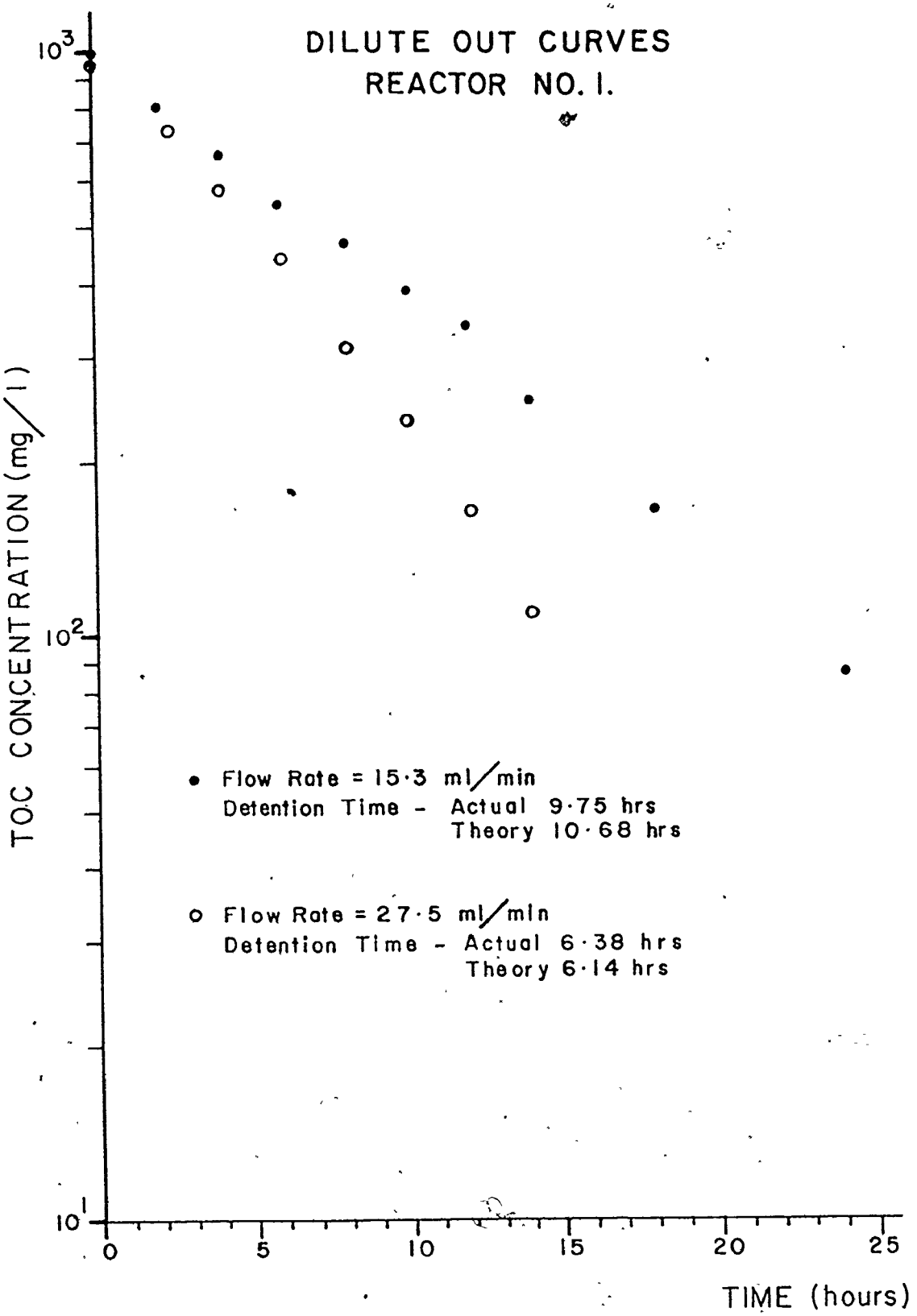

DILUTE OUT CURVES
REACTOR NO. 1.
TOC CONCENTRATION (mg/l)
10^3
10^2
10^1
0
5
10
15
20
25
TIME (hours)
• Flow Rate = 15·3 ml/min
Detention Time - Actual 9·75 hrs
Theory 10·68 hrs
o Flow Rate = 27·5 ml/min
Detention Time - Actual 6·38 hrs
Theory 6·14 hrs

FIGURE 27

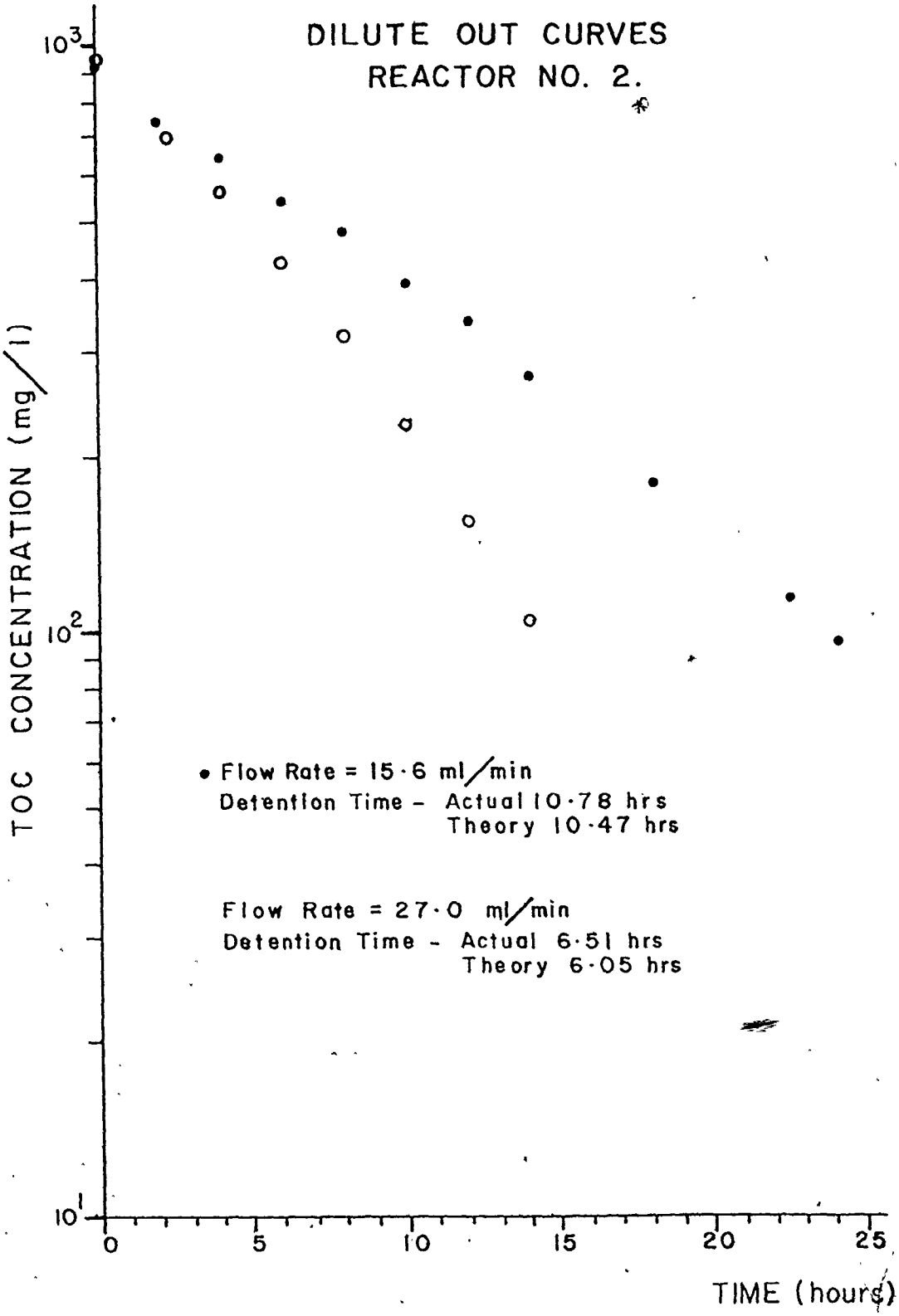

DILUTE OUT CURVES
REACTOR NO. 2.
TOC CONCENTRATION (mg/l)
10^3
10^2
10^1
0
5
10
15
20
25
TIME (hours)
• Flow Rate = 15·6 ml/min
Detention Time - Actual 10·78 hrs
Theory 10·47 hrs
Flow Rate = 27·0 ml/min
Detention Time - Actual 6·51 hrs
Theory 6·05 hrs

FIGURE 28

PROBABILITY DISTRIBUTION OF
INFLUENT NITRITE PLUS NITRATE

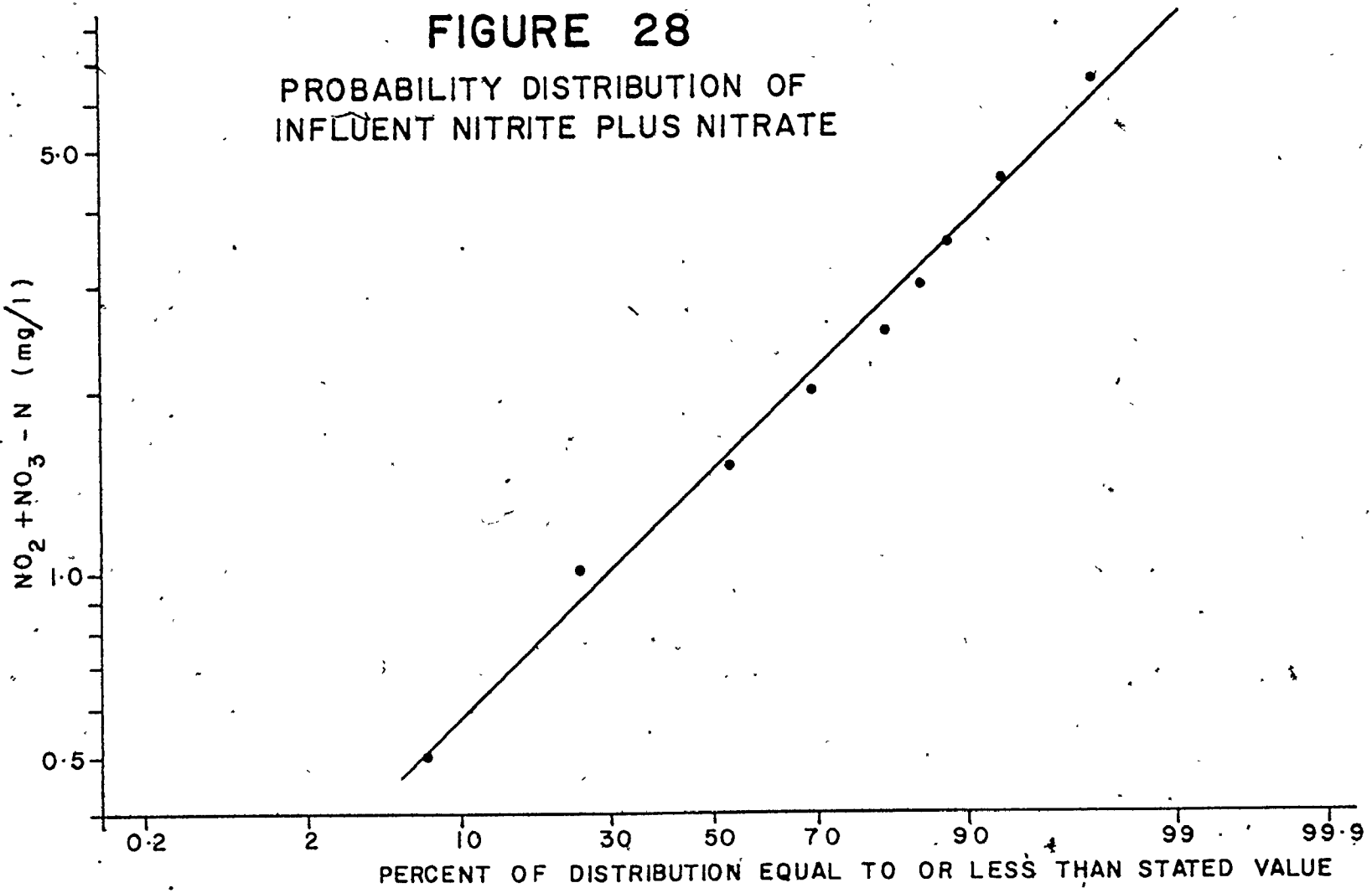


FIGURE 29

PROBABILITY DISTRIBUTION OF INFLUENT COD

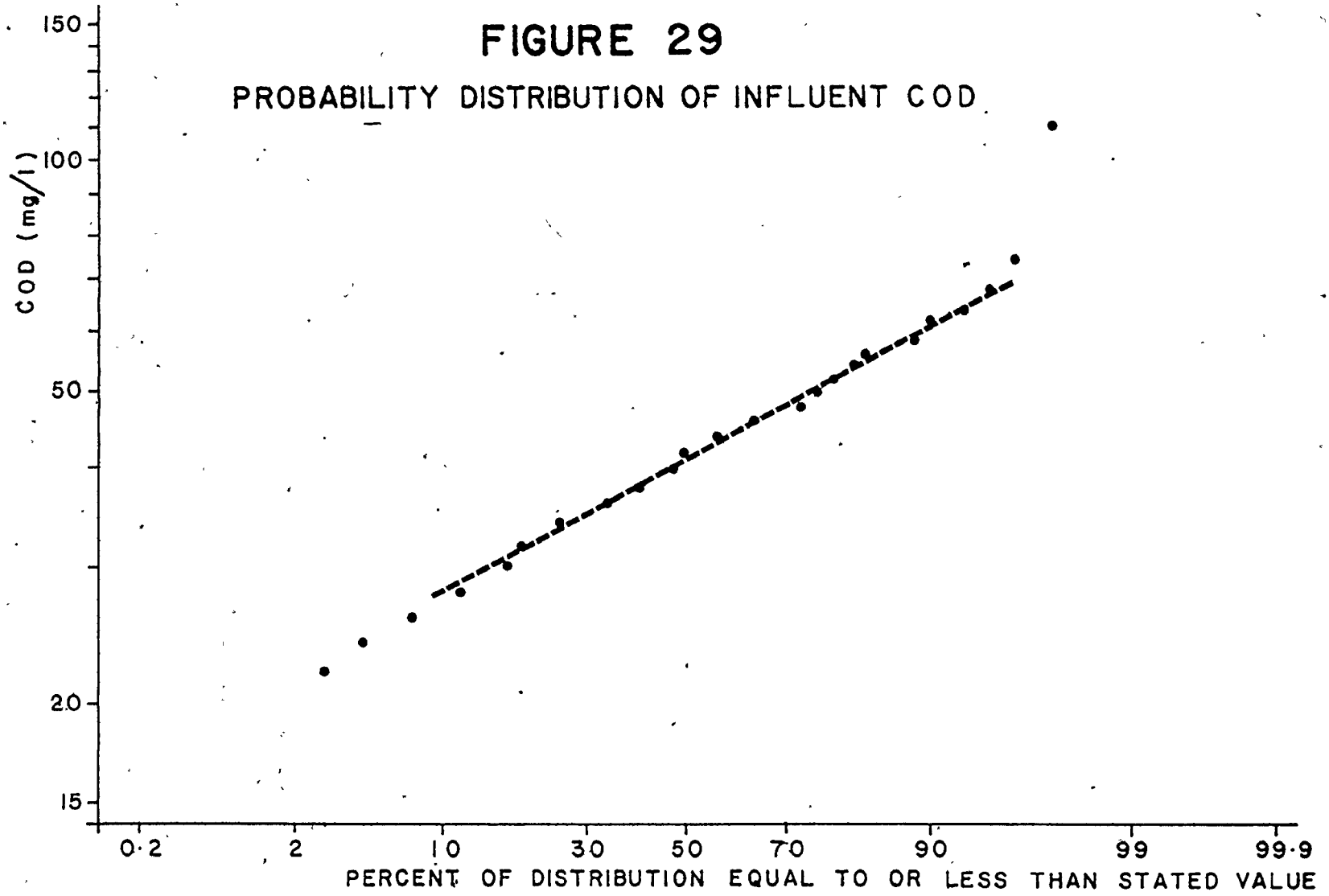


FIGURE 30

PROBABILITY DISTRIBUTION OF INFLUENT TOC

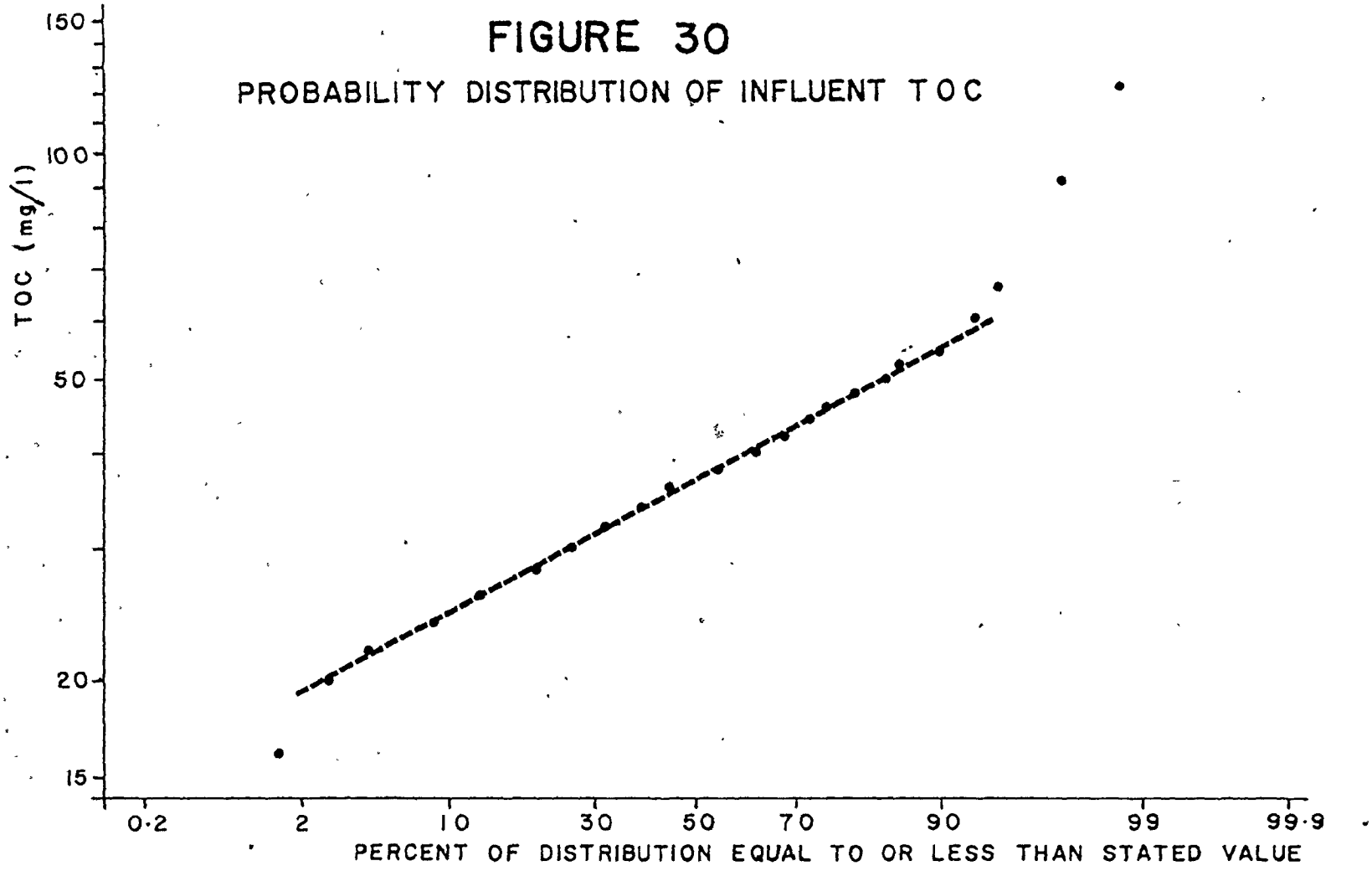
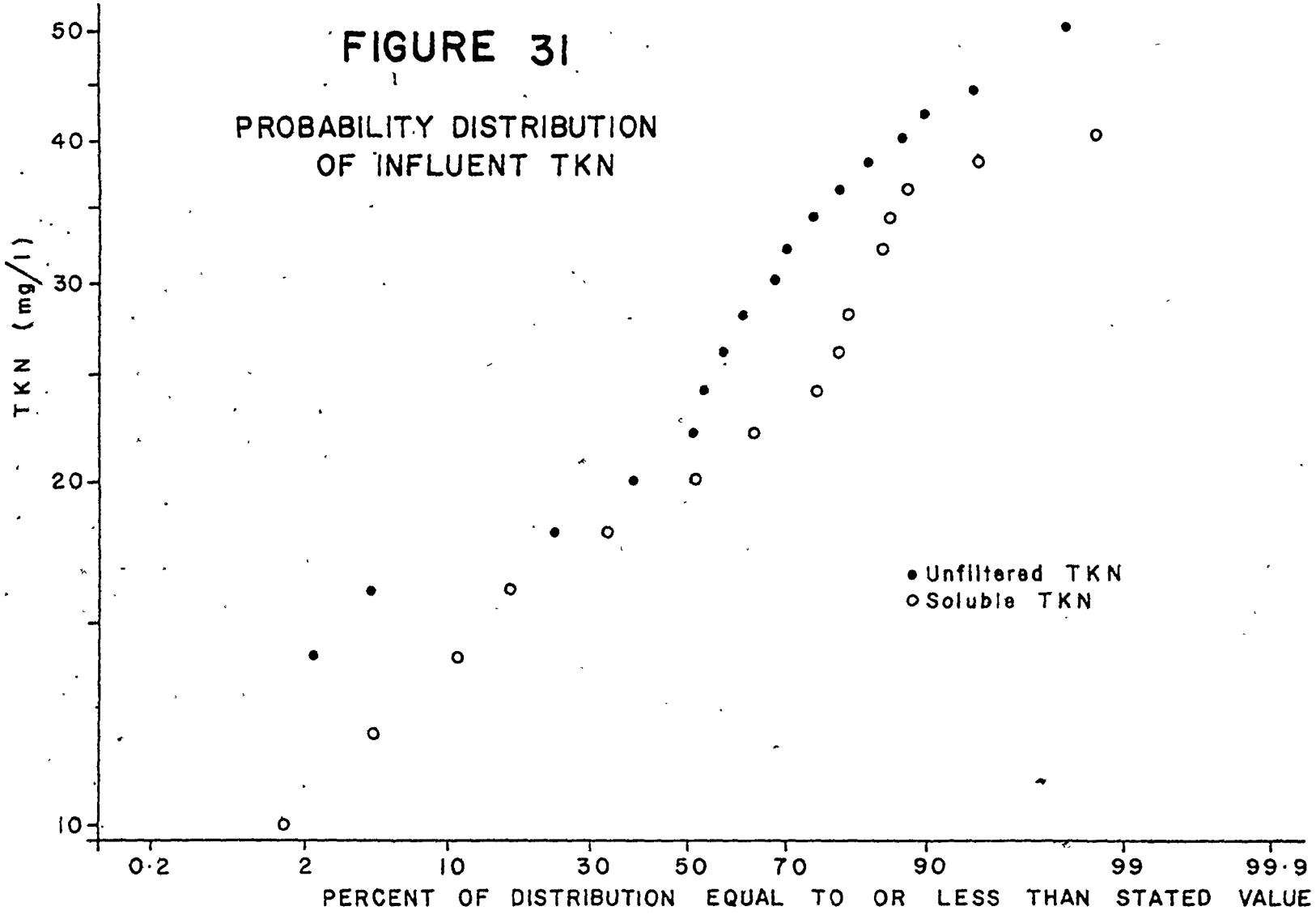


FIGURE 31

PROBABILITY DISTRIBUTION OF INFLUENT TKN



EXPERIMENTAL DESIGN

The experimental design was originally conceived as a full 3^2 factorial design. However external constraints dictated that the number of runs be reduced and a subset of the original factorial design was carried out. Replicate runs were done at the center point and the low temperature point to provide an estimate of pure error.

Three temperatures, 5°C, 12.5°C, and 20°C, and three sludge ages, 4 days, 8 days, and 12 days, were selected to provide a symmetrical design and because they represented values which might be encountered under Canadian climatic conditions. Identical sludge ages and temperatures were maintained in each reactor for each experimental run, the only difference being the dissolved oxygen level.

The effect of differences between the reactor systems was eliminated by randomly selecting which reactor would have the high dissolved oxygen level. Because of the biological nature of the system it was not possible to fully randomize the selection of either temperature or sludge age. However, parameter selection was random as possible.

Table A-1 summarizes the conditions of each run. Figure 32 illustrates graphically the experimental design.

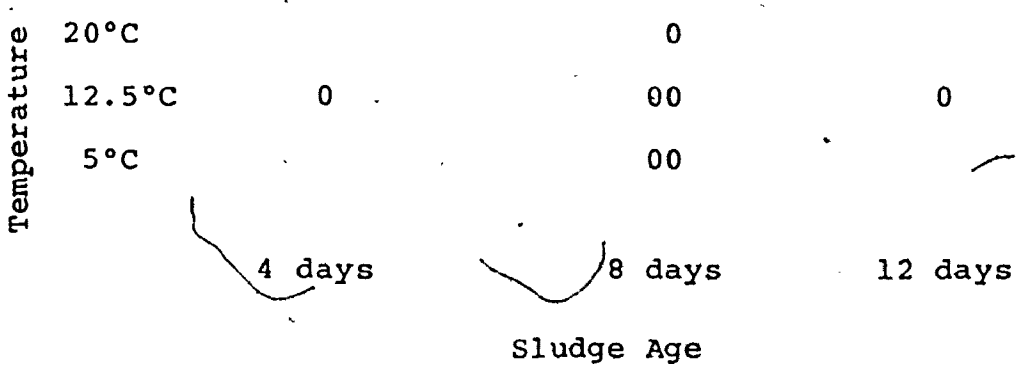
TABLE A-1

EXPERIMENTAL DESIGN

Run No.	Sludge Age (days)	Temperature (°C)	High D.O. Reactor
1	8	12.5	1
2	8	5.0	1
3	4	12.5	2
4	8	20.0	2
5	12	12.5	1
6	8	12.5	2
7	8	5.0	2

FIGURE 32

EXPERIMENTAL DESIGN



APPENDIX B

ANALYTICAL PROCEDURES

TOTAL KJELDAHL NITROGEN (TKN), (Ferrari, 1960)

The analyses for both filtered, (0.45u membrane filter), and unfiltered Total Kjeldahl Nitrogen were carried out on a Technicon Auto Analyzer utilizing Industrial Method 30-69A. Analyses were carried out in the range 0.1 mg/l to 40 mg/l. Samples with concentrations higher than 40 mg/l were diluted with ammonia-free distilled water until the concentration fell within the analytical range.

Standards were made from a stock solution of ammonium chloride, 100 mg/l as N (382 mg/l NH_4Cl).

To help ensure representative samples, air was pumped into the sample cup at the same time as the sample was aspirated. This provided enough agitation to completely mix the contents of the sample cup.

Samples were preserved by the addition of 1 ml of concentrated sulphuric acid per 300 ml of sample followed by storage at 5°C.

AMMONIA NITROGEN (NH_3-N)

Soluble (passing a 0.45u membrane filter) ammonia nitrogen was analyzed by bypassing the digestion process of the Auto Analyzer Industrial Method 30-69A. Samples were filtered immediately after they were obtained. Analyses were carried out in the range 0.1

mg/l to 40 mg/l. Samples containing more than 40 mg/l ammonia nitrogen were diluted with ammonia-free distilled water until the concentration fell within the analytical range.

Standards were prepared from the same stock as the Total Kjeldahl Nitrogen standards.

Samples were preserved by the addition of 1.0 ml of chloroform per 300 ml of sample. Samples were vigorously shaken and then stored at 5°C until they were analyzed.

NITRATE AND NITRITE NITROGEN (NO_3-N and NO_2-N)

All nitrate and nitrite analyses presented were analyzed by a Technicon Auto Analyzer utilizing Industrial Method 33-69W. Using this methodology, nitrate plus nitrite concentrations were first determined. The nitrite concentration of the sample was then ascertained and the nitrate concentration obtained by subtraction of the two results. Samples were analyzed in the range 0 to 20 mg/l as nitrogen. Both nitrate and nitrite standards were prepared in accordance with the Technicon methodology. Samples were preserved by addition of 1 ml of chloroform per litre of sample and storage at 5°C.

DISSOLVED NITROGEN GAS

A Fisher Scientific Clinical Gas Chromatograph,

Model 99, was used to measure the concentration of dissolved nitrogen gas.

Sample aliquots of 0.5 ml were obtained from inside the stem of the funnel in the saturator and from below the water surface of the outlet tube. A sample consisted of at least 3 aliquots. Sample size repeatability was maintained through the use of a Chaney adaptor on a Hamilton gas-tight syringe. No more than five seconds elapsed between taking the sample and injection into the apparatus.

The aliquots were injected into a glass chamber with a fritted glass disk. Helium carrier gas stripped all dissolved gases from the liquid. Water vapor was removed by passing the stream through one foot of ¼ inch I.D. Tygon tubing packed with 14 to 25 mesh $CaSO_4$ (Drierite). This drying column was replaced after six injections.

Oxygen and nitrogen gases were separated in the chromatograph's two columns, their quantities being detected by a pair of matched thermistors (Keulemans et al, 1957).

Samples of air-saturated water at different constant temperatures were used as standards.

The output from the chromatograph was recorded on a Phillips PM 8100 flat bed recorder receiving power through a Sola 120 VA constant voltage transformer. Re-

corder speed was 25 mm/min and sensitivity was 1 mV.

For a more detailed description of the analytical equipment and procedure see Swinnerton et al (1962), and Dawson (1971).

NUMBER OF NITRIFIERS

The number of nitrifiers was determined by a 5 tube most probable number technique.

Media Preparation

Winogradski's media (Selibera, 1962) was used to grow the autotrophic Nitrosomonas and Nitrobacter. The ingredients are listed below:

$(NH_4)_2SO_4$	2.0 g/l
K_2HPO_4	1.0 g/l
$MgSO_4 \cdot 7\ H_2O$	0.5 g/l
NaCl	2.0 g/l
$FeSO_4 \cdot 7\ H_2O$	0.4 g/l
$CaCO_3$	2.0 g/l
$MnCl_2 \cdot 4\ H_2O$	trace
distilled water	to make up volume
pH before autoclaving	7.2
after autoclaving	7.3

Five ml of Winogradski's media was pipetted into each of a large number of 50 ml test tubes. The tubes

were stoppered with foam rubber plugs and the tubes were autoclaved for 15 minutes at 120°C (248°F).

Buffer Preparation

The standard microbiological buffer solution as described in Standard Methods, page 650, was used to prepare the necessary dilutions. Ninety ml of dilute buffer (1.25 ml/l) was poured into a number of 180 ml capacity bottles and 250 ml of dilute buffer was poured into a number of 500 ml capacity erlynmeyer flasks. The bottles had plastic screw-type caps with rubber seals. These were put on loosely. The flasks were stoppered with nonabsorbent cotton and the mouth covered with tin foil. Both bottles and flasks were autoclaved for 15 minutes at 120°C (248°F).

Inoculation Procedure

Using a sterilized wide mouth 5 ml pipet, 2.5 ml of mixed liquor was pipetted directly into 250 ml of dilute buffer. (The pipets were sterilized at 170°C, (338°F), for 2 hours.) The mixture was blended for 15 minutes at the highest speed of a Waring "Futura 750" blender to break down the activated sludge flocs and prevent their reforming. This was also used to ensure a uniform concentration of microorganisms in the mixture.

Serial dilutions of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}

ml/ml activated sludge were prepared by pipetting 10 ml of the lower dilution into 90 ml of sterilized buffer. Sterilized 10 ml disposable plastic pipettes were used for this operation. The bottles were shaken vigorously for 2 to 3 minutes prior to making the next dilution.

Sterilized 1 ml disposable plastic pipettes were then used to transfer 1 ml of each dilution to each of 5 test tubes containing Winogradski's media. The bottles and flasks were vigorously shaken frequently during this operation to maintain as consistent a microorganism concentration as possible. This transfer to the test tubes was done in order from the highest dilution to the lowest dilution to avoid gross contamination of the inoculated media. The foam caps were replaced and each set of 30 test tubes, plus two uninocculated blanks, was incubated at 16°C for 30 days.

Nitrifer Determination Procedure

Three separate spot tests were used to detect the presence of the following:

1. ammonia nitrogen,
2. nitrite nitrogen, and
3. nitrite plus nitrate nitrogen.

Following the incubation period, a few drops from each test tube were placed in 3 depressions on a white glazed ceramic spot plate. The presence or absence of

each chemical species was determined by observing the color change (or lack of color change) which occured in each sample upon the addition of a small amount of the chemical(s) involved.

The table below outlines the chemicals used in each determination and the color change involved. In all cases, no color development indicated a lack of that particular form of nitrogen.

Form of Nitrogen	Chemicals Used	Color
NH_3	Nesslers Reagent (Koch and McMeekin) Fisher Chemical No. S_0-N-24	yellow to dark reddish brown
NO_2	Sulfanilic Acid Test Solution (Selibera, 1962) (see below) plus Alpha-Napthylamine Test Solution (Selibera, 1962) (see below)	pink to dark red
$NO_2 + NO_3$	Brucine Sulphate plus Concentrated Sulphuric Acid	yellow

Sulfanilic Acid Test Solution

Put 14.7 gm of glacial acetic acid in 15 ml of dis-

tilled water. Add 1.0 gm of sulfanilic acid. Heat to dissolve. Slowly add 270 ml of distilled water.

Alpha-Napthylamine Test Solution

Put 14.7 gm of glacial acetic acid in 25 ml of distilled water. Add 0.2 gm Alpha-Napthylamine. Heat to dissolve. Add 300 ml of distilled water.

The reactions involved in the formation of the colored reaction products are presented by Feigl and Anger, (1972).

ADENOSINE TRIPHOSPHATE (A.T.P.)

Firefly Extract Preparation

Firefly extract (Worthington Biochemical) is supplied in dessicated form in vials which, when reconstituted with 5 ml of distilled water, produces a suspension of pH 7.4 containing 0.05M potassium arsenate buffer and 0.02M magnesium sulfate. Before use, the reconstituted extract was diluted to 25:0 ml with a diluent made up of equal volumes of 0.1M sodium arsenate, (3.12 gm of $Na_2HASO_4 \cdot 7H_2O$ per 100 ml, pH 7.4), and 0.04M magnesium chloride, (0.81 gm of $MgCl_2 \cdot 6H_2O$ per 100 ml, pH 7.4). The extract was made on the day of the procedure. The diluted extract was kept in an ice bath during use.

A.T.P. Standards

100.0 mg of adenosine triphosphate disodium salt, (K and K Laboratories), was dissolved in 1.0 litres of 0.025M tris buffer (3.062 gm tris (hydroxymethyl) aminomethane per litre adjusted to pH 7.75 with concentrated HCl). This solution was pipetted in 25 ml aliquots into test tubes, parafilmed and frozen at -20°C until needed.

At the time of the A.T.P. assay, a test tube was thawed and the contents diluted with 0.025M tris buffer producing a stock of 10.00 mg/l. Standards were made by diluting this stock with 0.025M tris buffer.

Extraction Procedure

A.T.P. was extracted from the activated sludge by pipetting 5 ml of mixed liquor into centrifuge tubes containing 35 ml of 0.025M tris buffer, which was at a temperature of at least 95°C (Patterson et al, 1970). The centrifuge tubes were then held in a boiling water bath for 10 minutes with occasional shaking.

The centrifuge tubes were rapidly cooled by placing them in an ice bath and centrifuged, (Lourdes Instrument Company), for 15 minutes at 6000 rpm to bring down cell debris. The supernatent was then made up to 50 ml with 0.025M tris buffer.

A.T.P. was extracted from the standard solutions by pipetting 0.25 ml of standard solution into test tubes

containing 2.25 ml of 0.025M tris buffer at a temperature of at least 95°C. The test tubes were held in a boiling water bath for 10 minutes with occasional shaking and then allowed to cool to room temperature.

A.T.P. Assay Procedure

An American Instrument Company "Chem Glow" photometer (Cat. NO. 4-7441) was used to measure light emission. A Phillips PM 8100 flat bed recorder connected through a Sola 120 VA constant voltage transformer was used to measure the light intensity 30 seconds after initiation of the bioluminesence reaction. The recorder sensitivity was 50 mV and the chart speed was 10 mm per minute.

The analytical procedure used was the following:

1. pipette 0.2 ml of diluted firefly extract into a 6 mm x 50 mm culture tube,
2. start a stop watch,
3. pipette 0.4 ml of the solution being assayed into the culture tube after 5 seconds have elapsed,
4. place a piece of parafilm over the culture tube and mix the contents by inverting twice while shaking vigorously,
5. remove parafilm and place culture tube in photometer sample holder,
6. rotate the culture tube into the reaction chamber after total elapsed time of 35 sec-

onds (i.e., 30 seconds after initiation of the reaction), and record the light intensity.

The A.T.P. content of the activated sludge was determined from a calibration chart derived from A.T.P. standards analyzed the same day.

At least 3 aliquots of each standard and each sample were analyzed. Three separate samples were taken from each reactor.

Total Organic Carbon (T.O.C.)

Total Organic Carbon was calculated by subtraction of Total Inorganic Carbon from Total Carbon as determined by a Beckman Model 915 Total Organic Carbon Analyzer.

Samples were filtered through a 0.45u membrane filter, (Sartorius Cat. No. 11306), prior to injection. Twenty microliter sample aliquots were used.

Stock solutions for both Total Inorganic Carbon and Total Carbon were prepared according to Beckman instructions. Standards were prepared at least every 3 days.

Chemical Oxygen Demand (C.O.D.)

Chemical Oxygen Demand was determined by using a Technicon Auto-Analyzer, Industrial Method 27-69W, modified as outlined below.

A working digestion solution was prepared by diluting 30 ml of stock digestion mixture to one liter with

concentrated sulphuric acid. This allowed analysis of samples containing C.O.D. concentrations up to 50 mg/l. Samples containing higher C.O.D. concentrations were diluted with double distilled water until they fell within this range.

Dissolved Oxygen (D.O.)

Dissolved oxygen levels in the reactors were determined by using a Precision Scientific galvanic cell dissolved oxygen probe (Cat. No. 68850) attached to a microammeter (Armaco UM-3, Class 2.5).

The D.O. probes were calibrated by placing the probe in air-saturated distilled water and reading the current produced after approximately 5 minutes. This reading was plotted against the theoretical dissolved oxygen value obtained from Standard Methods (1971), corrected for atmospheric pressure. The resultant one point calibration curve was used to determine the dissolved oxygen concentration. The probes were replaced at least once a week.

Dissolved Oxygen Uptake Rates

The Precision Scientific galvanic cell dissolved oxygen probes were used to determine dissolved oxygen uptake rates. All oxygen uptake rates were carried out in-situ.

Immediately prior to the commencement of the test,

the feed flow to the reactors was cut off. In the low D.O. reactor, the dissolved oxygen concentration was raised to the level in the high D.O. reactor before the gas flow to the reactors was shut off. The falling D.O. level of each reactor was then monitored until a D.O. concentration of 1 to 2 mg/l was reached, at which time the oxygen supply and the feed flow to the reactors were again turned on.

Solids Determination

Suspended solids levels were determined by the method outlined in Standard Methods (1971). The filters and solids were dried for one hour at 103°C and weighed on an analytical balance.

Volatile suspended solids were determined by ignition in a muffle furnace at 550°C for 15 minutes.

Sartorius glassfibre filters (Cat. No. 13400) were used. All filters were dried at 103°C for one hour prior to use and stored in a dessicator until needed.

The glassfibre filters lost weight during ignition in the muffle furnace. Ten filters which had been previously dried at 103°C for one hour were placed in the muffle furnace for 15 minutes to determine the weight loss. Both the median and the average weight loss was 0.0040 gm. The range was 0.0036 gm to 0.0042 gm. This amount (0.0040 gm) was subtracted from the apparent filter and

solids weight loss when determining volatile suspended solids levels.

Hydrogen Ion Concentration (pH)

The pH in the reactors was monitored by a Fisher Scientific Accumet 230 pH/ion meter and a Fisher Scientific Standard Combination electrode (Cat. No. 13-639-90). The meter was calibrated at pH 7.0 and checked at pH 9.0 prior to use.

APPENDIX C

STATISTICAL PROCEDURES

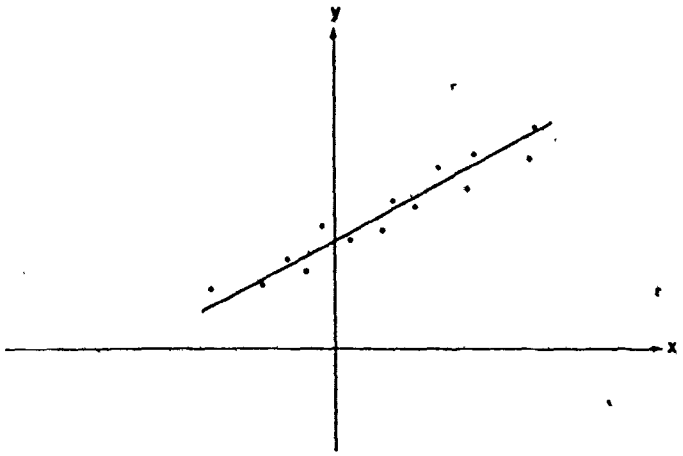
STATISTICAL PROCEDURES

General

A Hewlett-Packard HP-25 was used to carry out the statistical procedures for this work. The programs are outlined on the following eight pages.

CHAPTER 6 STATISTICS

CURVE FITTING–LINEAR REGRESSION



When investigating the relationship between two variables in the real world, it is a reasonable first step to make experimental observations of the system to gather paired values of the variables, (x, y). The investigator might then ask the question: What mathematical formula best describes the relationship between the variables x and y? His first guess will often be that the relationship is linear, i e , that the form of the equation is $y = a_1 x + a_0$, where a_1 and a_0 are constants The purpose of this program is to find the constants a_1 and a_0, which give the closest agreement between the experimental data and the equation $y = a_1 x + a_0$ The technique used is linear regression by the method of least squares.

The user must input the paired values of data he has gathered, (x_i, y_i), $i = 1, \ldots, n$ When all data pairs have been input, the regression constants a_1 and a_0 may be calculated A third value may also be found, the coefficient of determination, r^2 The value of r^2 will lie between 0 and 1 and will indicate how closely the equation fits the experimental data the closer r^2 is to 1, the better the fit.

Equations:

$$y = a_1 x + a_0$$

All summations below are performed for $i = 1, \ldots, n$.

Regression constants:

$$a_1 = \frac{\Sigma xy - \frac{\Sigma x \, \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

$$a_0 = \bar{y} - a_1 \bar{x}$$

where $\bar{y} = \frac{\Sigma y}{n}$

$$\bar{x} = \frac{\Sigma x}{n}$$

Coefficient of determination:

$$r^2 = \frac{\left[\Sigma xy - \frac{\Sigma x \Sigma y}{n}\right]^2}{\left[\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right]\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right]}$$

Note:

The values for a_0 and a_1 are stored in R_0 and R_1, respectively. After the calculation of a_0, a_1, and r^2, the estimated y value, $\hat{y}$, corresponding to any x-value may be calculated by $y = a_1 x + a_0$.

Programming Remarks:

The intermediate value $C = \Sigma xy - (\Sigma x \Sigma y/n)$ is first calculated at line 14 but is also needed near the end of the program to find r^2. Since all registers R_0 through R_7 are in use, the only place to save this value is in the stack. Hence C is preserved in one or more of the stack registers from lines 14 through 36, when it is used. It is due to the presence of C in the stack that users are warned not to disturb the contents of the stack after calculation of a_0 and a_1 (see step 4 of User Instructions).

DISPLAY LINE	DISPLAY CODE	KEY ENTRY	X	Y	Z	T	COMMENTS
00			y	x			Steps 1-7 for summation
01	31	1	y	y	x		
02	16 02	$g\,x^2$	y^2	y	x		
03	23 51 02	STO + 2	y^2	y	x		Σy^2
04	22	RI	y	x		y^2	
05	21	x⇄y	x	y		y^2	
06	26	Σ+	n	y		y^2	n, Σy, Σxy, Σx^2, Σx
07	13 00	GTO 00	n	y		y^2	
08	24 06	RCL 6	Σxy				
09	24 07	RCL 7	Σx	Σxy			
10	24 04	RCL 4	Σy	Σx	Σxy		
11	61	x	$\Sigma x\,\Sigma y$	Σxy			
12	24 03	RCL 3	n	$\Sigma x\,\Sigma y$	Σxy		
13	71	+	$\Sigma x\,\Sigma y/n$	Σxy			
14	41	-	C				$C = \Sigma xy - (\Sigma x\,\Sigma y/n)$
15	24 06	RCL 6	Σx^2	C			
16	24 07	RCL 7	Σx	Σx^2	C		
17	16 02	$g\,x^2$	$(\Sigma x)^2$	Σx^2	C		
18	24 03	RCL 3	n	$(\Sigma x)^2$	Σx^2	C	
19	71	+	$(\Sigma x)^2/n$	Σx^2	C	C	
20	41	-	D	C	C	C	$D = \Sigma x^2 - [(\Sigma x)^2/n]$
21	71	+	a_1	C	C	C	$a_1 = C/D$
22	23 01	STO 1	a_1	C	C	C	
23	24 07	RCL 7	Σx	a_1	C	C	
24	61	x	$a_1\,\Sigma x$	C	C	C	
25	32	CHS	$-a_1\,\Sigma x$	C	C	C	
26	24 04	RCL 4	Σy	$-a_1\,\Sigma x$	C	C	
27	61	+	$\Sigma y - a_1\,\Sigma x$	C	C	C	
28	24 03	RCL 3	n	$\Sigma y - a_1\,\Sigma x$	C	C	
29	71	+	a_0	C	C	C	$a_0 = \bar{y} - a_1\,\bar{x}$
30	23 00	STO 0	a_0	C	C	C	
31	74	R/S	a_0	C	C	C	Halt to display a_0
32	24 01	RCL 1	a_1	a_0	C	C	
33	74	R/S	a_1	a_0	C	C	Halt to display a_1
34	21	x⇄y	a_0	a_1	C	C	
35	22	RI	a_1	C	C	a_0	
36	61	x	$a_1\,C$	C	a_0	a_0	
37	24 02	RCL 2	Σy^2	$a_1\,C$	C	a_0	
38	24 04	RCL 4	Σy	Σy^2	$a_1\,C$	C	
39	16 02	$g\,x^2$	$(\Sigma y)^2$	Σy^2	$a_1\,C$	C	
40	24 03	RCL 3	n	$(\Sigma y)^2$	Σy^2	$a_1\,C$	
41	71	+	$(\Sigma y)^2/n$	Σy^2	$a_1\,C$	$a_1\,C$	
42	41	-	E	$a_1\,C$	$a_1\,C$	$a_1\,C$	$E = \Sigma y^2 - [(\Sigma y)^2/n]$
43	71	+	r^2	$a_1\,C$	$a_1\,C$	$a_1\,C$	$r^2 = a_1\,C/E$
44	13 00	GTO 00	r^2	$a_1\,C$	$a_1\,C$	$a_1\,C$	
45							
46							
47							
48							
49							

REGISTERS	
R_0	a_0
R_1	a_1
R_2	Σy^2
R_3	n
R_4	Σy
R_5	Σxy
R_6	Σx^2
R_7	Σx

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS				OUTPUT DATA/UNITS
1	Key in program						
2	Initialize		f	REG	f	PRGM	
3	Perform for i = 1,..., n						
	Input x-value and y-value	x_i	f				
		y_i	R/S				i
4	Compute regression constants		GTO	08	R/S		a_0*
			R/S				a_1*
5	Compute coefficient of deter						
	mination		R/S				r^2
6	To calculate a projected y-value,						
	input the x-value	x	RCL	1	x	RCL	
			0	+			ŷ
7	Perform step 6 as many times as						
	desired						
8	For a new case go to step 2						
	* The contents of the stack						
	should not be disturbed at these						
	points.						

Example:

An eccentric professor of numerical analysis wakes up one morning and feels feverish. A search through his medicine cabinet reveals one oral thermometer which, unfortunately, is in degrees centigrade, a scale he is not familiar with. As he stares disconsolately out his window, he spies the outdoor thermometer affixed to the windowframe. This thermometer, however, will not fit comfortably into his mouth. Still, with some ingenuity....

The professor suspects that the relationship is $F = a_1 C + a_0$. If he can get a few data pairs for F and C, he can run a linear regression program to find a_1 and a_0, then convert any reading in °C to °F through the equation. So tossing both thermometers into a sink of lukewarm water, he reads the following pairs of temperatures as the water cools:

C	40.5	38.6	37.9	36.2	35.1	34.6
F	104.5	102	100	97.5	95.5	94

If the relationship is indeed $F = a_1 C + a_0$, what are the values for a_1 and a_0? What is the coefficient of determination?

COVARIANCE AND CORRELATION COEFFICIENT

For a set of given data points $\{(x_i, y_i), i = 1, 2, \ldots, n\}$, the covariance and the correlation coefficent are defined as:

$$\text{covariance } s_{xy} = \frac{1}{n-1}\left(\Sigma x_i y_i - \frac{1}{n}\,\Sigma x_i \Sigma y_i\right)$$

$$\text{or } s_{xy}' = \frac{1}{n}\left(\Sigma x_i y_i - \frac{1}{n}\,\Sigma x_i \Sigma y_i\right)$$

$$\text{correlation coefficient } r = \frac{s_{xy}}{s_x s_y}$$

where s_x and s_y are standard deviations

$$s_x = \sqrt{\frac{\Sigma x_i^2 - (\Sigma x_i)^2/n}{n-1}} \qquad s_y = \sqrt{\frac{\Sigma y_i^2 - (\Sigma y_i)^2/n}{n-1}}$$

Note:

$-1 \leq r \leq 1$

DISPLAY LINE	DISPLAY CODE	KEY ENTRY
00		
01	31	↑
02	15 02	$g\,x^3$
03	23 51 02	STO + 2
04	22	R↓
05	21	$x \rightleftarrows y$
06	25	Σ+
07	13 00	GTO 00
08	24 05	RCL 5
09	24 04	RCL 4
10	24 07	RCL 7
11	61	×
12	24 03	RCL 3
13	71	+
14	41	−
15	24 03	RCL 3
16	01	1
17	41	−
18	23 00	STO 0
19	71	+
20	23 01	STO 1
21	74	R/S
22	24 00	RCL 0
23	61	×
24	24 03	RCL 3

DISPLAY LINE	DISPLAY CODE	KEY ENTRY
25	71	+
26	74	R/S
27	14 22	$1/x$
28	23 71 01	STO + 1
29	24 02	RCL 2
30	24 04	RCL 4
31	15 02	$g\,x^3$
32	24 03	RCL 3
33	71	+
34	41	−
35	24 00	RCL 0
36	71	+
37	14 02	$f\sqrt{x}$
38	23 71 01	STO + 1
39	24 01	RCL 1
40	13 00	GTO 00
41		
42		
43		
44		
45		
46		
47		
48		
49		

REGISTERS
R_0 n − 1
R_1 Used
R_2 Σy^2
R_3 n
R_4 Σy
R_5 Σxy
R_6 Σx^3
R_7 Σx

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS				OUTPUT DATA/UNITS
1	Key in program						
2	Initialize		f	PRGM	f	REG	
3	Perform this step for i = 1, 2,...n	x_i	f				
		y_i	R/S				i
4	Compute covariance s_{xy}		GTO	08	R/S		s_{xy}
5	Compute s_{xy}'		R/S				s_{xy}'
6	Compute correlation coefficient		R/S				r
7	For new case go to step 2						

Example:

x_i	26	30	44	50	62	68	74
y_i	92	85	78	81	54	51	40

Solution:

$s_{xy} = -354.14$

$s_{xy}' = -303.55$

$r = -0.96$

PAIRED t STATISTIC

Given a set of paired observations from two normal populations with means μ_1, μ_2 (unknown)

x_i	x_1	x_2	...	x_n
y_i	y_1	y_2	...	y_n

let

$$D_i = x_i - y_i$$

$$\overline{D} = \frac{1}{n} \sum_{i=1}^{n} D_i$$

$$s_D = \sqrt{\frac{\Sigma D_i^2 - \frac{1}{n}(\Sigma D_i)^2}{n-1}}$$

$$s_{\overline{D}} = \frac{s_D}{\sqrt{n}}$$

The test statistic

$$t = \frac{\overline{D}}{s_{\overline{D}}},$$

which has $n - 1$ degrees of freedom (df), can be used to test the null hypothesis

$$H_0: \mu_1 = \mu_2.$$

DISPLAY LINE	DISPLAY CODE	KEY ENTRY
00		
01	41	-
02	25	$\Sigma+$
03	13 00	GTO 00
04	14 22	f s
05	24 03	RCL 3
06	14 02	f $\sqrt{x}$
07	71	+
08	14 21	f $\bar{x}$
09	21	$x \rightleftarrows y$
10	71	+
11	74	R/S
12	24 03	RCL 3
13	01	1
14	41	-
15	13 00	GTO 00
16		
17		
18		
19		
20		
21		
22		
23		
24		

DISPLAY LINE	DISPLAY CODE	KEY ENTRY
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		
46		
47		
48		
49		

REGISTERS
R_0
R_1
R_2
R_3 n
R_4 Used
R_5 Used
R_6 ΣD_i
R_7 ΣD_i^2

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS				OUTPUT DATA/UNITS
1	Key in program						
2	Initialize		f	REG	f	PRGM	
3	Perform for i = 1,..., n						
	Input one pair of observations	x_i	f				
		Y_i	R/S				i
4	Delete erroneous data	x_k	f				
		Y_k	-	f	$\Sigma-$		
5	Compute t and df		GTO	04	R/S		t
			R/S				df
6	For new case, go to step 2.						

"t" Test Results

The following values of "t" were calculated from the program on page 186 and 187 .

Nitrogen balance:

i) concentration difference t = -.516

df = 13

t(95%) = 1.771

ii) % difference t = -1.214

df = 13

t(95%) = 1.771

Daily Nitrate effluent concentration:

Run 3 t = 5.399

df = 6

t(95%) = 1.943

Run 4 t = 5.850

df = 3

t(95%) = 2.353

Run 5 t = 4.141

df = 10

t(95%) = 1.812

Run 6 t = -.614

df = 2

t(95%) = 2.920

Run 7 t = .591

df = 5

t(95%) = 2.015

"Instantaneous COD" effluent concentration:

t = 0.407

df = 13

t(95%) = 1.771

"Instantaneous" COD removal rate:

t = -0.110

df = 12

t(95%) = 1.782

Daily total flow through reactors:

t = -1.081

df = 56

t(95%) = 1.645

MLSS

i) temperature

5°C	t = -0.19	df = 17	t(95%) = 2.110
12.5°C	t = +0.92	df = 38	t(95%) = 1.96
20°C	t = +1.25	df = 7	t(95%) = 2.365

ii) sludge age

4 days	t = 2.03	df = 14	t(95%) = 2.145
8 days	t = -1.05	df = 38	t(95%) = 1.96
12 days	t = -1.43	df = 10	t(95%) = 2.228

MLVSS

i) temperature

5°C	t = 0.479	df = 16	t(95%) = 2.120
12.5	t = -0.093	df = 31	t(95%) = 1.96
20°C	t = -1.736	df = 6	t(95%) = 2.447

ii) sludge age

4 days	t = 0.805	df = 10	t(95%) = 2.2228
8 days	t = -0.948	df = 34	t(95%) = 1.96
12 days	t = 1.193	df = 9	t(95%) = 2.262

Polynomial Model

The following polynomial model was generated from the experimental design:

$$y = B_o + B_1x_1 + B_2x_2 + B_{11}x_1^2 + B_{22}x_2^2 + B_3x_3 + B_{13}x_1x_3 + B_{23}x_2x_3$$

or

$$Y = BX$$

The values of plus one, zero and minus one were assigned to the various levels of sludge age, temperature and dissolved oxygen level as follows to facilitate matrix manipulation:

Assigned Value	-1	0	+1
Sludge age (days)	4	8	12
Temperature (C)	5	12.5	20
Dissolved Oxygen level (mg/l)	2		8

The X matrix generated by the experimental design was as follows:

Run	D.O. Level	x_o	x_1	x_2	x_1^2	x_2^2	x_3	x_1x_3	x_2x_3
1	High	+	0	0	0	0	+	0	0
	Low	+	0	0	0	0	-	0	0
2	High	+	-	0	+	0	+	-	0
	Low	+	-	0	+	0	-	+	0
3	High	+	0	-	0	+	+	0	-
	Low	+	0	-	0	+	-	0	+
4	High	+	+	0	+	0	+	+	0
	Low	+	+	0	+	0	-	-	0
5	High	+	0	+	0	+	+	0	+
	Low	+	0	+	0	+	-	0	-
6	High	+	0	0	0	0	+	0	0
	Low	+	0	0	0	0	-	0	0
7	High	+	-	0	+	0	+	-	0
	Low	+	-	0	+	0	-	+	0

The "$\hat{B}$"'s were calculated from the following:

$$B = (X'X)^{-1} X'Y$$

where: X' is the transpose of X.

This equation was solved by hand methods.

Where daily composite data only was available the first four rows of the above X matrix were not used.

As an example, the following values were calculated for the TOC effluent quality model:

$$\hat{B}_o = 18.48$$

$$\hat{B}_1 = 1.30$$

$$\hat{B}_2 = 11.46$$

$$\hat{B}_{11} = 0.84$$

$$\hat{B}_{22} = 9.70$$

$$\hat{B}_3 = 0.89$$

$$\hat{B}_{13} = 0.91$$

$$\hat{B}_{22} = 1.42$$

APPENDIX D

ANALYTICAL RESULTS

TABLE D-1

DAILY ANALYTICAL RESULTS

DATE	TOC	COD	UNF TKN	SOLUBLE TKN	NO_2&NO_3	EFFLUENT HIGH D.O. TOC	EFFLUENT HIGH D.O. NO_2&NO_3	EFFLUENT LOW D.O. TOC	EFFLUENT LOW D.O. NO_2&NO_3	TOTAL FLOW HIGH D.O.	TOTAL FLOW LOW D.O.
Feb. 14-15	58.0	57.3		19.4	2.6	25.0		37.3		32	32
15-16	40.6	43.1		20.8	6.1	25.7		26.4		35	36
16-17	37.9	29.4		18.7	0.9	25.6		29.0		32	33
17-18	41.0	-		31.5	1.3	28.0		29.5		34	32
18-19	32.7	29.5		9.9	3.7	18.9		16.9		29	29
19-20	51.8	54.8		18.3	1.4	22.3		18.3		24	25
20-21	64.7	66.6		19.2	2.3	37.2		37.6		19	19
21-22	47.9	47.1		15.6	0.6	37.6		33.5		22	23
22-23	36.0	33.7		22.9	5.3	36.9		25.6		20	22
23-24	26.1	26.6		15.9	1.5	25.9		26.6		22	24
24-25	14.2	22.9		10.9	2.9	21.0		18.2		20	21.5
25-26	44.2	46.8		11.6	4.3	25.6		33.0		24	26
26-27	52.8	51.4		22.1	2.7	34.9		40.1		21	22
27-28	49.3	53.0		17.9	1.7	31.2		25.7		24	24
Feb. 28-Mar.1	59.9	73.7		22.3	2.3	68.1		48.0		21	21
Mar. 1-2	38.1	38.0		19.1	1.6	43.5		35.9		22	22
2-3	28.5	33.5		18.7	1.5	33.9		32.8		17	17
3-4	53.1	57.5		19.4	1.4	37.0		37.3		32	32
4-5	39.5	46.1		19.0	1.7	26.7		26.0		24	24
5-6	37.4	43.3		20.4	2.4	-		-		20.5	20.5
6-7	52.3	62.3	22.6	19.6	2.1	24.6		26.5		-	-
7-8	33.9	45.7	26.2	13.5	0.5	-		-		25	25
8-9	35.8	39.4	20.0	17.1	0.7	22.5		29.4		23	23
9-10	24.3	26.9	18.3	16.3	0.9	10.7	18.2	10.8	9.5	25	25
10-11	36.4	-	33.5	23.3	13.7	19.7	29.5	10.0	18.1	21.5	22
11-12	49.5	56.6	27.9	21.1	0.3	25.2	16.3	13.7	13.0	22	24
12-13	46.1	63.0	28.6	25.4	5.5	-	-	17.7	13.8	-	20
13-14	44.8	61.0	24.9	20.1	2.3	-	-	13.3	11.0	-	23
14-15	33.5	43.1	17.5	20.4	0.8	9.5	18.3	11.8	11.8	22	19.5

ANALYSIS NOT CARRIED OUT DURING THIS PERIOD

ANALYSES CARRIED OUT DURING THIS PERIOD CONSIDERED UNRELIABLE

ANALYSES CARRIED OUT DURING THIS PERIOD CONSIDERED UNRELIABLE

Table D-1 cont'd...

DATE	INFLUENT TOC	INFLUENT COD	INFLUENT UNF TKN	INFLUENT SOLUBLE TKN	INFLUENT NO_2&NO_3	EFFLUENT HIGH D.O. TOC	EFFLUENT HIGH D.O. NO_2&NO_3	EFFLUENT LOW D.O. TOC	EFFLUENT LOW D.O. NO_2&NO_3	TOTAL FLOW HIGH D.O.	TOTAL FLOW LOW D.O.
Mar. 15-16	25.5	35.7	21.1	19.0	0.5	18.6	12.9	20.2	9.9	22	22
16-17	23.0	30.0	16.9	22.9	0.5	12.8	12.0	17.4	7.7	32	32
17-18	43.9	57.5	24.5	23.5	0.6	24.7	12.7	27.7	6.4	30	27
18-19	39.05	50.9	41.4	38.5	1.6	-	-	-	-	-	-
19-20	27.9	34.0	35.2	37.3	1.8	12.3	33.0	12.8	4.8	-	-
20-21	29.6	27.4	42.3	32.9	3.4	12.0	37.3	19.7	12.5	-	-
21-22	42.7	32.6	43.6	34.5	1.7	13.5	47.0	20.3	11.3	23	22
22-23	22.9	28.5	48.8	24.5	1.8	10.5	48.7	18.2	15.3	25	23
23-24	36.1	44.3	55.6	45.4	1.9	10.3	52.0	12.0	26.5	25	23
24-25	35.2	21.1	45.8	45.2	3.2	6.7	48.0	5.3	31.3	22	21
25-26	18.1	24.7	46.4	45.8	4.5	-	46.3	-	29.5	22	22
26-27	22.3	25.3	47.1	44.6	2.5	7.8	44.3	7.6	33.3	-	-
27-28	27.8	32.7	16.0	-	2.3	-	-	-	-	-	-
28-29	28.65	39.4	19.6	-	1.5	4.0	12.7	16.6	9.1	-	-
29-30	24.2	34.7	20.5	14.3	1.4	12.4	15.0	6.3	6.3	18	19
30-31	27.85	34.3	18.4	16.9	1.5	7.3	22.0	8.0	15.5	18	19
Mar. 31-Apr.1	30.6	36.5	18.2	18.1	1.5	15.0	19.5	17.2	18.4	19	20
Apr. 1-2	38.75	46.5	17.6	15.6	1.0	13.0	18.9	14.0	18.3	16	17
2-3	91.75	110.0	32.5	30.5	0.9	13.0	21.9	25.5	10.7	17	19.5
3-4	91.35	45.4	33.5	31.0	1.1	15.2	30.4	21.1	8.6	15	16
4-5	123.5	172.5	48.5	39.9	1.3	12.9	23.5	24.2	6.0	15	16
5-6	41.45	38.3	34.1	21.9	2.3	5.6	39.0	19.1	14.1	17	18
6-7	32.9	41.3	44.2	37.3	1.5	37.0	55.5	39.5	30.0	20	20
7-8	38.85	38.7	42.9	32.3	1.7	13.1	41.3	15.2	34.9	14	14
8-9	33.85	35.9	43.0	23.7	0.7	10.7	31.7	17.0	16.7	20	20
9-10	20.4	21.5	15.4	12.8	1.1	13.6	25.3	14.9	17.0	20	20
10-11	31.35	37.7	17.7	13.5	1.4	11.7	12.7	12.6	14.2	19.5	19
11-12	26.75	28.5	17.0	13.2	1.0	12.0	14.3	11.1	13.1	17	16
12-13	34.6	42.0	21.5	21.3	1.3	12.0	17.0	12.0	-	24	24
13-14	35.1	49.3	18.8	18.6	1.2	9.7	-	11.6	15.6	22	22
14-15	28.1	34.7	16.8	16.6	1.4	15.1	12.3	14.5	13.6	20.5	20.5

Table D-1 cont'd ....

DATE	INFLUENT TOC	INFLUENT COD	INFLUENT UNF TKN	INFLUENT SOLUBLE TKN	INFLUENT NO_2&NO_3	EFFLUENT HIGH D.O. TOC	EFFLUENT HIGH D.O. NO_2&NO_3	EFFLUENT LOW D.O. TOC	EFFLUENT LOW D.O. NO_2&NO_3	TOTAL FLOW HIGH D.O.	TOTAL FLOW LOW D.O.
Apr. 15-16	42.05	53.5	19.7	17.7	1.7	13.8	14.1	13.6	12.3	14	14
16-17	30.9	37.0	17.5	16.9	0.6	18.4	7.3	18.4	8.0	21	21
17-18	47.25	47.3	16.7	17.5	1.0	-	-	-	-	-	-
18-19	30.3	37.0	13.7	17.4	0.1	17.5	8.4	15.7	6.7	24	25
19-20	35.95	43.3	18.2	16.3	1.6	22.2	7.2	19.9	7.4	22	21
20-21	40.2	48.5	21.8	21.5	1.2	44.7	10.4	25.2	9.4	18	18
21-22	37.5	47.5	20.3	17.4	1.2	19.0	7.0	16.0	8.6	21	20.5

NOTE: Total Flow in litres per day; all other results in mg/l.

TABLE D-2

"INSTANTANEOUS" ANALYTICAL RESULTS

CONDITIONS	PARAMETERS	INFLUENT	HIGH D.O. REACTOR	HIGH D.O. EFFLUENT	LOW D.O. REACTOR	LOW D.O. EFFLUENT
Feb. 18, 1975	D.O.		10.0		1.1	
12.5°C	pH		7.35		7.05	
S.A. = 8 days	MLVSS		1085		1140	
	COD	36.8		17.0		34.7
	TOC	36.4		10.7		14.0
	TKN-UF	33.7		23.8		21.9
	TKN-F	28.0		22.1		21.6
	NH_3	25.4		22.8		20.0
	NO_3	0.2		13.5		10.5
	NO_2	0.0		0.6		0.5
	N_2	0.0		0.0		0.0
Feb. 20, 1975	D.O.		8.1		2.0	
12.5°C	pH		6.75		6.75	
S.A. = 8 days	MLVSS		1235		1205	
	COD	66.5		20.5		20.0
	TOC	54.5		16.3		17.1
	TKN-UF	21.9		9.4		9.2
	TKN-F	19.9		6.4		6.8
	NH_3	10.5		2.9		3.9
	NO_3	2.0		13.1		14.0
	NO_2	0.3		2.2		1.5
	N_2	0.0		0.0		0.0

Table D-2 cont'd...

Mar. 1, 1975	D.O.		8.0		2.8	
5°C	pH		7.25		7.50	
S.A. = 8 days	MLVSS		950		990	
	COD	88.4		76.3		61.5
	TOC	67.1		53.3		45.8
	TKN-UF	25.8		22.8		23.5
	TKN-F	21.2		18.9		17.3
	NH_3	13.0		13.0		15.2
	NO_3	3.7		5.4		6.8
	NO_2	0.5		0.4		1.8
	N_2	trace		0.0		0.0
Mar. 3, 1975	D.O.		10.85		2.4	
5°C	pH		-		-	
S.A. = 8 days	MLVSS		885		905	
	COD	35.5		22.5		18.5
	TOC	30.3		20.5		18.5
	TKN-UF	20.5		16.8		16.3
	TKN-F	18.5		17.1		16.1
	NH_3	18.0		16.8		15.5
	NO_3	2.5		7.4		7.4
	NO_2	0.2		0.4		0.3
	N_2	trace		trace		0.0
Mar. 16, 1975	D.O.		10.9		2.2	
12.5°C	pH		6.75		7.1	
S.A. = 4 days	MLVSS		690		670	
	COD	58.0		42.0		44.0
	TOC	27.9		20.7		32.5
	TKN-UF	20.0		10.7		13.8
	TKN-F	19.2		9.5		11.5
	NH_3	20.2		8.5		12.0
	NO_3	trace		7.1		4.9
	NO_2	trace		0.8		1.4
	N_2	0.0		0.0		0.0

Table D-2 cont'd ...

Mar. 18, 1975	D.O.		14.3		1.5		
12.5°C	pH		6.85		7.05		
S.A. = 4 days	MLVSS		550		470		
	COD	47.1		29.9		27.0	
	TOC	38.7		19.1		24.9	
	TKN-UF	26.4		18.0		20.6	
	TKN-F	21.0		11.4		20.4	
	NH_3	26.3		17.9		20.5	
	NO_3	trace		5.6		4.0	
	NO_2	trace		3.2		2.8	
	N_2	0.0		0.0		0.0	
Mar. 25, 1975	D.O.		8.2		1.1		
20°C	pH		6.2		6.7		
S.A. = 8 days	MLVSS		695		760		
	COD	18.6		12.5		-	
	TOC	22.1		11.8		2.7	
	TKN-UF	44.9		3.5		22.3	
	TKN-F	37.6		3.5		18.1	
	NH_3	48.1		2.7		19.7	
	NO_3	2.2		44.4		28.2	
	NO_2	0.5		0.5		1.1	
	N_2	0.0		0.0		0.0	
Mar. 27, 1975	D.O.		8.3		0.9		
20°C	pH		6.8		6.85		
S.A. = 8 days	MLVSS		575		655		
	COD	-		14.3		12.7	
	TOC	12.5		7.3		18.5	
	TKN-UF	45.6		22.1		26.5	
	TKN-F	29.6		-		21.3	
	NH_3	42.7		21.1		23.0	
	NO_3	0.6		26.5		22.0	
	NO_2	2.6		0.3		0.5	
	N_2	0.0		0.0		0.0	

Table D-2 cont'd ...

Apr. 6, 1975	D.O.		10.2		2.0	
12.5°C	pH		5.95		7.0	
S.A. = 12 days	MLVSS		1285		1265	
	COD	33.4		15.0		15.0
	TOC	32.4		8.6		17.8
	TKN-UF	47.5		2.6		31.3
	TKN-F	42.5		1.7		30.1
	NH_3	37.4		0.6		26.8
	NO_3	1.3		54.3		21.6
	NO_2	0.4		0.5		0.5
	N_2	0.0		0.0		0.0
Apr. 8, 1975	D.O.		8.7		1.0	
12.5°C	pH		6.1		6.8	
S.A. = 12 days	MLVSS		1250		1130	
	COD	56.0		27.0		24.0
	TOC	36.6		13.1		15.2
	TKN-UF	36.5		7.1		14.6
	TKN-F	32.0		-		13.1
	NH_3	31.9		trace		11.3
	NO_3	8.2		41.2		32.0
	NO_2	0.1		0.7		0.4
	N_2	0.0		0.0		0.0
Apr. 13, 1975	D.O.		10.7		1.0	
12.5°C	pH		6.6		6.7	
S.A. = 8 days	MLVSS		830		925	
	COD	40.0		19.0		19.0
	TOC	43.8		14.2		15.0
	TKN-UF	17.3		1.3		4.0
	TKN-F	17.7		1.8		2.0
	NH_3	15.2		0.1		0.4
	NO_3	0.4		14.0		13.9
	NO_2	0.7		0.2		0.3
	N_2	0.0		0.0		0.0

Table D-2 cont'd....

Apr. 15, 1975	D.O.		9.9		3.1	
12.5°C	pH		6.6		6.75	
S.A. = 8 days	MLVSS		890		1015	
	COD	40.5		19.5		19.0
	TOC	32.1		24.3		14.2
	TKN-UF	13.8		1.8		2.0
	TKN-F	15.2		1.8		2.0
	NH_3	13.0		0.2		trace
	NO_3	trace		10.0		10.2
	NO_2	trace		0.3		0.7
	N_2	0.0		0.0		0.0
Apr. 20, 1975	D.O.		16.3		2.2	
5°C	pH		6.3		6.95	
S.A. = 8 days	MLVSS		895		855	
	COD	48.4		19.0		18.3
	TOC	42.7		27.4		21.1
	TKN-UF	14.3		6.2		4.2
	TKN-F	16.3		5.9		4.0
	NH_3	11.1		3.5		2.3
	NO_3	1.4		8.6		10.5
	NO_2	0.2		0.1		0.3
	N_2	0.0		0.0		0.0
Apr. 22. 1975	D.O.		10.2		1.8	
5°C	pH		6.9		7.3	
S.A. = 8 days	MLVSS		890		810	
	COD	55.0		28.0		28.7
	TOC	41.1		21.5		18.7
	TKN-UF	18.2		10.5		13.4
	TKN-F	16.1		10.1		9.3
	NH_3	14.1		6.7		6.5
	NO_3	1.0		6.5		6.1
	NO_2	0.4		0.1		0.2
	N_2	0.0		0.0		0.0

TABLE D-3

DAILY MLSS AND MLVSS RESULTS

DATE	HIGH D.O. MLSS	HIGH D.O. MLVSS	LOW D.O. MLSS	LOW D.O. MLVSS
Feb. 14	2000	-	2000	-
15	2070	-	1795	-
16	1760	1350	1850	1405
17	1710	-	1670	-
18	1500	1085	1615	1140
19	1435	965	1740	1140
20	1690	1325	1600	1225
21	1720	1275	1565	1090
22	1625	-	1445	-
23	1390	1000	1265	915
24	1360	1145	1190	925
25	1185	850	1135	835
26	1115	870	1170	885
27	1125	970	1165	965
Feb. 28	1350	1040	1120	910
Mar. 1	1160	920	1175	1015
2	1060	915	1050	880
3	1065	875	1030	915
4	930	855	1050	855
5	830	710	980	720
6	720	635	840	680
7	785/1880	-/1420	1045/1765	-/1465
8	1725	1320	1870	1395
9	1385	1085	1420	1100
10	1035	810	1085	835
11	960	770	935	725
12	810	-	935	-
13	620	-	910	-
14	785	720	745	650
15	710	580	735	535
16	680	510	650	420
17	565	-	545	410
18	640	550	645	490
19	810	630	850	585
20	845	660	800	655
21	-	-	-	-
22	1395	1070	1385	1115
23	1230	-	1185	845
24	1110	735	1120	785
25	995	680	1005	750
26	970	655	885	665
27	790	545	775	650
28	865	620	670	425
29	-/1975	-/1415	-/2075	-/1455
30	1960	1345	1915	1360

TABLE D-3 cont'd....

DATE	HIGH D.O. MLSS	HIGH D.O. MLVSS	LOW D.O. MLSS	LOW D.O. MLVSS
Mar. 31	1720	1280	1875	1430
Apr. 1	1800	-	1720	-
2	1735	1155	1570	1220
3	1775	1395	1615	1150
4	1740	1265	1600	1260
5	1755	1370	1510	1225
6	1580	1255	1560	1280
7	1565	1360	1600	1375
8	1425	1215	1415	1045
9	1340	1085	1490	1230
10	1190	1035	1255	1105
11	1095	835	1170	875
12	980	860	1020	870
13	1105	820	1045	940
14	1130	1000	1125	915
15	1005	850	1070	1050
16	965	800	1070	865
17	1005	810	1105	1025
18	985	780	855	755
19	975	930	1070	880
20	1005	885	1010	845
21	1065	705	1105	1000
Apr. 22	1075	950	930	745

NOTE: All results expressed in mg/l

TABLE D-4

RESULTS OF MPNN SPOT TESTS

FOR NITRITE PLUS NITRATE NITROGEN

RUN	D.O. LEVEL	DILUTION	TUBE 1	2	3	4	5	MPNN
1	High	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	+	+	+	+	+	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	2,300,000/ml
		Blank	-					
1	Low	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	+	+	+	+	+	
		10^{-3}	+	+	+	-	+	
		10^{-2}	+	+	+	+	+	430,000/ml
2	High	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	-	+	+	-	-	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	-	+	56,000/ml
		Blank	-					

TABLE D-4 cont'd...

RUN	D.O. LEVEL	DILUTION	TUBE 1	2	3	4	5	MPNN
2	Low	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	+	-	-	-	
		10^{-4}	+	-	+	+	+	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	1,700,000/ml
3	High	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	-	-	-	-	+	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	330,000/ml
		Blank	-					
3	Low	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	-	-	-	-	-	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	230,000/ml

TABLE D-4 cont'd...

RUN	D.O. LEVEL	DILUTION	TUBE 1	2	3	4	5	MPNN
4	High	10^{-7}	-	-	-	-	-	
		10^{-6}	+	-	+	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	+	+	+	-	+	
		10^{-3}	+	-	+	+	+	
		10^{-2}	+	+	+	+	+	350,000/ml
		Blank	-					
4	Low	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	+	+	+	+	-	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	1,300,000/ml
5	High	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	+	
		10^{-4}	-	+	+	+	+	
		10^{-3}	+	+	-	+	+	
		10^{-2}	+	+	+	+	+	350,000/ml
		Blank	-					

TABLE D-4 cont'd...

RUN	D.O. LEVEL	DILUTION	TUBE 1	2	3	4	5	MPNN
5	Low	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	+	+	+	-	+	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	1,300,000/ml
6	High	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	+	+	+	+	-	
		10^{-4}	+	+	+	+	+	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	
		Blank	-					
6	Low	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	+	-	
		10^{-4}	+	-	+	-	+	
		10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	
7	High	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	+	+	+	+	+	

TABLE D-4 cont'd....

RUN	D.O. LEVEL	DILUTION	TUBE 1	2	3	4	5	MPNN
7	High	10^{-3}	+	+	+	+	+	
		10^{-2}	+	+	+	+	+	2,300,000/ml
		Blank	-					
7	Low	10^{-7}	-	-	-	-	-	
		10^{-6}	-	-	-	-	-	
		10^{-5}	-	-	-	-	-	
		10^{-4}	+	-	+	-	+	
		10^{-3}	+	+	+	+	+	
		10^{-2}	-	+	+	+	+	64,000/ml

TABLE D-5

DISSOLVED OXYGEN UPTAKE DATA

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
1	Feb. 18	High	0.0	10.0
			1.0	9.2
			4.0	8.2
			8.0	7.2
			12.33	6.2
			18.0	5.2
			23.0	4.2
			31.25	3.15
			38.0	2.15
1	Feb. 18	Low	0.0	10.2
			1.75	9.6
			6.5	8.8
			11.25	7.9
			19.5	6.75
			24.5	6.15
			33.0	5.3
			42.0	4.4
			49.0	3.55
			58.5	2.7
			70.75	1.8

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
1	Feb. 20	High	0.0	8.2
			1.5	7.0
			6.75	6.0
			9.25	5.2
			11.5	3.95
			16.75	3.15
			22.75	2.15
1	Feb. 20	Low	11.75	7.0
			19.5	6.15
			25.5	5.3
			32.0	4.3
			37.75	3.55
			45.5	2.5
			50.5	1.8
2	Mar. 1	High	0.0	15.35
			12.0	15.0
			30.5	13.1
			55.0	11.2
			82.0	9.1
			98.0	7.4
			128.0	5.55
			152.0	3.9
			180.0	1.9

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
2	Mar. 1	Low	0.0	17.9
			12.0	17.5
			33.0	15.5
			56.0	13.1
			82.0	10.9
			98.0	8.9
			128.0	6.5
			152.0	4.55
			180.0	2.4
2	Mar. 3	High	0.0	15.2
			28.0	15.0
			52.0	12.7
			76.0	9.7
			120.0	5.55
			141.0	3.9
			162.0	2.6
			172.0	2.05
2	Mar. 3	Low	0.0	18.8
			28.0	17.05
			52.0	14.85
			76.0	13.1
			120.0	9.1
			141.0	7.55
			162.0	5.6
			172.0	4.55

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
3	Mar. 16	High	0.0	14.2
			14.0	13.1
			25.0	12.85
			40.0	12.75
			50.0	12.2
			61.0	11.55
			70.0	11.1
			80.0	10.9
			97.0	9.5
			105.0	8.4
			120.0	5.6
3	Mar. 16	Low	0.0	15.2
			14.0	14.2
			25.0	12.7
			40.0	10.4
			50.0	7.6
			61.0	5.2
			70.0	3.0
			80.0	1.7
3	Mar. 18	High	0.0	16.6
			15.0	14.5
			29.0	13.05
			43.0	11.0
			63.0	8.9
			72.0	7.95

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
3	Mar. 18	High	90.0	5.95
			104.0	4.4
			117.0	3.4
			136.0	1.55
3	Mar. 18	Low	6.0	13.2
			15.0	11.8
			29.0	10.3
			43.0	8.7
			63.0	6.3
			72.0	5.1
			90.0	3.05
			104.0	1.65
4	Mar. 25	High	0.0	9.0
			4.5	8.75
			9.5	8.1
			14.0	7.6
			19.0	6.7
			25.5	5.85
			30.0	4.65
			35.0	3.6
			57.0	1.8
			60.0	1.2
			65.0	0.75

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
4	Mar. 25	Low	0.0	10.1
			4.5	8.5
			9.5	6.35
			14.0	6.2
			19.0	1.8
			25.5	0.9
4	Mar. 27	High	Probe Not Working	
4	Mar. 27	Low	0.0	11.1
			10.0	7.9
			14.0	6.2
			18.0	4.8
			22.5	3.3
			25.5	2.35
			31.5	0.85
5	Apr. 6	High	0.0	15.7
			7.5	12.8
			11.25	11.5
			17.25	9.9
			21.5	8.8
			28.5	7.45
			37.5	6.05
			42.5	5.7
			47.0	4.95
			57.0	3.7

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
5	Apr. 6	High	61.0	3.6
			65.75	2.8
			71.75	2.45
5	Apr. 6	Low	0.0	13.1
			7.5	11.45
			11.25	10.55
			17.25	8.8
			21.5	7.7
			28.5	5.3
			37.5	3.45
			42.5	2.4
			47.0	1.3
5	Apr. 8	High	0.0	17.7
			5.5	17.0
			12.0	15.75
			19.5	14.8
			23.5	14.0
			27.5	13.5
			31.5	12.8
			37.75	12.2
			41.0	11.7
			44.0	11.0
			49.5	10.45
			54.0	9.9
			58.5	9.3

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
5	Apr. 8	High	72.5	7.2
			77.5	6.3
			83.0	5.43
			95.5	3.7
			113.0	1.55
5	Apr. 8	Low	0.0	16.0
			5.5	14.7
			12.0	12.3
			19.5	9.6
			23.5	8.0
			27.5	6.7
			31.5	5.3
			37.75	3.1
			41.0	2.0
			44.0	1.3
6	Apr. 13	High	0.0	10.5
			4.25	9.55
			9.0	9.3
			17.5	8.0
			24.0	7.3
			29.0	6.6
			37.0	5.55
			44.5	5.2
			53.5	4.1
			62.5	2.9

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
6	Apr. 13	High	73.0	2.4
			76.0	1.8
6	Apr. 13	Low	0.0	12.3
			4.25	11.3
			9.0	10.6
			17.5	9.7
			24.0	8.7
			29.0	8.4
			37.0	7.3
			44.5	6.3
			53.5	5.8
			62.5	4.6
			73.0	3.6
			76.0	3.3
			84.0	2.3
			89.0	2.1
6	Apr. 15	High	0.0	11.2
			4.0	10.8
			11.0	10.2
			22.0	9.2
			38.0	7.8
			45.0	7.2
			70.0	5.3
			76.0	4.8
			82.0	4.2

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
6	Apr. 15	High	91.0	3.7
			97.5	2.85
			107.0	2.5
			118.5	1.4
6	Apr. 15	Low	0.0	12.2
			4.0	11.8
			11.0	10.9
			22.0	9.9
			38.0	9.7
			45.0	8.8
			70.0	7.55
			76.0	7.45
			82.0	7.3
			91.0	6.4
			97.5	5.9
			107.0	5.2
			118.5	4.7
			130.5	3.6
			141.0	2.45
			162.5	2.0
7	Apr. 20	High	0.0	13.2
			8.0	12.0
			18.5	10.5
			27.0	9.0
			39.5	7.5

TABLE D-5 cont'd...

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
7	Apr.	High	53.5	5.7
			71.0	3.0
			87.0	1.35
7	Apr. 20	Low	0.0	18.2
			8.0	16.4
			18.5	14.2
			27.0	12.8
			39.5	10.0
			53.5	6.9
			71.0	4.2
			87.0	2.2
7	Apr. 22	High	0.0	9.0
			7.0	8.5
			21.0	7.2
			37.0	5.8
			53.0	4.5
			71.0	3.0
			83.0	2.25
			93.0	1.5
7	Apr. 22	Low	0.0	23.6
			7.0	23.0
			21.0	21.8
			37.0	19.5
			53.0	17.7

TABLE D-5 cont'd....

Run	Date	D.O. Level	Time (minutes)	D.O. Concentration (mg/l)
7	Apr. 22	Low	71.0	15.5
			83.0	13.7
			93.0	12.8