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THE MECHANISM OF WASTE TREATMENT AT LOW TEMPERATURE

PART A: MICROBIOLOGY

By

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George D. Boone, and Kirke L. Martin

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THE MECHANISM OF WASTE TREATMENT AT LOW TEMPERATURE

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ABSTRACT

THE MECHANISM OF WASTE TREATMENT

AT LOW TEMPERATURE

PART A. MICROBIOLOGY

Low temperatures adversely affect wastewater treatment efficiency, partly by decreasing rates of biological oxidation. Because of the importance of adequate treatment efficiency, the feasibility of inoculating psychrophiles into a wastewater treatment system to increase biological oxidation rates at low temperatures was investigated.

Three psychrophiles capable of relatively rapid growth or oxygen uptake in sterilized sewage were selected from water and soil isolates collected in the Fort Collins vicinity. Of 97 Alaskan psychrophiles screened for maximum activity as indicated by growth rate and rate of reduction of methylene blue thiocyanate, three were selected for use in the waste stabilization experiments.

In studies with heat sterilized sewage, the effects of number of cells inoculated and culture age of the three psychrophiles (local) on percent BOD removals at different temperatures were analyzed. The effects of temperature and number of cells inoculated were the most significant. Percent BOD removals decreased as temperature decreased, and the large inoculum (10^{10} cells/200 ml) removed more BOD than the small inoculum (10^{8} cells/200 ml).

The effects of organisms used as inocula and of culture age of inocula were also significant. At 15 and 20 C, the psychrophile inocula removed less BOD than the inocula of mesophiles, but at 5 and 10 C, the psychrophiles were more efficient than the mesophiles. The 1-day old cells generally removed more percent BOD than 7-day cells, except when 10^{8} cells were inoculated at 5 C. When the small inoculum was used at 5 C, the 7-day cultures were more efficient than the 1-day cultures. It was thought that this phenomenon was the result of cold shock that affects physiologically young cells, but not stationary growth phase cells.

Using raw settled sewage as substrate at 5 C, the Alaskan psychrophiles removed substantially more BOD within a 72 hr detention period

than untreated controls; however, within a 120 hr period, BOD removals never averaged greater than 31.3%. In raw sewage studies at 2 C, it was found that the sewage inoculated with locally isolated psychrophiles had 19% BOD removals within 5 hr, but the mesophile inoculated and non-inoculated raw sewage had 15% and 14% removals, respectively. Although this difference was statistically significant, it was observed that a bacterial inoculum appreciably added to the initial BOD of the raw sewage, resulting in a higher final BOD concentration after 5 hr despite the psychrophiles ability to more rapidly oxidize wastewater nutrients.

These observations add to our knowledge concerning biological treatment as a method of wastewater stabilization; however, the system investigated appears to be too inefficient for practical use, even though it showed a statistically significant advantage over untreated or mesophilic inoculated controls.

INTRODUCTION

Modern sanitary engineering technology is largely responsible for the development of biological wastewater treatment processes capable of efficient operation in temperate regions. Although these treatment facilities have been adequately designed for efficient waste removal in temperate regions, their efficiency at low temperatures is usually not sufficient to meet current demands of pollution control agencies. In regions where very low temperatures are encountered for extended periods of time, such as the high mountain communities of Colorado or the far north, wastewater treatment presents a series of unique problems which must be resolved before waste removal criteria can be met.

Unequal loading presents a problem in communities and resort areas where weekends and holidays can introduce large increases in population and resulting waste to be treated. The principal problem encountered in low temperature wastewater treatment is decreased biological activity in the system. The secondary process of most conventional waste treatment systems is largely based on physiocochemical floculation and settling and bio-oxidation of the organic materials in the waste. The organisms responsible for the biochemical activity are usually mesophilic, having temperature optima between 20 and 40 C and, by definition, exhibiting very little activity below 10 C.

Methods of improving low temperature waste water stabilization may be approached by modifying the engineering design or improving biological activity. The engineer might design new facilities which minimize the adverse effect of low temperatures or find inexpensive methods of modifying present facilities to work more efficiently at low temperatures. However, since sewage degradation is primarily a biological phenomenon, the biologist may be able to alter the biota and enhance the biological rate of waste stabilization at low temperatures.

The approach of the study undertaken at Colorado State University was to isolate psychrophilic bacteria and evaluate their potential as inocula into sewage to increase the efficiency of waste stabilization at low temperatures. This approach could be used in already existing facilities or incorporated into the operation of newly designed facilities.

The specific objectives of this study were:

1) Isolation, identification and selection of psychrophilic bacteria capable of relatively high activity at low temperatures.
2) To determine if mutant strains with increased growth rates at low temperatures could be developed.
3) To analyze the effect of combined and pure culture inocula of different size and age on BOD and COD removals in sterile sewage at temperatures between 5 and 20 C.
4) A determination of BOD removal in raw settled sewage by the treatment organisms at low temperatures.
5) To compare the exponential growth rates of the psychrophiles with oxygen uptake rates at 5, 10, 15, and 20 C.
6) To investigate other factors which could conceivably increase the efficiency of BOD removals at low temperatures.

LITERATURE REVIEW

The prolonged persistence of organic pollution and of disease producing organisms in cold regions (1) necessitates adequate treatment of domestic wastewaters. Present treatment facilities, designed for use in temperate regions, are inefficient at low temperatures and must be improved.

The adverse effect of cold temperature on plant efficiency differs among treatment processes. Decreased temperature exerts a more adverse effect on anaerobic processes than on aerobic ones (1): thus, aerobic treatment seems better suited to cold wastewaters. Of the aerobic processes, activated sludge is less seriously affected by low temperature than is the trickling filter (39, 23, 1). Waste stabilization ponds are also adversely affected by low temperature, as winter BOD removals from ponds in Canada range from 0 to 55% compared to 65 to 90% in the summer (27). Aerated lagoons also cannot be depended upon to produce high quality effluents during winter unless provisions for process modifications which enhance efficiency are taken (7). Although aerated lagoon efficiency can be improved by having a number of lagoons in series to minimize short circuiting (99), the effects of temperature on the biological activity of the system remain. Although it has been suggested that it is possible to isolate and culture psychrophiles capable of degrading waste if inoculated into the wastewater, there is little information concerned with the design of such systems and no literature identifying capable species (1). Therefore, only literature indirectly related to the problem can be reviewed. The main topics of this review, then, are: 1) the effect of temperature on biological degradation of wastewater, 2) the properties of psychrophilic microorganisms, and 3) the feasibility of using selected strains of psychrophilic microorganisms to improve cold temperature treatment efficiency.

I. The effect of temperature on biological degradation of wastewater.

A. Biochemical reaction rates

Degradation of sewage is primarily biological, thus, inherently biochemical. Because rates of biochemical reactions vary with temperature according to the van't Hoff-Arrhenius relationship, rates of waste degradation would be expected to follow the equation

$$d(\ln K)/dT = E_a/RT^2 \qquad \text{equation 1}$$

in which E_a is the activation energy for the reaction (cal/mole), K is the reaction rate constant, R is the universal gas constant (1.99 cal/mole · ^oK), and T is the temperature (^oK). Integrating this equation yields a more useful form,

$$\ln K = -E_a/RT + C \qquad \text{equation 2}$$

or

$$\ln \frac{K_2}{K_1} = \frac{-E_a}{R}(1/T_2 - 1/T_1) \qquad \text{equation 3}$$

which shows that a decrease in temperature decreases reaction rate.

The integrated form also shows that a plot of the logarithm of the reaction rate versus the inverse of absolute temperature yields a curve with a slope of $-E_a/R$. If the activation energy is constant, the curve would be linear; but the curve would not be linear if the activation energy is also a function of temperature. Inorganic chemical reactions generally have activation energies which are changed only slightly by changes in temperature, and their Arrhenius plots are essentially linear. The effect of temperature on activation energies for biochemical reactions are more complex. Ware and Jex (99) found that the activation energy for the reduction of manure's BOD by a mixed microbial population was a linear function of temperature (^oC); thus, an Arrhenius plot would not be linear. Sawyer and Rohlich, however, cited numerous investigators who found that the temperature coefficient of reaction rates did not remain constant, but showed breaks at transition temperatures. Between transition temperatures,however, the coeficients were constant. Arrhenius plots of growth rates of a psychrophilic Vibrio grown in a mineral salts-glucose medium showed a temperature range where the curve was clearly linear, but at temperatures outside this linear range, the rate of growth decreased drastically (37).

The apparent discrepancy between the findings of Ward and Jex (99) and the findings reported by Sawyer and Rohlich (80) and Hanus and Morita (37) might be explained on the basis of an inherent

difference in their investigations. Ward and Jex investigated the effect of temperature on a mixed microbial population metabolizing a heterogeneous source of nutrients; whereas, the other investigators studied only single species. It is possible that the gradually changing slope (E_a not constant) of an Arrhenius plot of the rate of a complex biological reaction brought about by a heterogenous microbial population is due to 1) a change in the E_a of specific reactions as the relative abundances of the various species of the mixed population shift as temperature changes, and 2) a change in nutrient substrates being utilized as the population shifts with changes in temperature.

Although a biologist would expect an Arrhenius plot to have a temperature range where the curve is linear, a non-linear van't Hoff-Arrhenius relationship probably depicts the expected effect of temperature on biological removal of BOD from wastewater more accurately. Research has variously shown that process efficiency is: 1) independent of temperature, 2) dependent on temperature, or 3) relatively independent of temperature with temperature changes resulting in a shift of the relative importance of biological and physical processes (51). The discrepancies reported could be because the effect of temperature on treatment efficiency varies among treatment processes.

Hawkes (39) stated that seasonal temperature fluctuations have little effect on activated sludge efficiency. Keefer (48) compiled data over a twenty year period from an activated sludge plant in Baltimore and compared percent BOD and suspended solids removal to temperature. When the average flow was between 18.0 and 22.0 mgd, the average BOD removal was 86.5% at 54 F (11.7 C) and 91.4% at 79 F (26 C), but, when the flow was between 12.0 and 14.0 mgd, the average BOD removal was 89.5% at 54 F (12.2 C) and 91.5% at 76 F (24.3 C). The effect of temperature on suspended solids removal when the flow was between 18 and 22 mgd was similar to the temperature effect on BOD removal. At 53 F (11.7 C) the suspended solids removed was 80.0% and at 79 F (26 C) the removal was 92.5%.

Hunter, Genetelli, and Gilwood (40) studied the effects of temperature on BOD and suspended matter removals in a batch operated

activated sludge system with a 16 hour retention time. The results of their research are tabulated as follows:

Temperature (^{o}C)	%BOD removed	% suspended matter removed
4	79.1	81.5
20	92.3	91.8
28	90.1	83.6
35	91.1	86.6
45	93.7	93.3
55	83.6	81.3

Sawyer (78) studied the effect of temperature and sludge concentration on BOD removal in a batch operated laboratory activated sludge. He used wide mouth two-gallon jars filled with sewage and 1,000 mg/l activated sludge. The jars were placed in constant temperature baths ranging from 10 to 25 C, aerated with diffused air for ten hours and settled for one hour. The supernatant was siphoned off (the effluent) and replaced with fine screened sewage. The average percent BOD removed was 90.5, 94.3, 94.5, and 94.6 at 10, 15, 20, and 25 C, respectively. The percent BOD removal varied with sludge concentration. At 800, 1600, 2400, and 3200 ppm sludge, the BOD removals at 10 C were 84.0, 95.9, 97.2, and 96.8%, respectively.

It appears that the effect of temperature on activated sludge efficiency does not always truly follow the van't Hoff-Arrhenius relationship, because BOD removals often showed only a slight or no relation to temperature. The data of Keefer (48) showed only a 5% increase in BOD removal by activated sludge when the temperature increased from 12 to 26 C, and when the flow was relatively low, there was only 2% increase in BOD removal when the temperature increased from 12 to 24 C. The BOD and suspended solids removal data for activated sludge of Hunter et al. (40) showed no relation to temperature in the range of 20 to 45 C, although outside this range there was a definite temperature effect. Sawyer's (78) data also indicated no temperature effect on BOD removals by activated sludge between 15 and 25 C.

B. Temperature effects

Low temperatures affect equilibrium constants and saturation values of various solids and gases which may be dissolved in sewage (1) and cause a general reduction in the rates of almost all biological, chemical, and physical reactions (1, 16). Different parameters are affected by low temperatures to different degrees in each treatment system, but the overall effect is uniformly undesirable.

Conventional methods of waste disposal in the soil, including the use of leaching pits, septic tank disposal fields, the pit privy, etc., are useless in very cold regions, since decomposition and assimilation do not occur, and accumulation rather than disposal results. This problem is particularly acute in far north permafrost regions where the soil remains frozen year round (20).

When waste disposal in the soil is ineffective, one alternative is disposal into rivers and streams. However, before this can be considered feasible, adequate secondary treatment of the wastewater will be necessary. The secondary treatment process of most conventional wastewater treatment systems is a biological treatment in which organic material present in the primary effluent is microbially oxidized and removed (69) after being adsorbed on a biological floc or slime. According to Hawkes, the organisms actively responsible for this oxidation are mesophilic, having optima between 20 C and 40 C depending upon the species (39), and this is the primary reason that many conventional wastewater treatment processes are unable to do an adequate job of waste stabilization at low temperatures.

Secondary treatment in the activated sludge system is composed of physical adsorption onto the biological floc followed by aerobic degradation of the adsorbed organic materials and anaerobic digestion of the excess sludge formed by the settled floc and removed in the secondary sedimentation basin. The aerobic portion of the activated sludge process has been described as being slightly, but significantly, affected by low temperatures (11, 39, 48, 51, 101). Actual oxygen demand removals drop from the ninety percentile range

at 25 C to 30 C into the eighty percentile range at 5 C to 10 C, a difference in efficiency of 5 to 10 percent (11, 51, 52). Although little mention is usually made of detention times in activated sludge systems, a decrease in operating temperature is almost certain to require extended detention.

Pipes (72) pointed out that there are three essential biological requirements for the activated sludge process:

1) A mixed population of aerobic microorganisms must be capable of degrading the noxious components of the waste.
2) The required population must be able to grow in the aeration tank.
3) The organisms must grow in a form that will settle out in the secondary clarifier.

In describing these microorganisms as mesophiles, Hawkes indirectly provides a reason for one to suspect that Pipes' first two requirements would not be met at low temperatures. Ludzack's findings that low temperatures generally resulted in an inferior floc formation (51) indicates that the last of Pipes' biological requirements may be inadequate at low temperatures. Part of the effects of the depressed biological activity in the activated sludge process are offset by extending the detention time in the aerator to increase oxygen demand removals (11).

Fair, Geyer, and Okun describe anaerobic digestion as a process occurring from mesophilic and thermophilic activity, with the greatest efficiency occurring at approximately 55 C by the thermophilic microorganisms and very slow digestion occurring below 20 C (23). According to Warren (100), thermophilic digestion at temperatures of 43 to 54 C can digest volumes within days that would normally require months at temperatures below 15 C. At low temperatures little digestion is going to be accomplished. After a study of wastewater disposal facilities at DEW line radar stations in Greenland, Reed and Tobiason concluded that without some form of artificial stimulation, low temperature anaerobic systems appear to serve as little more than storage devices (73).

The trickling filter is much more adversely affected by temperature than other aerobic biological wastewater treatment

systems. This is probably due to the fact that the trickling filter relies upon a microbial film for its activity, and this layer's efficiency of organic removal generally increases with an increase in temperature and decreases with a decrease in temperature (10, 58). This same effect is reflected in the statements that trickling filters mature in a few weeks in the summer, but only in several months in the winter (23) and that winter recirculation of wastes (which results in cooling) may cause an efficiency drop up to 20 percent compared to summer (10).

The trickling filter process requires the exposure of the wastewater to a large surface area rendering the system more susceptible to freezing. In severely cold areas, both the rotating distributors and the beds of trickling filters have been known to freeze solid, reducing the effectiveness of the plant to that of a primary settling basin (10).

The viscosity of the influent also results in a lengthening of the necessary detention time in a trickling filter. The detention time has been described mathematically by the formula

$$t_d = ch \frac{\nu}{g}^{\frac{1}{3}} \frac{A/V}{Q}^{\frac{2}{3}} \qquad \text{equation 4}$$

where t_d = detention time, seconds
h = filter depth, feet
ν = kinematic viscosity of the wastewater, ft^2/sec
g = acceleration of gravity, 32.2 ft/sec^2
Q = volume/(unit area) (unit time), ft/sec
A/V = surface area/unit volume of bed, ft^{-1}
c = a dimensionless constant that reflects film buildup (23).

According to Lof, Ward and Hao (50) it can be shown that

$$c = Kk^{\frac{1}{3}} \qquad \text{equation 5}$$

where K = the fraction of the pore volume occupied by the liquid, dimensionless
k = dimensionless coefficient = 5

Furthermore, equation 4 strictly applies at an air flow rate of zero. Air flowing countercurrent to the downward liquid flow would increase the detention time in the filter. Designating this increase in detention time as Δt, the total detention time, t, could be represented by the formula

$$t = t_d + \Delta t \qquad \text{equation 6}$$

They also showed that, for a G/L ratio of less than about 0.4,

$$\Delta t = 400\ (G/L) \qquad \text{equation 7}$$

where G/L = weight ratio of air-water.
Accordingly, the total detention time in a trickling may be expressed by the semi-empirical formula

$$t = Kk^{\frac{1}{3}} h \frac{\nu}{g}^{\frac{1}{3}} \frac{A/V}{Q}^{\frac{2}{3}} + 400\ (G/L) \qquad \text{equation 8}$$

If all other parameters in equation 8 remain constant, an increase in viscosity would result in an increase in detention time. This is important because water viscosity increases as temperature decreases so that water is twice as viscous at 0 C than at 25 C (77). If increased viscosity due to low temperatures results in a sufficiently extended detention time, problems could conceivably arise in loading, especially in high-rate trickling filters which recirculate secondary effluents.

Although several different types of sewage lagoons exist, the aerobic lagoon is undoubtedly the most common type found in cold regions. These systems have a relatively high dissolved oxygen content throughout their entire volume, due usually to mechanical aeration. The oxidation of organics is done biochemically by the microbial flora present in the lagoon.

According to Dawson and Grainge, short retention lagoons with detention times of two to four days characteristically produce 70 percent BOD removals in the summer, but only remove 30 percent of the BOD in the winter (17), consistent with removals obtained by primary settling (57, 69). Halvorson described a similar lagoon located in Winnipeg, Canada, which yielded an 87 percent BOD removal during the summer, but removed only 24 percent during the winter (35).

If contact times are extended for sufficiently long periods, BOD removals of 68 to 90 percent can be obtained (33, 59, 74). The time necessary to obtain any given BOD removal varies with the temperature of the wastewater being treated. Reduction of BOD as high as 88 percent at a temperature of 3 C, with a 9.3 day detention, have been reported in an aerated lagoon by McKinney and Edde (59). In contrast, an aerated lagoon operating in a high altitude Colorado ski area has been described as requiring a 32 day detention at 0.25 C to obtain a BOD removal of only 65.6 percent (see report Section B). Apparently, contact times in excess of 20 days for low temperature aerated lagoons are not unusual (17, 75).

In spite of the long detentions inherent in the low temperature sewage lagoon, it is considered to be a promising system for wastewater treatment in cold climates, and some Alaskan areas as well as mountain communities are presently making use of it (13, report Section B). The sewage lagoon is an attractive treatment system because it is inexpensive (4) and because fairly high BOD removals can be obtained as long as loading rates are low enough or enough lagoons are utilized in series to allow sufficient detention.

II. The properties of psychrophilic microorganisms

The word "psychrophile" is the result of a combination of the two Greek roots "psychros", meaning cold, and "philos", meaning to love. Taken together the word is somewhat of a misnomer, as pointed out by Ingraham and Stokes (45), since the bulk of psychrophilic organisms do not "love" cold as much as they endure or adapt to it.

Biological activity at near 0 C temperatures, characteristic of high altitude or polar region wastewater, is restricted to psychrophilic organisms. Although there is no precise definition of "psychrophile", it usually refers to a microorganism capable of forming macroscopically visible colonies within one week on solid media at 0 C (87). However, other definitions are in the literature (45, 61, 25, 26).

According to Ingraham and Stokes (45), most psychrophilic bacteria belong to the genus Pseudomonas and, to a lesser extent, Achromobacter, Flavobacterium, Alcaligenes, and Micrococcus. Many other genera of psychrophiles, however, have been isolated. These include Vibrio (63),

Serratia (34), and Cytophaga (56). Most psychrophiles are aerobic, but some anaerobic strains have been isolated (83, 96).

Differences between psychrophiles and mesophiles reportedly include range of growth temperature, temperature characteristic of growth, temperature coeficient, and endogenous respiration (90).

Psychrophiles usually have an optimum growth range of 20 to 30 C with a maximum between 30 to 40 C, while mesophiles generally grow optimally at 30 to 40 C with a maximum of about 50 C and a minimum of about 10 C (88). Obligate psychrophiles have an optimum growth temperature below 20 C (87). The majority of obligate psychrophiles show rapid thermal death at temperatures greater than 20 C (38), and exposure to melted agar is sufficient to render certain obligate psychrophiles nonviable (62). Psychrophiles also have a shorter lag period over the temperature range in which growth of both groups occurs (15).

A plot of generation time versus temperature is similar for mesophiles and psychrophiles. The major difference is that the psychrophile has a generation time minimum at a temperature lower than that of the mesophiles (41). Greater differences are seen when Arrhenius plots, logarithm of growth rates vs. inverse temperature, are compared. The maximum growth rate for the psychrophile occurs at a lower temperature than the maximum for the mesophile, and the slope of the linear region is less negative for the psychrophile than for the mesophile (42). The slope of the linear region is the negative temperature characteristic (μ) for growth; thus, on the basis of an Arrhenius plot of Ingraham's data, the psychrophile can be distinguished from a mesophile because of it's characteristic of low temperature growth. This comparison between psychrophiles and mesophiles is best when the temperature characteristic is determined between 15 and 25 C (6).

Hanus and Morita (37), however, reported that psychrophiles could not be distinguished from mesophiles on the basis of the temperature characteristic as they found that reported values of μ showed no consistant differences between mesophiles and psychrophiles. These discrepancies reported by Hanus and Morita may by due to species variation rather than to basic differences between psychrophiles and mesophiles, since μ for a mesophilic mutant of a psychrophile Micrococcus cryophilus, was higher than μ for the parent psychrophile (91).

Psychrophiles reportedly can also be distinguished from mesophiles on the basis of their temperature coefficient of glucose oxidation (temperature coefficient = $Q_{10} = (K_2/K_1)^{10/dT}$ where K is the rate of reaction and dT is the change in temperature, C). Psychrophiles exhibit a lower temperature coefficient of glucose oxidation, with rates being based on oxygen uptake in a glucose substrate (15,44). The temperature coefficient for acetate and formate oxidation was also less for psychrophiles than for mesophiles (44). Temperature coefficients, however, differed when reference temperatures are changed, and the groups were best distinguished from one another only when reference temperatures were set at 10 and 30 C (91).

Baxter and Gibbons (8) reported that a psychrophilic yeast, Candida, had a greater endogenous respiration rate than a mesophile up to 30 C. Endogenous respiration comparisons of a psychrophilic bacterium, Micrococcus cryophilus and its mesophilic mutant gave greater rates for the mesophile (91), indicating that differences in endogenous respiration were not attributable to the ability to grow at low temperatures.

Comparisons between psychrophiles and mesophiles on the basis of temperature characteristic, temperature coefficient (Q_{10}), and rates of endogenous respiration are unreliable in distinguishing the two groups. By definition the groups can be distinguished on the basis of temperature range of growth; psychrophiles are able to function at low temperature, but mesophiles cannot. Rose (76) believed that low temperatures have two effects on the physiology of microorganisms: 1) on lipid content, and 2) on the regulation of metabolism. As temperature is lowered, the proportion of unsaturated fatty acids in the lipids of the cytoplasmic membrane of Escherichia coli increases (54). These changes in the fatty acids of the cytoplasmic membrane may change the architectural nature of the membrane and prevent permease action (25). Psychrophiles, presumably,resist structural changes as temperature decreases.

The fatty acid composition of a psychrophilic Candida remained nearly constant during growth, but that of a mesophile, Candida lypolytica changed during growth (47). Although this change was not conclusive evidence as to why mesophiles cease to grow at low temperature, it did show there was a difference in lipid metabolism between psychrophiles and mesophiles.

In addition to affecting lipid content and cell structure, low temperatures may alter metabolism. There are numerous reports of temperature changing the metabolic end products (76). Usually, only a proportional decrease in the end products occurred with decreased temperature, but occasionally the ratio of metabolic products changed or even new products could be formed (68). There was often an increase in polysaccharide synthesis at low temperature (76). Psychrophiles are capable of a wide range of metabolic activity at low temperatures, exhibiting proteolytic, lipolytic and saccharolytic abilities to varying extents (32, 35, 65, 70, 86). Most psychrophiles appear to be able to hydrolyze urea extremely well and at very high rates (35, 86). In testing 437 psychrophilic strains, Stefaniak found that 86.5 percent were capable of urea decomposition, 69.8 percent could ammonify peptones, 62 percent were capable of nitrate reduction, 23.1 percent were capable of degrading starch, and 19 percent were able to hydrolyze gelatin. It is interesting to note that, although psychrophilic nitrate reduction has been observed (12, 86), the process seems to stop short of actual dentrification. Also, in spite of highly aerobic environments, low temperatures appear to inhibit nitrification (14, 33, 60, 78).

Many enzymes and enzyme forming systems of psychrophiles are abnormally sensitive to heat (88), and this sensitivity could explain the adverse effect of temperatures above the optimum for growth (61). Formic hydrogenlyase of a psychrophilic, gram-negative, rod shaped bacterium was not synthesized above 20 C, and, once formed, is inactivated at 45 C. A mesophile, Escherichia coli, however, synthesized the enzyme at 45 C, and this enzyme is not denatured until 70 C (97).

The L-serine dehydrase of a psychrophilic Pseudomonas aeruginosa is less affected by temperatures below 15 C than is the same enzyme of a mesophilic Escherichia coli (85). Psychrophiles are apparently endowed with enzyme systems that are more temperature sensitive than those of mesophiles, accounting for the psychrophile's inability to function at relatively moderate temperatures. These enzymes function better at lower temperatures enabling them to grow at relatively low temperatures.

Other effects of temperature on enzymes exist: effects on feedback inhibition (43), induction and repression of enzyme synthesis (76), and changes in catalytic activity due to shifts in the conformation of the enzymes (93) caused by increased intra- and inter-molecular hydrogen bonding at lower temperatures (9, 53).

Psychrophily is the result of a cell structure and metabolism which allows the transport and breakdown of an exogenous nutrient supply to obtain energy for growth at 0 C. Although both cell structure and metabolism account for psychrophily, cell structure is the primary factor enabling growth at low temperature. Brown (9) and Ingraham and Bailey (44) showed that glucose oxidation rates differ between mesophiles and psychrophiles only when whole cell suspensions were used. With sonicated suspensions, the rates of glucose oxidation of the two groups were similar. These findings do suggest that cell structure determines the ability of an organism to grow at low temperature.

III. The feasibility of using selected strains of psychrophilic microorganisms to improve cold temperature treatment efficiency.

The inoculation of psychrophiles into a wastewater treatment system to improve efficiency at low temperature has been suggested, but there is little information found in the literature pertaining directly to such an approach (1). Although Boyd and Boyd (13) suggested that "starter" cultures could be developed and added to the sewage to increase mineralization at low temperatures, they did not attempt to isolate or test strains of bacteria capable of relatively high degradative ability at low temperature. The feasibility is interesting and open to conjecture. The biological degradation of wastes involves a myriad of ecological relationships; consequently, considerations for improving the biological removal of wastes at low temperature should follow an ecological approach. The objectives of an ecological study of waste are to determine what organisms are beneficial to the system and to determine how to design and operate the process so growth of the beneficial organisms is encouraged (72). The organisms most useful in stabilizing waste's organic matter, however, are not really known.

Dias and Bhat (19) felt that the most abundant organisms in their study, Zoogloea and Comamonas, were the most important, although they recognized that it was doubtful that a single bacterial species could utilize all the organic substances that occur in sewage and bring about its complete stabilization. Unz and Dondero (95), on the other hand, felt that the heterogeneous bacteria associated with nondescript flocs and zoogloeas were most important in waste stabilization, and the roles

played by branched zoogloeas and possibly Zoogloea spp. were relatively minor. The predominant genera of bacteria reported present in activated sludge varies among authors, but Pipes (72) assimilated the findings of different investigators and determined that Achromobacter, Alcaligenes, Flavobacterium,Pseudomonas, and Bacillus were the most common. The enteric bacteria present in domestic wastes were generally ignored by the various workers.

Any bacterial strain capable of increasing waste treatment efficiency at low temperature should be able to: 1) utilize the organic matter of the waste at low temperature and 2) for trickling filter or activated sludge, form an adsorptive slime or settleable adsorptive floc.

A microorganism able to utilize waste at low temperatures would likely be psychrophilic and would probably be a strain representative of the genera commonly found in sewage. Pseudomonas, Achromobacter, Alcaligenes and Flavobacterium, genera common to wastewater, have psychrophilic representatives (45). It is, thus, likely that bacteria exist that can utilize waste at low temperatures.

Because a sludge with good settling qualities is necessary for an activated sludge process to be effective, organisms capable of forming flocs are important to the treatment system. The significant bacteria in activated sludge must be able to flocculate by themselves, be flocculated by other organisms, or be able to attach themselves to larger particles which do settle out (72). Although the specific organisms most capable of forming floc are not known (30), many genera are evidently capable of flocculation. Zoogloea was once considered the most important floc former, but many other genera, including some strains of Escherichia, Alcaligenes, Bacillus, and Pseudomonas, have been isolated that readily flocculate in pure cultures (72). Unz and Dondero (95) isolated 20 strains of gram negative bacteria that were capable of forming flocs when grown in a nutrient broth. It is, thus, possible that psychrophilic bacteria are also capable of flocculation.

Because bacteria probably exist that are capable of utilizing waste at low temperatures and also capable of settling as flocs, improvement of treatment efficiency at low temperatures by using inocula of selected bacteria appears feasible. The only other alternative to biological treatment in cold environments seems to be housing and heating the entire treatment system (16).

MATERIALS AND METHODS

The methods and materials used in this study will be presented under the following headings:

I. Isolation and selection of psychrophilic organisms
   A. Local isolates
   B. Alaskan Isolates
II. Ultraviolet irradiation of sewage and selected local isolates
III. Biochemical oxygen demand determinations
IV. Psychrophilic treatment of sterile sewage with local isolates
V. Psychrophilic treatment of raw settled sewage
   A. Local isolates
   B. Alaskan isolates
VI. Psychrophilic treatment of concentrated synthetic sewage with Alaskan isolates
VII. Analysis of raw settled sewage effluent
VIII. Growth rate determinations on local isolates
IX. Oxygen uptake studies on local isolates

I. Isolation and selection of psychrophilic organisms

A. Local isolates

Water and soil samples were taken from the Fort Collins, Colorado, vicinity; the sampling locations are identified in Table 1. Either 1-ml or 0.1-ml aliquots of the water samples were spread with a glass rod on regrigerated Bacto Plate Count Agar (Difco, PCA) plates, and, for soil samples, 1 g of the soil was suspended in a 9-ml sterile buffer blank, shaken thoroughly, and either 1 or 0.1-ml aliquots were spread on regrigerated PCA plates. All plates were kep at near 4 C. Colonies which became visible within a week were picked and assigned a Roman numberal (designating the sampling location) and an Arabic number (designating the colony picked from the plate) (Table 1).

The isolates that were obtained from soil, water, irradiated sewage, and from the irradiation of previously selected isolates were inoculated into 9 ml of m-PCB contained in screw-cap tubes and placed in a test tube rack fixed at an angle of approximately

45° on a gyrotory shaker. The shaker was placed in a walk-in refrigerator (near 0 C), and the cultures were shaken at 150 rpm for 3 days. Twenty isolates, visually demonstrating the most dense growth, were transferred to and maintained on refrigerated PCA slants.

The number of isolates to be maintained was further decreased by selecting 5 isolates that grew best in heat sterilized sewage at near 0 C. Two loopfuls each, of the twenty isolates, were inoculated into 10 ml of heat sterilized sewage and incubated for 24 hr in the walk-in refrigerator with shaking. Serial dilutions were then made and spread on PCA plates which were incubated at 20 C for 48 hr. Although there were 20 cultures, only five were tested at a time, and the 1 or 2 isolates from each group yielding the highest colony counts were maintained.

Each of these five isolates was inoculated into respective 10-ml aliquots of filter sterilized sewage and incubated in a household refrigerator (4 C) for 48 hr. The density of each of the five cultures was adjusted to a percent transmittance of 80 at wavelength of 450 nm with a Spectronic 20 spectrophotometer. Because the percent transmittance of all five cultures was less than 80, adjustment with sterile deionized water to 80% was made. One ml of each adjusted suspension was inoculated into respective 100-ml aliquots of heat sterilized sewage contained in 250-ml erlenmeyer flasks, and incubated on the gyrotory shaker (150 rpm) for 24 hr in the walk-in refrigerator. Serial dilutions were plated on PCA and incubated for 48 hr at 20 C. The three isolates (XVI-4, XXII-1, XXIV-1) yielding the greatest number of colonies were maintained by monthly transer on refrigerated PCA slants. Cultures XIV-4 and XXIV-1 were selected for use in the experimental studies; the third isolate (T28B) used was from soil and was selected on the basis of its rapid oxygen uptake at 10 C.

Table 1. Sampling locations and identification of colonies which became visible within a week on PCA at refrigerator temperature.

Sample Number	Site	Number of Colonies Picked
IV	Sugar beet waste	1
V	Sugar beet waste	2
VI	Effluent-Fort Collins Trickling Filter Plant	3
VII	Effluent-Fort Collins Trickling Filter Plant	3
VIII	Effluent-Fort Collins Trickling Filter Plant	1
XVI	Poudre River-Mountain Park	4
XVII	Poudre River-Mountain Park	8
XVIII	Poudre River-Mountain Park	6
XX	Poudre River-Watson Lake	10
XXI	Watson Lake	7
XXII	Watson Lake	5
XXIII	Raw sewage-Fort Collins Trickling Filter Plant	3
XXIV	Raw sewage irradiated 5 sec	2
XXV	Raw sewage irradiated 10 sec	4

B. Alaskan isolates

A total of 97 psychrophilic isolates taken from the Chena, a subarctic Alaskan river, were obtained from Ronald C. Gordon, Ph.D., of the Environmental Protection Agency, College, Alaska. Each was subcultured into 5 ml quantities of half strength Bacto m-Plate Count Broth (½ m-PCB) in screw-cap tubes upon arrival at the laboratory and were incubated stationary at 1 C for 5 days. At the end of this five-day incubation, the culture tubes were each shaken vigorously on a Vortex mixer and 0.1 ml of the resulting cell suspension from each tube was spread plated on PCA. All plates were incubated at 1 C for 5 days and were inspected daily for signs of growth. The ten cultures exhibiting the fastest growth during this incubation period were set aside for further screening.

Gram stain indicated that the most rapidly growing organisms were gram negative rods of approximately the same size, so organism RW-25 was arbitrarily chosen as the "standard" for preparing a turbidimetric cell determination curve. A 200 ml volume of ½-m PCB was placed in a 500 ml erlenmeyer flask and autoclaved for 15 minutes at 124 C. After cooling, this medium was inoculated with organism RW-25 from the broth culture described above and incubated for 24 hours at 20 C with shaking at 113 rpm. The culture was then centrifuged in a refrigerated centrifuge at 9750 x g for 20 minutes. The supernatant was discarded and the cell pack resuspended in deionized water. Various dilutions of this cell suspension were made, each microscopically counted for total cell number per ml in an Improved Neubauer Levy Ultra Plane counting chamber and turbidity was read in a Klett Summerson Photoelectric Colorimeter using a blue No. 42 Klett filter (400-450 nm). The standard curve was a plot of the turbidity against cell numbers.

Fresh raw sewage was obtained from the Fort Collins #1 trickling filter plant immediately following grit clarification, was supplemented with 0.5 gram per liter Bacto Yeast Extract and autoclaved. After cooling, this sterile supplemented sewage was centrifuged for 20 minutes at 12,100 x g and the supernatant vacuum filtered simultaneously through a double thickness of fiber-glass prefilters and a 0.45 membrane filter. The filtrate was again autoclaved and refrigerated at 5 C.

A methylene blue thiocyanate solution was prepared by dissolving an 8.23 mg tablet of the dye in 50 ml boiling deionized water and brought to a volume of 200 ml by adding cool deionized water. The solution was refrigerated at 5 C in a foil covered flask.

The ten cultures obtained from the primary screening procedure were inoculated into 10 ml quantities of sterile supplemented sewage in sterile screw-cap test tubes. All tubes were incubated at 5 C for 4 days after which they were vigorously shaken on a Vortex mixer and each cell suspension adjusted to a total cell concentration of 1.5×10^8 cells per ml (Klett value of 10) by adding deionized water.

The final screening step involved the use of two incubation systems. The first was composed of eleven test tubes to each of which

had been added 8 ml of the sterile supplemented sewage, 2 ml of cell suspension and 1 ml of the methylene blue thiocyanate. The last tube (control) contained no psychrophilic cell suspension. The second system was identical to the first except that 8 ml of raw sewage which had been allowed to settle in the 1 C incubator for one hour was used as substrate instead of the sterile supplemented sewage. Both systems were incubated at 5 C for 5 days after which methylene blue reduction was determined visually from no reduction (++++) to complete methylene blue reduction (0). Both systems were compared and the three psychrophiles demonstrating greatest methylene blue reduction in both systems were selected as treatment organisms. These, designated B-6, B-39, and C, were maintained, from this point on, in sterile supplemented sewage as stock cultures.

Determination of pH effects on treatment organisms was done using an acetate buffer at pH values of 4 and 5 according to the method of Walpole (98), a tris-maleate buffer and pH values of 6, 7 and 8 according to the method of Gomori (31), and a carbonate-bicarbonate buffer at pH values of 9.2, 10, and 10.7 according to the method of Delory and King (18). One ml of buffer at each pH value was placed individually into three sterile screw-cap test tubes. Sterile supplemented sewage in 8 ml quantities was then added to all tubes. The three tubes buffered at each pH value were inoculated with 1 ml of the stock culture cell suspensions of each treatment organism, one organism to a tube. This was done so that at each pH value, three tubes would be represented, each tube containing a different treatment organism. Klett turbidity and pH measurements were made on all mixtures at the beginning and at the end of a three day 5 C incubation. Initial and final pH values were averaged together and recorded along with the difference between initial and final Klett turbidity measurements. The data presented graphically gave expression of the effect of pH on growth.

The effect of pH on substrate oxidation was experimentally determined using the same experimental design with the exception

that to each tube was added and thoroughly mixed, 1 ml of the methylene blue thiocyanate solution described above. At the end of the incubation period, all tubes were compared to a set of visual standards made by using 0.0041, 0.0033, 0.0025, 0.0016, 0.0008, 0.0004 and 0 mg per 1 of methylene blue thiocyanate in deionized water. Amount of methylene blue reduction (substrate oxidation) was recorded in mg per 1 methylene blue which compared most closely to the appropriate standard. Percent reduction was calculated and used for plotting.

II. Ultraviolet irradiation of sewage and selected local isolates

Approximately 20 ml of either raw sewage or broth cultures of the locally isolated psychrophiles were placed in clean glass petri dishes and irradiated from a distance of 37.5 cm with a 15 watt General Electric ultraviolet light source (2537 Å wavelength). The raw sewage samples were irradiated for 2, 5, or 10 seconds, and the isolates for 1 min. One millileter of the irradiated sample was then spread with a glass rod on PCA plates and incubated near 0 C in the walk-in refrigerator. Colonies which became visible within 4 days were isolated for possible use as inocula in the experimental studies.

III. Biochemical oxygen demand determinations

All standard 5-day, 20 C biochemical oxygen demands (BOD) were determined by the azide modification of the iodometric Winkler method described in Standard Methods for the Examination of Water and Wastewater (2). All dilutions were made directly in 300-ml BOD bottles by adding a known volume of sewage plus a 0.3 ml (equivalent to 0.1%) raw sewage seed into the BOD bottle and then siphoning aerated dilution water into the bottle until filled. The concentration of sewage added was determined by dividing the volume of sewage added by 300 ml. Only 5-day, 20 C BODs were determined.

IV. Psychrophilic treatment of sterile sewage with local isolates

Either 10 ml (large inoculum) or 0.1 ml (small inoculum) of the 24-hr or 7-day old culture suspension was inoculated into a 1000-ml flask containing 600 ml of sterilized sewage. A known volume (usually 3-6 ml) was aseptically withdrawn and placed into a 300-ml BOD bottle

for determination of the initial 5-day, 20 C, BOD. This inoculated sewage was decanted equally into three sterile 500-ml erlenmeyer flasks and incubated at the appropriate temperature (5, 10, 15, or 20 C) for 23 hr in an incubator shaker with aeration accomplished by shaking (60 rpm). After the 23-hr aeration period, the sewage was decanted into sterile 250-ml flasks and allowed to settle for 1 hr at the same temperature used when aerated. From this settled sewage, the final BODs were determined. The percent BOD removed was used as the parameter of treatment efficiency, and the data was analyzed by a four-factor analysis of variance. It was necessary to modify the three-factor analysis described in Snedecor and Cochran (84) to fit four treatments.

With combined culture inocula the experimental method was essentially the same as described for single cultures. Here, however, only large inocula (10 ml), each consisting of one of the four possible combinations of the three isolates, cultured and harvested as described previously, were used. These combinations were: 1) 5 ml XVI-4 + 5 ml T28B, 2) 5 ml XVI-4 + 5 ml XXIV-1, 3) 5 ml XXIV-1 + 5 ml T28B, and 4) 3.3 ml XVI-4 + 3.3 ml XXIV-1 + 3.4 ml T28B. The incubation conditions and BOD determinations were the same as described previously. The data were analyzed together with the large inocula data of the single culture experiment using a three-factor analysis of variance described by Snedecor and Cochran (84).

Two mixed, mesophilic populations were employed in this study as controls. The first control consisted of an inoculum obtained from a raw sewage culture and the method was the same as that used in single culture inocula studies. The data from this control were analyzed together with the data from the single culture inocula studies. The second control consisted of the raw sewage with no added inoculum. Approximately 200 ml of the freshly collected raw sewage was added to a clean 500-ml flask and the initial BOD was determined as described earlier, except seeding was not necessary. The sewage was incubated concurrently with the single and combined culture inoculated sewage and the final BOD was determined. The percent BOD removed was calculated, but the data was not analyzed statistically with the other data. This control was also used as the seed material for determining BODs on the heat sterilized sewage.

V. Psychrophilic treatment of raw sewage

A. Local isolates

A loopful of each of the three psychrophilic isolates, or 1 ml of raw sewage, was inoculated into a 250-ml erlenmeyer flask containing 100 ml of heat sterilized sewage supplemented with 1/5 strength m-PCB, and incubated at room temperature (20-25 C) on a gyrotory shaker at 150 rpm for 1 day (18-24 hr) or for 7 days. The cultures were centrifuged in a refrigerated centrifuge and the cell pellet was suspended in sterile deionized water so that a 1:50 dilution gave a reading of 50 Klett units as measured by a Klett-Summerson photoelectric colorimeter using the blue filter (400-450 nm). This cell suspension contained approximately 1.2×10^{10} cells/ml (Appendix 1).

Freshly collected raw sewage was decanted as 1000-ml aliquots into each of three 2000-ml flasks and placed into a constant temperature bath at 2 C. After the sewages equilibrated with the temperature of the bath (approximately 2 hr), one was inoculated with 5 ml of a suspension containing equal amounts of the three psychrophiles. The second flask was inoculated with 5 ml of a mixed, mesophilic population suspension, and the third flask was not inoculated. A volume of 7.5 ml was withdrawn from each flask for determining initial BOD concentrations (no seed was required). Each of the three sewages was aerated by bubbling compressed air, first into a flask containing water equilibrated with the temperature of the bath to filter and cool the incoming air and then into each of the three sewages. After a 4-hr aeration period, three 250-ml flasks were filled with each of the three sewages and allowed to settle for 1 hr before two 9-ml volumes from each flask were withdrawn for duplicate determinations of BOD. Percent BOD removed was calculated as the parameter of treatment efficiency and the data were analyzed statistically by a two-way analysis of variance (14).

B. Alaskan isolates

One hundred ml of sterile supplemented sewage was placed in each of three sterile 250 ml erlenmeyer flasks, inoculated with

each of the treatment organisms and incubated in a shaker at 5 C for 2-3 days, shaking at 92 rpm. When relatively heavy growth became visually apparent, turbidity was determined and converted to total cell number per ml. A sufficient amount of inoculum was placed in 50 ml centrifuge tubes to yield approximately 3×10^{10} total cells. These suspensions were centrifuged at 12,000 x g for 20 minutes, the supernatant discarded and the cell packs resuspended in 10 ml deionized water by vigorous shaking. This wash cycle was repeated once and the 10 ml suspensions were ready for use as inocula.

Several liters of raw sewage were obtained immediately following grit clarification and allowed to settle for one hour at 1 C, after which the supernatant was drawn off and 300 ml quantities placed in one-liter flasks. These were inoculated with a 10 ml cell suspension described above and 5 ml of centrifuge wash water were added; the control contained 15 ml of deionized water. The inoculation of treatment organisms gave a total psychrophilic cell count of approximately 1×10^{8} cells per ml. All treatment flasks were incubated in the temperature controlled shaker at 5 C for 5 days at 92 rpm.

Treatment flasks were sampled initially and at 24 hour intervals for 5 days for BOD determinations (3). D_1 and D_2 values for the BOD were determined in duplicate for each treatment and averaged to yield a single BOD value at each sampling period.

Initially and at 24 hour intervals for 5 days appropriate dilutions of samples from each of the psychrophilic treatment systems as well as of the control system were made, using sterile buffered water blanks. Each dilution was spread plated in 0.1 ml amounts in duplicate on PCA. All plates were incubated for 2 weeks at 1 C in the walk-in cold incubator and plates showing 30 to 300 colonies were counted and duplicates averaged.

VI. Psychrophilic treatment of concentrated synthetic sewage with Alaskan isolates

Psychrophilic inoculum preparation in this experiment was identical to that described earlier for Alaskan isolates. A fecal coliform which failed to exhibit visual evidence of growth for 2 weeks at 5 C

was used as a mesophilic control. This organism was inoculated into a 100 ml volume of sterile supplemented sewage, incubated for 24 hours at 37 C without shaking, harvested and used in the same manner as the psychrophilic inocula.

The synthetic sewage of Pipes (71) with the modification that boron was not added as one of the constituents was used. This synthetic material was made in triple strength and used in the same manner as the raw settled sewage described earlier with one modification, tris-maleate was used to buffer the flasks, containing organisms B-6, B-39 and control at pH 7.0 to 7.2 and the treatment flask containing organism C to a pH of 7.4 to 7.6. Dow Corning Antifoam A was used periodically to control excessive foaming.

BOD determinations were identical to those previously described and the procedure for psychrophilic counts was the same as described earlier with the following exceptions: 1) dilutions of 10^{-2} of the fecal coliform control were made and 0.1 ml plated in duplicate and 2) psychrophilic treatment plates were incubated at 15 C for 60 hours.

VII. Analysis of the raw settled sewage effluent

Inoculum preparation was identical to that described earlier in this paper for Alaskan isolates, except that the inoculum was adjusted to yield a cell count of 6×10^8 cells for each treatment organism.

Substrate preparation was also identical to that described earlier, with the exception that 2 liter flasks were used to contain 600 ml of the raw settled sewage. Initially and at 24 hour intervals, 90 ml quantities were removed from each flask, vacuum filtered simultaneously through a double thickness of prefilters and a 0.45 μ membrane filter. Aliquots of the filtrate from each treatment were used for analysis.

A. Analysis for nitrate-nitrite nitrogen

Aliquots of 20 ml from each treatment filtrate were colorimetrically analyzed for nitrate-nitrite nitrogen by the method of Schall and Hatcher (81), with the modification that the protein precipitation was not carried out. All colorimetric tests in the effluent analyses were read for optical density in a Spectronic 20 spectrophotometer.

B. Analysis for sugar

One ml aliquots from each treatment filtrate were colorimetrically analyzed for sugar by the method of Morris (64).

C. Analysis for orthophosphate

Aliquots of 35 ml from each treatment filtrate were colorimetrically analyzed for orthophosphate by the Vanado-Molybdate methods as described in Standard Methods for the Examination of Water and Wastewater (3).

D. Analysis of viscosity

Viscosity measurements of all treatment filtrates were made using a Gilmont V-2100 falling ball viscosimeter.

VIII. Growth rate determinations

Each of the three selected local isolates were inoculated into respective 200 ml portions of heat sterilized sewage contained in 500 ml erlenmeyer flasks, incubated in a shaker at 25, 20, 15, 10, or 5 C, and shaken at 100 rpm. Standard plate count determinations were made in triplicate for each culture four times, either once hourly, when incubated at 25 C, or once every other hour, when incubated at lower temperatures, while the cultures were in exponential growth (16-24 hr after inoculation). Growth rates were determined using the equation:

$$\frac{\log_{10} N_{T_2} - \log_{10} N_{T_1}}{0.301} \times \frac{1}{T_2 - T_1} = \text{generations/unit time} \qquad \text{equation 9}$$

where:

N_{T_2} = number of colonies counted at time T_2

N_{T_1} = number of colonies counted at time T_1

$0.301 = \log_{10} 2$

IX. Oxygen uptake studies on local isolates

The determination of oxygen uptake was performed in a Precision Warburg Apparatus as developed by Unbreit, Burris, and Stauffer (94).

The main compartment of each Warburg vessel received 2 ml of heat sterilized sewage and the side arm received 0.5 ml of a cell suspension (prepared as described previously for local isolates). The center well contained 0.2 ml of a 20% KOH solution and a fluted piece of filter paper (approximately 1 cm^2) to absorb CO_2.

The flasks were equilibrated to bath temperature before they were tipped and the cells mixed with the sewage. Readings were recorded at 15-min intervals for the first half-hour and then at 30 min intervals for the duration of the 6 hr incubation period. Uptakes were determined at 25, 20, 15, 10 and 5 C for each of the three isolates cultured in either ½ strength m-PCB or sterilized sewage supplemented with 1/5 strength m-PCB for less than 24 hr or for 7 days. Since all suspensions did not contain the same number of cells, all readings were corrected to a standard cell concentration (1.2×10^9 cells/ml) using the relationship of cell number to Klett reading (Appendix 1). It was assumed that the rate of oxygen utilization was proportional to the concentration of cells present in the reaction vessel.

RESULTS

Three locally isolated psychrophiles, two from aquatic environments and the other from soil, were selected as test organisms for experiments in raw and sterilized sewage. Improved growth rates at low temperatures of these and other isolates were not accomplished by ultraviolet irradiation. Of the Alaskan isolates, three were selected for use as inocula in raw sewage and concentrated synthetic sewage to determine the potential of psychrophiles used to increase wastewater stabilization at 5 C.

The results obtained in this study will be presented under the following headings:

I. Analysis of the effect that single and combined local culture inocula of different size and of different age have on BOD removals in sterilized sewage at temperatures ranging from 5 to 20 C.

II. Comparison of the BOD removal ability of psychrophiles inoculated into raw sewage to the removal ability of raw sewage's indigenous flora.
   A. With local isolates.
   B. With Alaskan isolates.

III. Comparison of the ability of Alaskan psychrophilic isolates to stabilize a concentrated synthetic sewage, measured by BOD and percent BOD reduction, with the stabilization ability of a known mesophile (fecal coliform) in the same substrate.

IV. Comparison of the effects of inoculated Alaskan psychrophiles on the chemical and physical composition of the wastewater substrate with effects exhibited by the raw sewage's indigenous flora.

V. Comparison between growth rates and oxygen uptake rates of the three local psychrophilic isolates in heat sterilized sewage.

VI. The effect of pH on the growth and oxidative ability of the Alaskan isolates in sterile sewage supplemented with 0.05 percent yeast extract.

I. Analysis of the effect that single and combined local culture inocula of different size and of different age have on BOD removals in sterilized sewage at temperatures ranging from 5 to 20 C.

The first experimental design involved determining what effect age and size of inocula of the three local psychrophilic isolates and of a mesophilic population had on percent BOD removals from sterilized sewage at temperatures ranging from 5 to 20 C. The composite results obtained with the four treatments are presented in Table 2 and the analysis of variance, shown in Table 3, revealed that the effects of all four treatments and their interactions were significant at the 0.5% level.

The most significant two-way interactions were size-age, temperature-age, and organism-temperature; the other two-way interactions were not as significant. The only three-way interactions significant at the 0.5% level were temperature-size-age and organism-temperature-age.

The results of BOD removals obtained by the single treatment variables are shown in Tables 4 through 7. The data in these tables were derived from Table 2 by averaging the percent removal values of the separate variables of each single treatment over all other identical variables. The BOD removal for the significant interactions, obtained similarly to the single treatment variables, are shown in Table 8 through 14 and Figures 1 and 2.

Table 4 reveals that the percent BOD removed varied among isolates. The difference among isolates was relatively slight although statistically significant (Table 3).

The differences in percent removals among temperatures (Table 5) were greater than differences among organisms; thus, the effect of temperature was greater than the effect of organism used as the inoculum, a fact shown by the larger F value for the temperature treatment. There was little difference between removals at 5 and 10 C, although the differences between successively higher test temperatures were large.

Table 6 shows that a large inoculum (10^{10} cells) removed more BOD than a small inoculum (10^{8} cells). As seen by the large difference between the two size variables and by the F value from Table 3 for the size treatment, this treatment had the greatest effect on BOD removals.

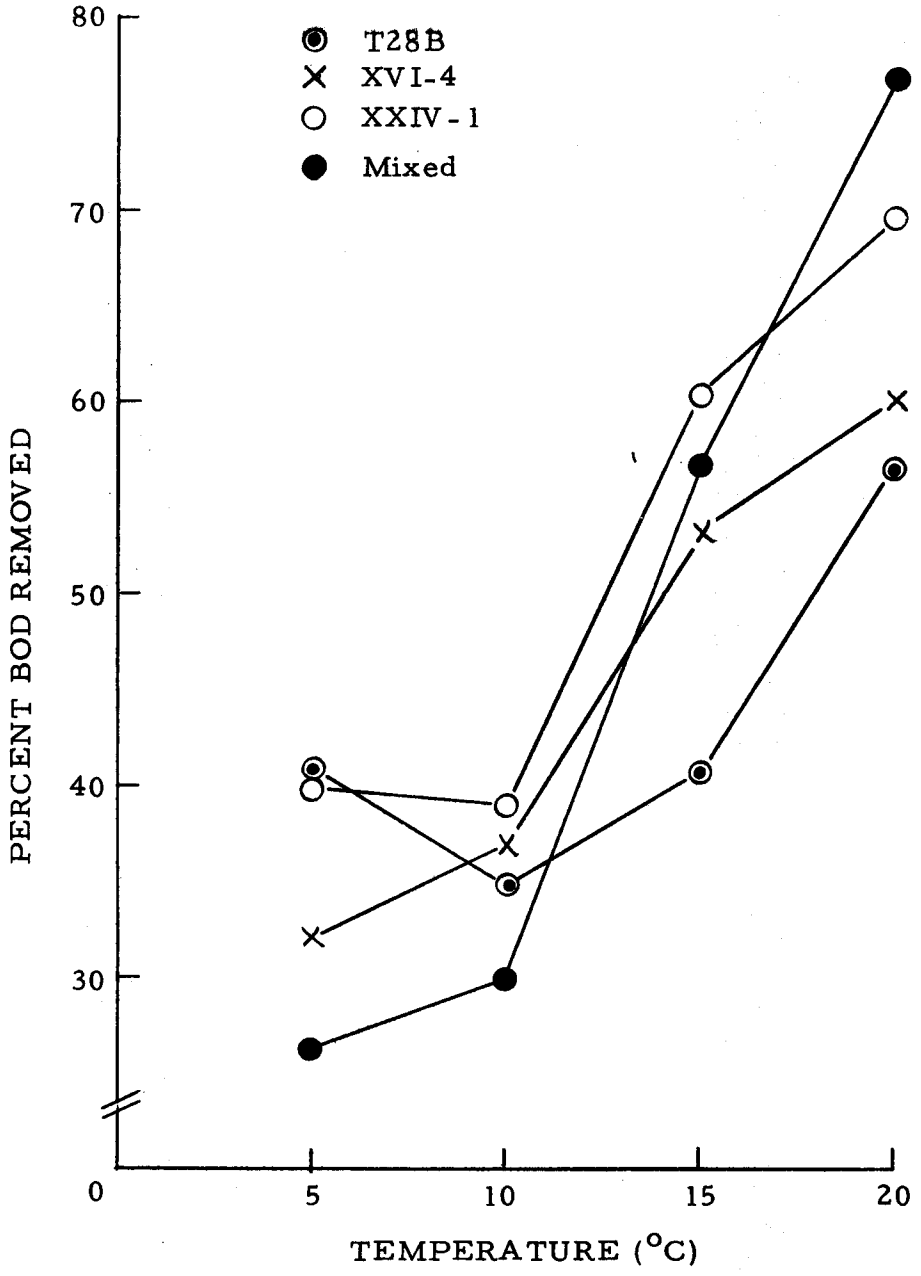


Figure 1. Average percent BOD removals by the four inocula at different temperatures.

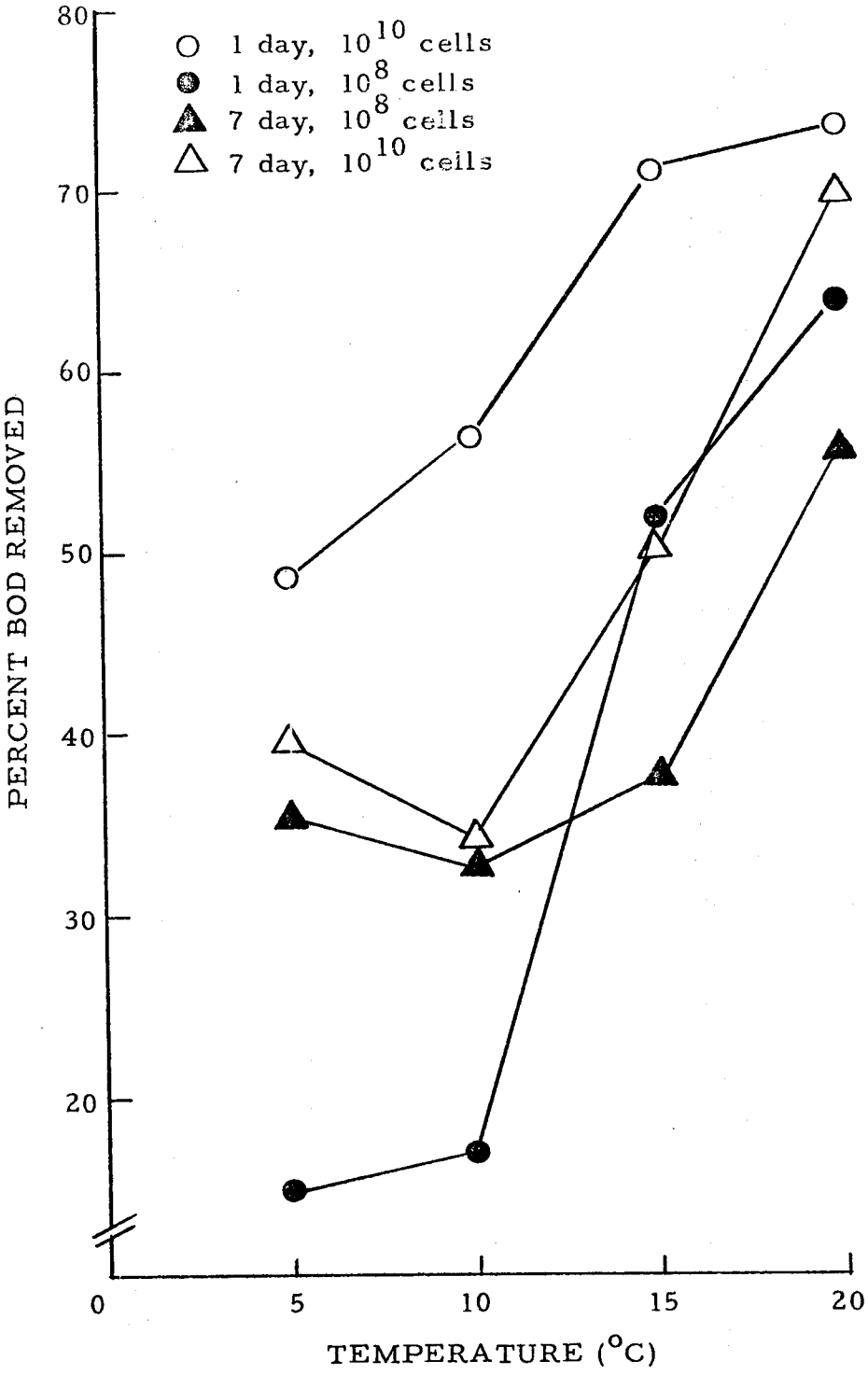


Figure 2. Average percent BOD removals by large and small quantities of 1 and 7 day old cultures of inocula at different temperatures.

Table 2. Percent BOD removal data, from heat sterilized sewage, for different age and size of four inocula at 5, 10, 15 and 20 C.

Temp. °C	Age	Organism							
		XVI-4		XXIV-1		T28B		MIXTURE	
		A	B	A	B	A	B	A	B
5	1 day	57.4	17.6	56.5	7.2	50.7	23.6	39.0	8.7
		51.0	13.5	50.9	17.6	52.5	19.3	32.3	8.2
		48.5	13.5	50.9	23.8	52.5	15.0	42.4	9.9
		(52.3)*	(14.9)	(52.8)	(16.2)	(51.9)	(19.3)	(37.9)	(8.8)
	7 days	35.4	20.4	51.0	35.9	50.7	38.1	20.4	37.9
		32.7	34.8	47.8	40.6	53.6	38.1	14.3	29.4
		32.7	25.8	44.5	49.9	50.7	41.8	35.1	35.2
		(33.6)	(27.0)	(47.8)	(42.2)	(51.7)	(39.3)	(23.3)	(34.2)
10	1 day	64.5	33.1	56.5	77.8	44.9	31.0	61.4	16.8
		58.7	9.7	59.6	66.1	46.2	4.8	58.8	10.9
		55.3	27.6	57.5	18.0	50.3	17.0	62.2	19.6
		(59.5)	(23.5)	(57.9)	(10.6)	(47.2)	(17.6)	(60.8)	(15.8)
	7 days	34.6	27.1	47.8	37.1	40.6	38.3	30.4	21.3
		11.0	60.8	47.8	46.7	39.0	32.7	32.5	1.5
		24.6	35.9	46.3	35.1	39.0	32.7	18.0	23.6
		(23.4)	(41.2)	(47.3)	(39.6)	(39.5)	(34.6)	(27.0)	(15.4)

Table 2. Percent BOD removal data, from heat sterilized sewage, for different age and size of four inocula at 5, 10, 15 and 20 C.

Temp. °C	Age	Organism							
		XVI-4		XXIV-1		T28B		MIXTURE	
		A	B	A	B	A	B	A	B
15	1 day	83.3	46.8	79.5	47.7	66.0	39.8	77.1	64.8
		47.4	53.2	78.7	51.0	60.9	38.6	86.0	63.6
		49.2	38.2	85.1	69.3	56.9	46.2	80.5	63.6
		(60.0)	(46.1)	(81.1)	(56.0)	(61.3)	(41.5)	(81.2)	(64.0)
	7 days	58.5	48.5	62.5	43.9	33.9	26.4	41.6	35.2
		57.8	49.4	69.5	39.5	29.0	27.1	51.5	36.1
		57.4	49.4	62.5	34.1	31.9	28.1	43.8	35.2
		(57.8)	(49.1)	(64.8)	(39.2)	(31.6)	(27.1)	(45.6)	(35.5)
20	1 day	69.9	72.4	76.0	79.4	62.8	55.9	85.2	52.9
		70.5	69.6	73.7	76.5	59.8	54.5	86.6	52.2
		71.0	63.2	76.9	78.9	61.0	55.3	86.6	55.5
		(70.4)	(68.4)	(75.3)	(78.3)	(61.2)	(55.2)	(86.2)	(53.2)
	7 days	63.3	45.3	70.8	67.7	57.6	45.6	84.4	79.0
		55.5	43.8	71.4	62.4	65.7	46.3	87.1	84.5
		52.6	44.5	74.8	27.7	67.2	44.0	85.5	79.6
		(57.1)	(44.6)	(72.3)	(52.6)	(63.5)	(45.3)	(85.6)	(81.0)

A - Initial inoculum of approximately 2×10^7 cells/ml

B - Initial inoculum of approximately 2×10^5 cells/ml

* - (Average value)

Table 3. Four-way analysis of variance of percent BOD removal data for different age and size of four inocula at 5, 10, 15, and 20 C.

SOURCE	d.f.	Sum Squares	Mean Square	F
Total	191	80,531.79		
Treatments	63	73,506.17		
Organisms (O)	3	2,133.40	711.13	12.96***
Temperature (T)	3	32,509.43	10,836.48	197.42***
Size (S)	1	13,227.71	13,227.71	240.99***
Age (A)	1	1,302.13	1,302.13	23.72***
Interactions				
O · T	9	5,867.90	651.99	11.88***
O · S	3	411.78	137.28	2.50
O · A	3	274.94	91.65	1.67
T · S	3	547.51	182.50	3.32**
T · A	3	3,291.58	1,097.19	19.98***
S · A	1	3,763.11	3,763.11	68.55***
O · T · S	9	1,327.98	147.55	2.69**
O · T · A	9	4,916.83	546.31	9.95***
O · S · A	3	499.74	166.58	3.03*
T · S · A	3	3,539.71	1,179.90	21.05***
O · T · S · A	9	1,194.55	132.73	2.42*
Residual	128	7,025.62	54.89	

Significance levels:
* 5.0%
** 2.5%
*** 0.5%

Table 4. Percent BOD removals from sterilized sewage by the psychrophile and mixed, mesophile inocula averaged over the other treatments.

Inoculum			
XVI-4	XXIV-1	T28B	Mixed Mesophile
45.6%	52.1%	43.0%	47.2%

Table 5. Percent BOD removals from sterilized sewage at different temperatures averaged over the other treatments.

Temperature (^{o}C)			
5	10	15	20
34.6%	35.1%	52.6%	65.7%

Table 6. Percent BOD removals from sterilized sewage by large and small quantities of inocula averaged over the other treatments.

Number of Cells Inoculated	
10^{10}	10^{8}
55.3%	38.7%

Table 7. Percent BOD removals from sterilized sewage by 1 and 7-day old cultures of the inocula averaged over the other treatments.

Culture Age of Inocula	
1 day	7 days
49.6%	44.4%

Table 8. Average percent BOD removals by the four inocula at different temperatures.

	Inoculum			
Temperature (^{o}C)	XVI-4	XXIV-1	T28B	Mixed
5	32.0%	39.7%	40.6%	26.0%
10	36.9%	38.9%	34.7%	29.7%
15	53.2%	60.3%	40.4%	56.6%
20	60.1%	69.6%	56.3%	76.6%

Table 9. Average percent BOD removals by large and small quantities of the four inocula.

Number of Cells Inoculated	Inoculum			
	XVI-4	XXIV-1	T28B	Mixed
10^{10}	51.8%	62.4%	51.0%	55.9%
10^{8}	39.6%	41.8%	35.0%	38.5%

Table 10. Average percent BOD removals by 1 and 7-day old cultures of the four inocula.

Culture Age	Inoculum			
	XVI-4	XXIV-1	T28B	Mixed
1 day	49.4%	53.5%	44.4%	51.0%
7 days	41.7%	50.7%	41.6%	43.5%

Table 11. Average percent BOD removals of large and small quantities of inocula at different temperatures.

Number of Cells Inoculated	Temperature (^{o}C)			
	5	10	15	20
10^{10}	43.9%	45.3%	60.4%	71.5%
10^{8}	25.2%	24.8%	44.8%	59.9%

Table 12. Average percent BOD removals by 1 and 7-day old cultures of inocula at different temperatures.

Culture Age	Temperature (^{o}C)			
	5	10	15	20
1 day	31.8%	36.6%	61.4%	68.6%
7 days	37.4%	33.5%	43.8%	62.8%

Table 13. Average percent BOD removals by large and small quantities of 1 and 7-day old cultures of inocula.

Number of Cells Inoculated	Culture Age of Inocula	
	1 day	7 days
10^{10}	62.3%	48.2%
10^{8}	36.9%	40.5%

Table 14. Average percent BOD removals by large and small quantities of 24 hour and 7 day old cultures of inocula at different temperatures.

Culture Age	Number of Cells Inoculated	Temperature (^{o}C)			
		5	10	15	20
1 day	10^{10}	48.7%	56.3%	70.9%	73.3%
	10^{8}	14.8%	16.9%	51.9%	63.9%
7 days	10^{10}	39.1%	34.3%	50.0%	69.7%
	10^{8}	35.7%	32.7%	37.7%	55.9%

The effect of culture age (Table 7) was relatively slight compared to the temperature and size treatments, but was still significant (Table 3).

The organism-temperature interaction data (Table 8, Figure 1) revealed that changes in temperature affected each organism's ability to remove BOD differently, with the mixed, mesophilic inoculum being affected the most and T28B the least. Although T28B removed the

least percent BOD over the entire temperature range studied, it removed the greatest percent at 5 C.

As seen in Table 9, showing the average percent BOD removed by two sizes each of four inocula, a smaller inoculum removed less BOD than a larger inoculum - a fact consistent with the data from Table 6. The effect of a smaller inoculum on BOD removed was similar for each organism, indicating that no organism-size interaction existed (a small, insignificant F value).

The data, presented in Table 12, for the temperature-age interaction showed that 7-day cultures removed more BOD at 5 C than did 1-day cultures, although, at 10, 15, and 20 C, 7-day cultures removed less than 24 hour cultures. The significance of this difference at 5 C was confirmed by the F value given for this interaction.

The size-age interaction disclosed that a small inoculum of 7-day old cultures removed more BOD than 1-day old cultures, but a large inoculum of 7-day old cultures removed much less percent BOD than old cultures. The significance of these findings, which opposed the general tendency of lower removals by 7-day cultures, could also be seen by the large F value.

The average percent BOD removed by two ages of two inoculum sizes at different temperatures, the temperature-size-age interaction, are shown in Table 14 and Figure 2. At 5 and 10 C, 1-day cultures of small inocula removed less BOD than corresponding 7-day cultures; however, at 15 and 20 C, 1-day cultures of small inocula removed more BOD. Relationships found in the two-way interactions (Table 11-13) could also be verified as shown in Table 14.

Table 15 shows percent BOD removals by 1 and 7-day old inocula of combinations of the three isolates at 5, 10, 15, and 20 C. Because only the large inoculum (10^{10} cells) was used, the data of Table 15 and the data for the large inoculum of Table 2 were statistically analyzed together. The results of the analysis (Table 16) disclosed that all treatments and their interactions were statistically significant. Table 17 shows that there was little detectable difference in percent removals between single and combined culture inocula.

The effect of temperature on percent BOD removals by the indigenous flora of raw sewage is given in Table 18. Low temperature (5 C) had

Table 15. Percent BOD removal data, from heat sterilized sewage for different aged combinations of inocula at 5, 10, 15, and 20 C.

Temp. (°C)	Age (days)	Organisms			
		A	B	C	D
5	1	50.4	11.2	34.2	68.5
		52.3	12.6	33.2	66.5
		51.4	9.7	31.1	63.6
		(51.4)*	(11.2)	(32.8)	(66.2)
	7	18.7	31.3	22.4	41.3
		21.5	27.8	16.7	36.2
		25.6	25.5	31.9	56.4
		(21.9)	(28.2)	(23.7)	(44.6)
10	1	58.7	58.7	73.3	70.8
		40.6	61.6	72.0	68.2
		46.3	57.8	89.2	68.9
		(48.5)	(59.3)	(78.2)	(69.3)
	7	45.6	47.3	34.2	36.1
		55.5	49.1	36.2	26.0
		50.0	46.4	34.2	30.5
		(50.4)	(47.5)	(34.8)	(30.9)
15	1	52.5	62.8	60.9	61.0
		49.1	61.0	59.3	50.0
		46.9	60.1	60.1	64.6
		(49.5)	(61.3)	(60.1)	(58.6)
	7	54.7	54.8	66.8	68.0
		54.1	55.7	62.4	65.0
		56.1	55.7	65.0	66.5
		(55.0)	(55.4)	(64.7)	(66.5)
20	1	54.9	61.7	71.8	70.6
		58.8	66.6	72.4	70.0
		83.6	63.0	73.0	71.6
		(65.8)	(63.8)	(62.4)	(70.7)
	7	67.4	76.1	74.6	73.9
		69.1	76.6	72.9	70.0
		70.2	76.6	74.6	71.6
		(68.9)	(76.4)	(74.0)	(71.8)

A - XVI-4 & T28B, B - XVI-4 & XXIV-1, C - T28B & XXIV-1,
D - XVI-4 & T28B & XXIV-1.
* - (average value)

Table 16. Three-way analysis of variance of the percent BOD removal data for different aged combinations of inocula at 5, 10, 15 and 20 C.

SOURCE	d.f.	Sum Squares	Mean Squares	F
Total	191	60,232.67		
Treatments	63	56,674.59		
Organisms	7	3,339.36	477.05	17.16***
Temperature	3	26,704.03	8,901.34	320.19***
Age	1	5,075.34	5,075.34	182.47***
Interactions				
O T	21	9,624.41	458.31	16.49***
O A	7	2,344.17	334.88	12.05***
T A	3	3,200.26	1,066.75	38.37***
O T A	21	6,387.02	304.14	10.94***
Residual	128	3,558.08	27.80	

Significance Levels: * 5.0%
** 2.5%
*** 0.5%

a pronounced adverse effect on percent removals, as removals at 5 C were much lower than at the other temperatures. There was little difference between removals at 10 and 15 C, but removals at 20 C were appreciably greater. It should be noted, however, that random selection of sewage samples provided initially higher oxygen demands for the 20 C samples. This may have influenced the percent BOD removals slightly.

II. Comparison of the BOD removal ability of psychrophiles inoculated into raw sewage to the removal ability of raw sewage's indigenous flora.

A. With local isolates.

Previous experiments had been performed using heat sterilized sewage, instead of raw sewage, so that the data represented BOD

Table 17. Percent BOD removals by two culture ages of single and combined culture inocula averaged over all temperatures.

Inoculum Type	Average Percent BOD Removed
Single culture	(55.1)*
XVI-4	51.8
XXIV-1	62.4
T28B	51.0
Combined culture	(54.2)*
XVI-4 & T28B	51.4
XVI-4 & XXIV-1	50.4
XXIV-1 & T28B	55.1
XVI-4 & XXIV-1 & T28B	59.8

*(Average)

removals attributable to treatment effects rather than to the indigenous flora effects. To determine whether an inoculum of psychrophiles could increase BOD removals in raw sewage at low temperatures, tests at 2 C on raw sewage inoculated with psychrophiles were compared to removal by a mesophilic inoculum and to raw sewage with no inoculum.

As shown in Table 19, the sewage inoculated with psychrophiles removed more BOD (%) than mesophile-inoculated or non-inoculated raw sewage. As seen in Table 20, this difference was statistically significant at the 0.5% level; however, the variance among replications was greater. Although the psychrophile-inoculated sewage had a greater percent BOD removed than the mixed or non-inoculated sewage, Table 21 shows that the initial and final BOD concentrations in inoculated sewage were greater than in non-inoculated sewage.

B. With Alaskan isolates

The effects of inoculated and indigenous flora on BOD over a five day holding period are expressed graphically in Figure 3, 5,

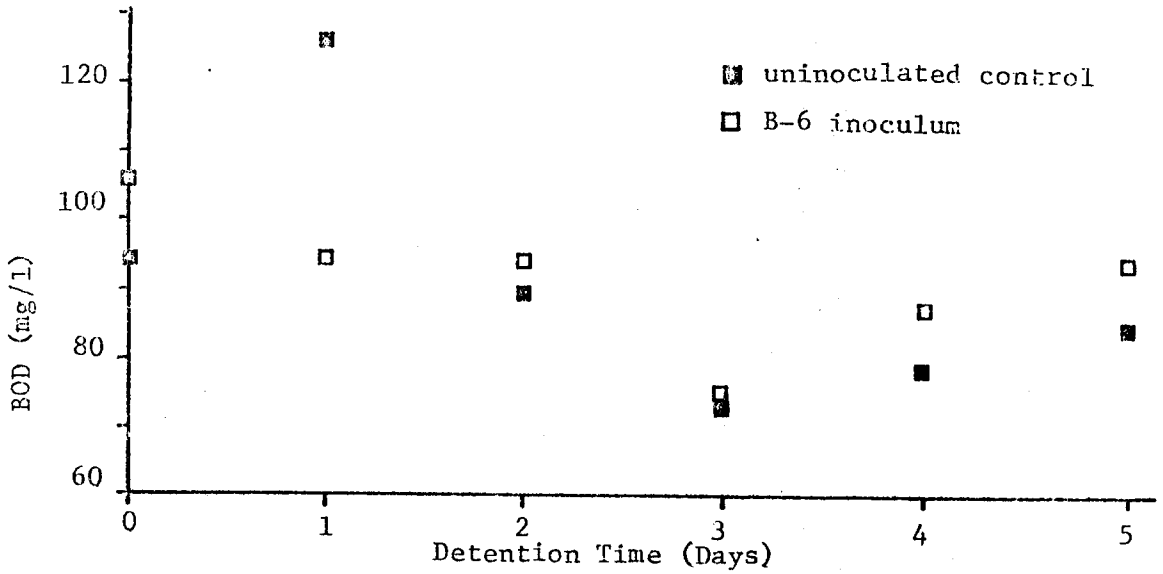


Figure 3. A plot of BOD in treated and untreated raw settled sewage at 5 C expressed as a function of time.

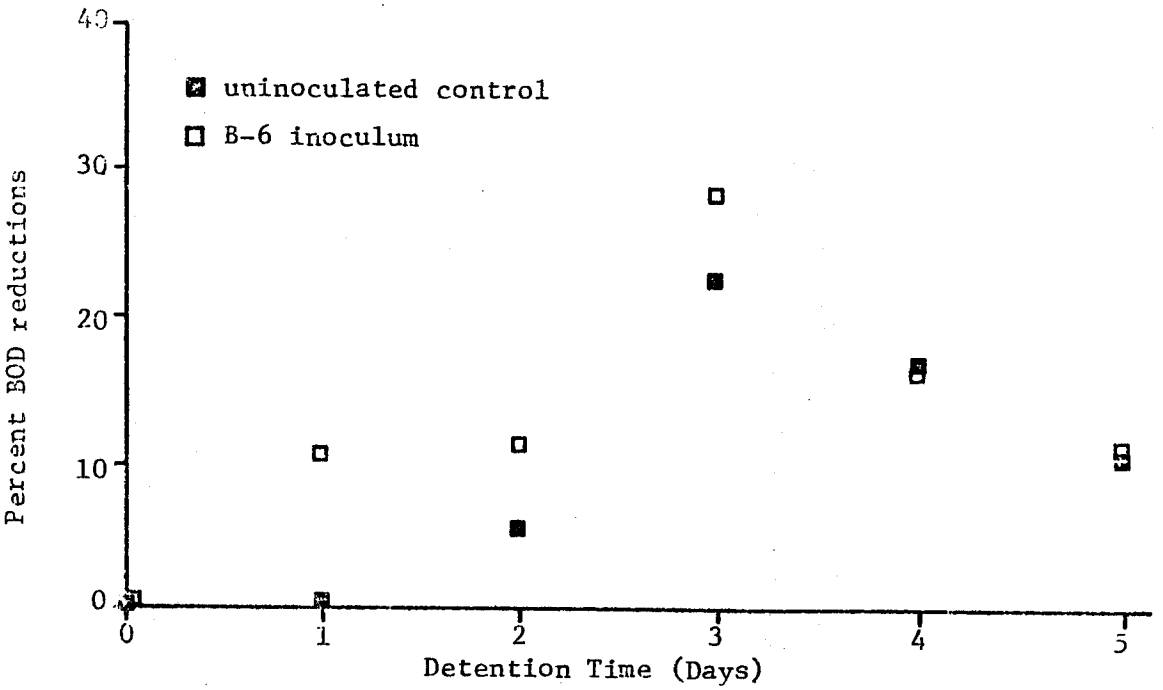


Figure 4. A plot of percent BOD reductions in treated and untreated raw settled sewage at 5 C expressed as a function of time.

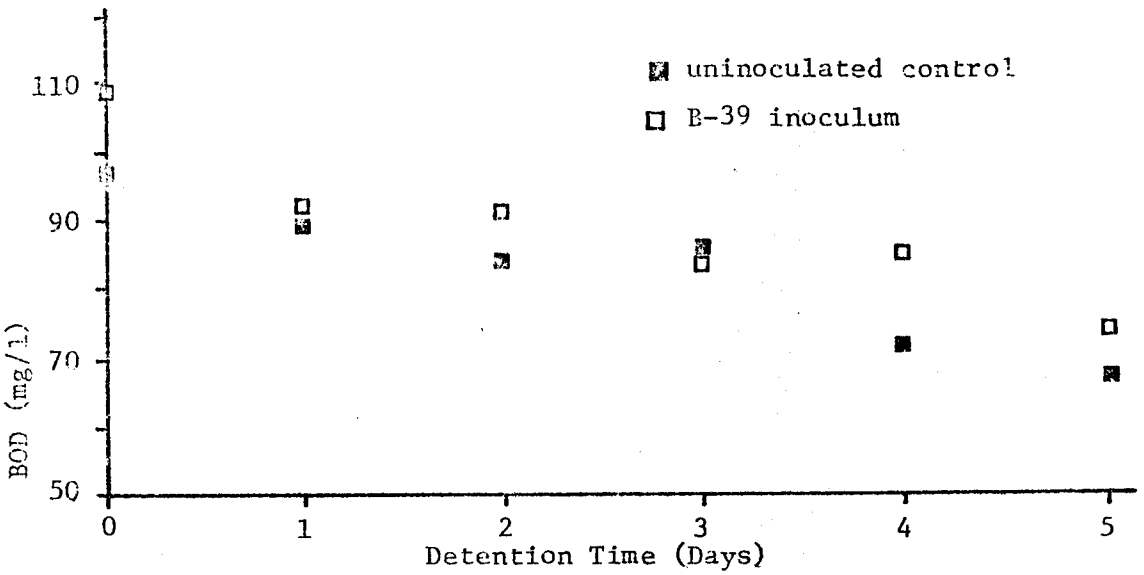


Figure 5. A plot of BOD in treated and untreated raw settled sewage at 5 C expressed as a function of time.

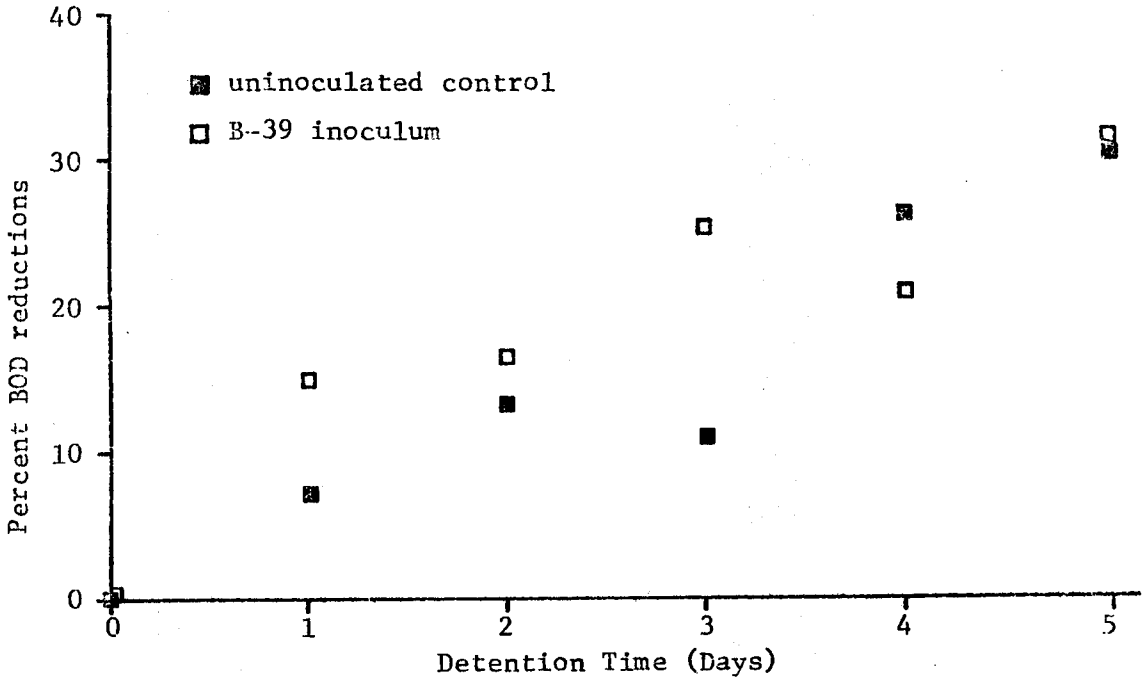


Figure 6. A plot of percent BOD reductions in treated and untreated raw settled sewage at 5 C expressed as a function of time.

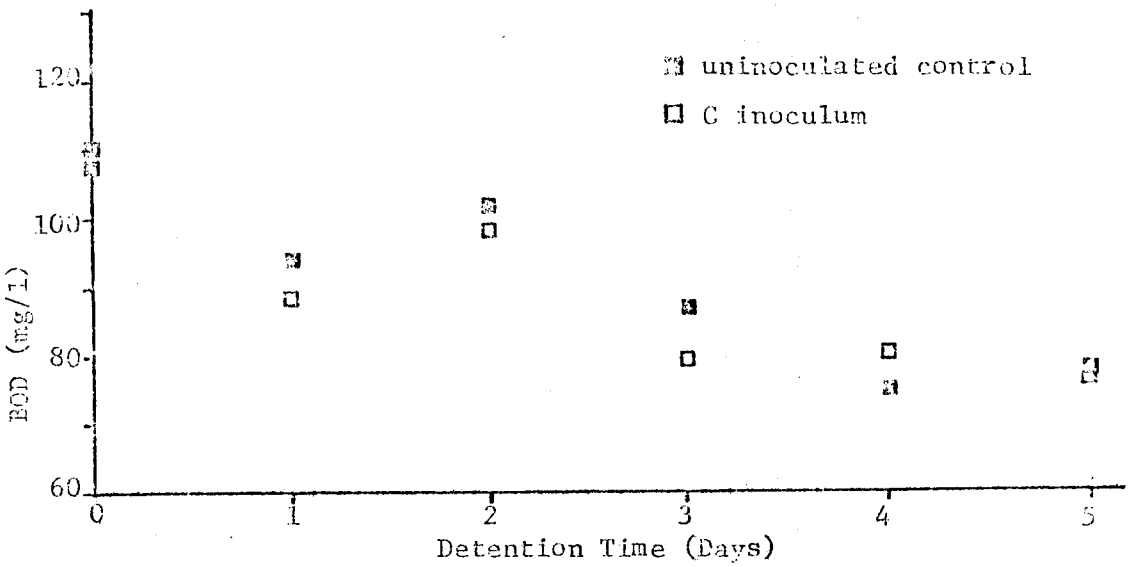


Figure 7. A plot of BOD in treated and untreated raw settled sewage at 5 C expressed as a function of time.

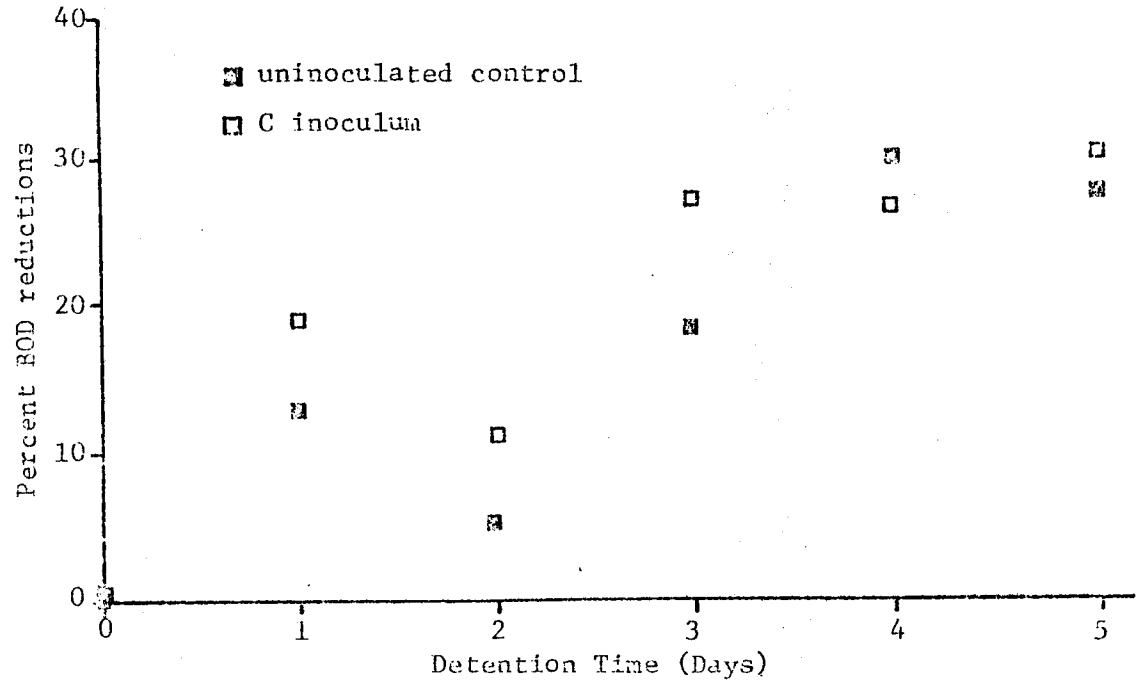


Figure 8. A plot of percent BOD reductions in treated and untreated raw settled sewage at 5 C expressed as a function of time.

Table 18. Initial BOD, change in BOD, and percent BOD removed for the raw sewage control (no inoculum) at 5, 10, 15, and 20 C.

Temp. (°C)	Initial BOD		Change in BOD		Percent BOD removed	
5	125.0	190.0	62.5	62.0	50.0	32.6
	140.0	240.0	32.5	136.0	23.2	56.8
	180.0	155.0	98.0	39.0	54.4	25.1
	240.0	185.0	124.0	65.0	51.7	35.1
	165.0	164.0	49.0	92.0	29.7	56.1
	115.0	222.9	43.0	134.9	37.4	60.5
	(176.8)*		(78.2)		(42.7)	
10	195.0	130.0	101.0	94.0	51.8	72.3
	175.0	205.0	95.0	165.0	54.3	80.5
	175.0	175.0	141.0	121.0	80.6	69.1
	165.0	190.0	105.0	150.0	63.6	79.0
	175.0	265.0	147.0	189.0	84.0	71.3
	190.0	195.0	148.0	205.0	77.9	69.5
	(194.6)		(138.4)		(71.2)	
15	150.0	200.0	104.0	104.0	69.3	74.0
	70.0	205.0	28.0	161.0	40.0	78.5
	170.0	140.0	122.0	115.0	71.8	82.1
	210.0	227.5	143.3	169.2	68.2	74.4
	160.0	275.0	106.0	213.0	66.2	77.4
	(180.8)		(131.0)		(70.2)	
20	350.0	330.0	298.0	301.7	85.1	91.4
	300.0	230.0	272.0	174.0	90.7	75.6
	579.0	260.0	561.0	216.0	96.7	83.1
	186.8	145.0	154.8	115.0	87.7	79.4
	180.0	165.0	142.0	115.0	78.9	69.7
	210.0	235.0	158.0	171.0	75.1	72.8
	(264.2)		(223.2)		(81.8)	

*(Average)

Table 19. Percent BOD removals from raw sewage at 2 C using no inoculum, a mixed (mesophilic) inoculation, and a psychrophilic inoculum.

Replications	Inoculum Type					
	None		Mixed		Psychrophilic	
1	24.4 23.0	(23.7)*	15.9 17.4	(16.7)	23.7 27.4	(25.6)
2	13.0 9.4	(11.2)	10.7 12.4	(11.5)	15.9 25.1	(20.5)
3	17.8 15.6	(16.7)	5.9 5.9	(5.9)	23.6 14.9	(19.3)
4	17.7 7.2	(12.5)	7.6 9.6	(8.6)	12.5 14.6	(13.5)
5	2.6 2.6	(2.6)	2.8 2.8	(2.8)	18.7 14.7	(16.7)
6	17.8 22.2	(20.0)	24.8 24.8	(24.8)	22.9 20.8	(21.9)
7	0.0 1.7	(0.9)	6.9 2.2	(4.5)	8.1 8.1	(8.1)
8	0.1 5.1	(2.6)	5.1 19.8	(12.5)	14.7 16.7	(15.8)
9	29.8 29.8	(29.8)	25.7 22.1	(28.6)	17.7 19.8	(18.8)
10	25.4 31.8	(28.6)	22.6 34.4	(28.6)	34.9 31.0	(33.0)
Sums	279.3	(14.0)	296.8	(14.8)	385.9	(19.3)

*(Average)

Table 20. Analysis of variance for the effect that a psychrophilic, a mesophilic, and no inoculum have on BOD removals from raw sewage within 5 hr at 2 C.

Source	df	Sum Squares	Mean Squares	F
Total	59	5,145.98		
Treatments (Inoculum)	2	327.12	163.56	6.55***
Replications	9	3,625.19	402.80	16.12***
Residual	48	1,193.67	24.87	

Significance levels: * 5.0%
** 2.5%
*** 0.5%

and 7. Each point on the graph was the arithmetic average of three experimental determinations. The trend exhibited by both inoculated psychrophiles and indigenous flora present in the uninoculated raw sewage was generally that of a gradual decline. The exception to this can be seen in Figure 3 where the BOD decreased up through the first three days of incubation then increased for the remaining two days. Differences between the BODs of inoculated and uninoculated systems were slight.

Percent BOD reductions represented in Figures 4, 6, and 8 indicate a trend toward increasing removals with increasing periods of detention. However, at no time during the five day incubation did any of the systems examined approach reductions to the 80 percent level, the minimum usually required. Differences between percent BOD reductions of inoculated and uninoculated systems were also slight as could be expected in view of the fact that differences between their BOD values were small.

Psychrophilic cell growth in the raw settled sewage substrate is described in Figures 9 through 11 for both inoculated microorganisms and those naturally present in the uninoculated sewage.

Table 21. Initial and final BOD concentrations (mg/1) raw sewage with and without inocula.

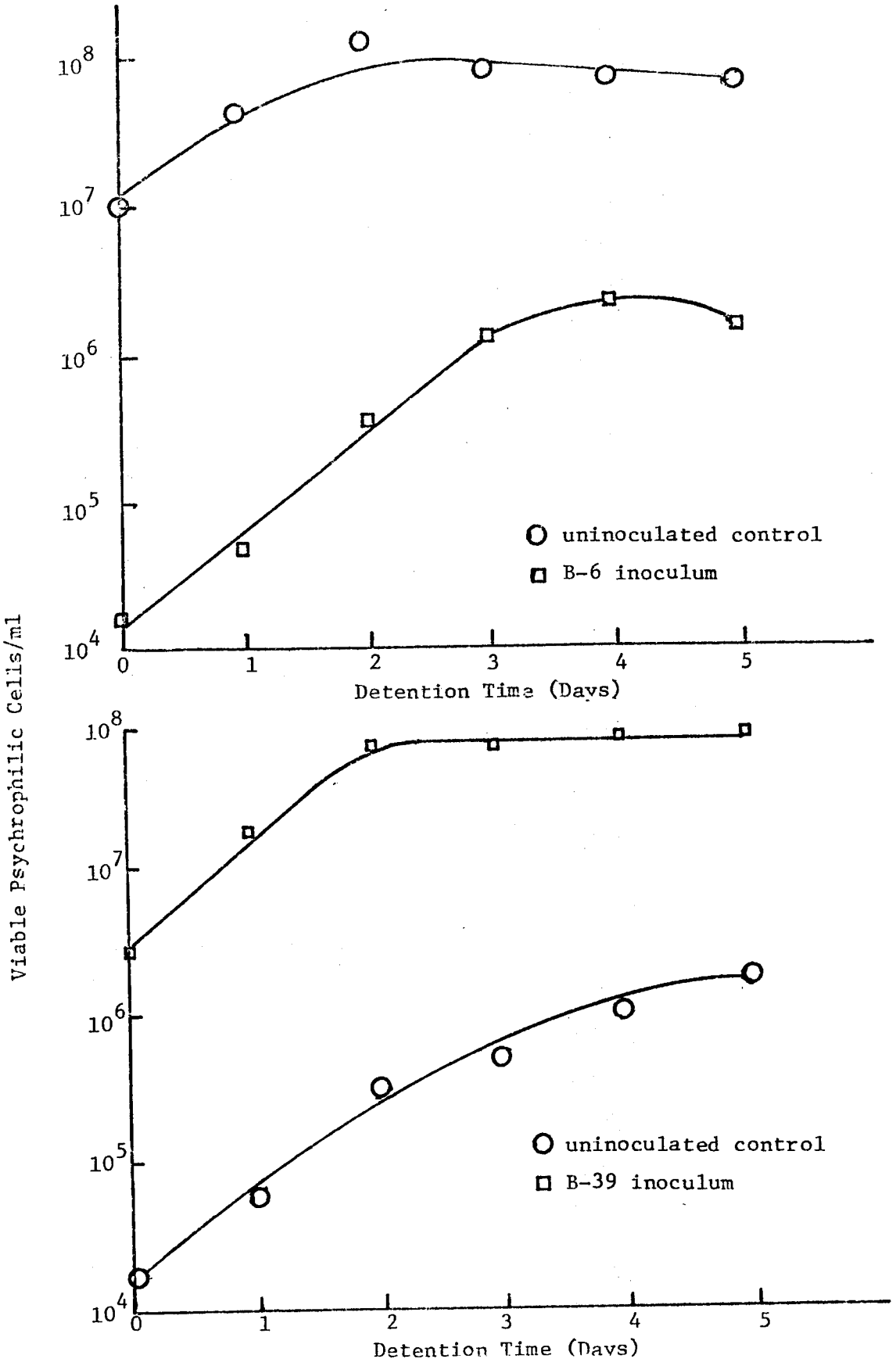
Mixed Inoculum		No Inoculum		Psychrophilic Inoculum	
Initial	Final	Initial	Final	Initial	Final
170	160 160	154	127 130	192	147 163
166	153 150	158	130 146	160	140 137
144	140 140	130	127 127	168	137 143
164	123 123	150	123 117	160	123 127
136	127 133	156	183 153	156	143 143
158	150 127	144	143 137	172	147 143
154	197 167	210	157 143	256	167 177
230	193 190	238	183 180	271	207 197
194	173 170	184	160 167	218	183 163
184	137 142	152	107 107	158	130 127
(180)	(153)	(168)	(142)	(191)	(152)

Figure 9

Viable psychrophilic cell number in treated and untreated raw settled sewage at 5 C expressed as a function of time.

Figure 10

Viable psychrophilic cell number in treated and untreated raw settled sewage at 5 C expressed as a function of time.


Viable Psychrophilic Cells/ml
10^8
10^7
10^6
10^5
10^4
0
1
2
3
4
5
Detention Time (Days)
uninoculated control
B-6 inoculum
10^8
10^7
10^6
10^5
10^4
0
1
2
3
4
5
Detention Time (Days)
uninoculated control
B-39 inoculum

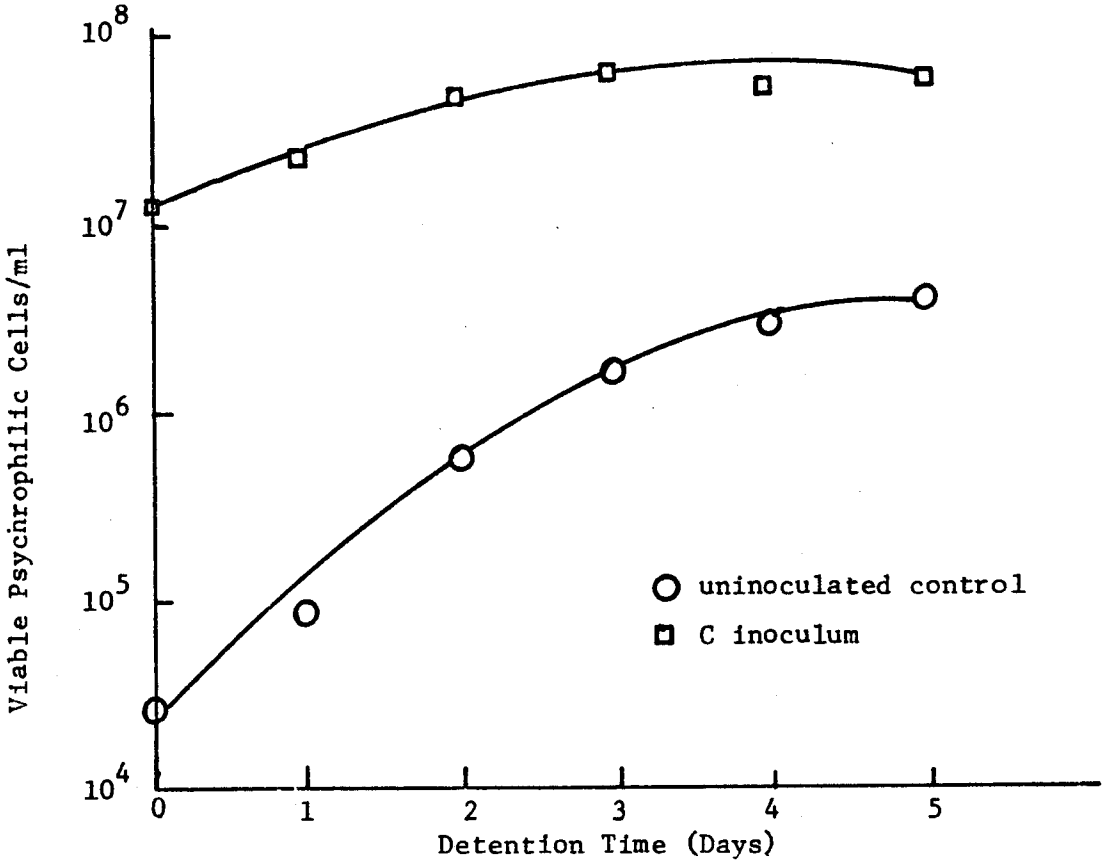


Figure 11. Viable psychrophilic cell number in treated and untreated raw settled sewage at 5 C expressed as a function of time.

These counts were made simultaneously with BOD determinations in an effort to correlate cell growth with BOD removals. All inoculated psychrophiles exhibited slow exponential growth for the first two days of detention, after which the cell numbers became stationary. However, with the exception of the uninoculated system shown in Figure 9, the psychrophilic cells indigenous to raw sewage never reached the stationary phase of growth. As could be expected, the indigenous flora of the uninoculated sewage was also found to be present in much lower numbers than inoculated psychrophiles. Viable cell numbers of three experimental runs were averaged to obtain each graphical value.

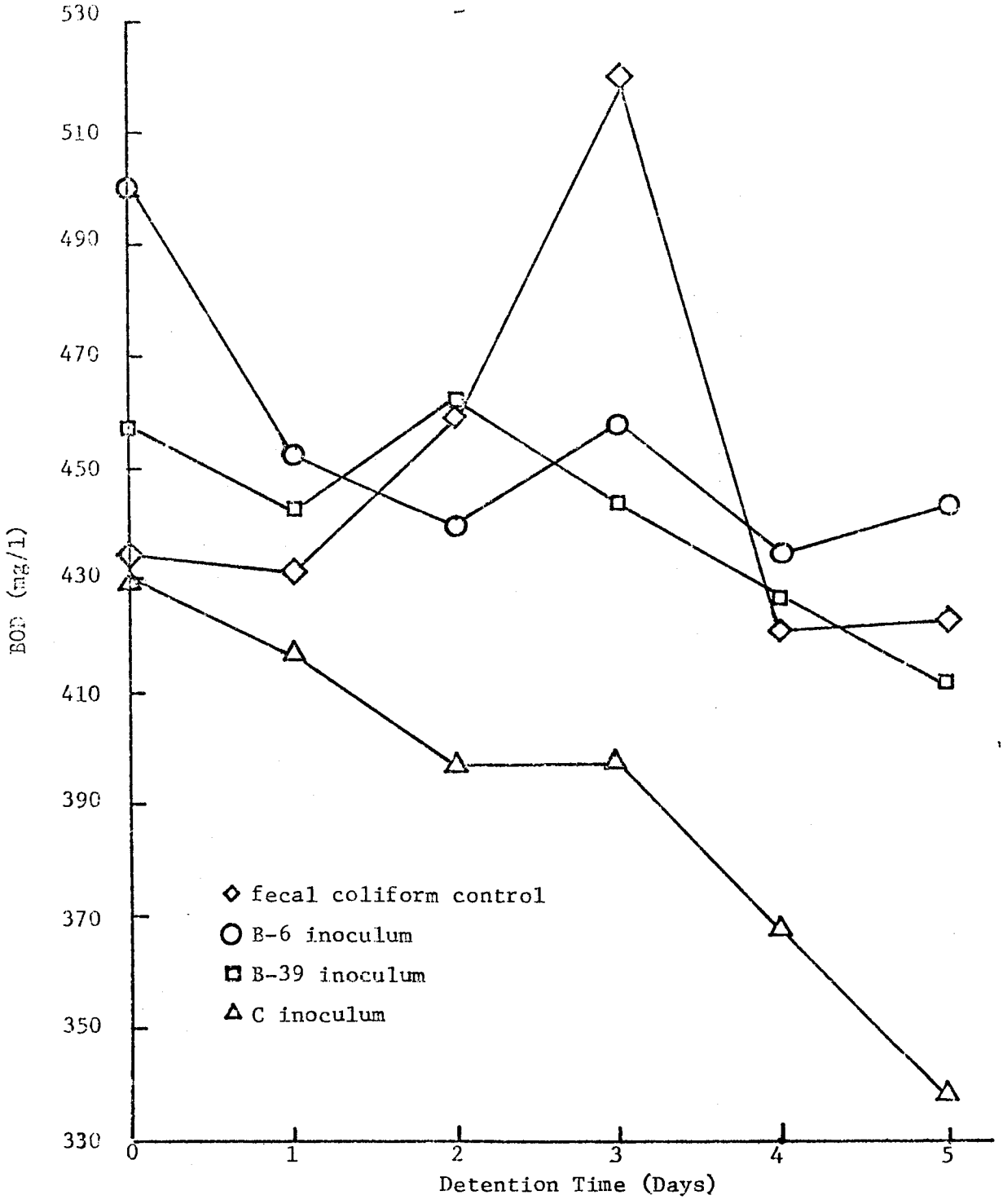


Figure 12. The effects of inoculated microorganisms on the BOD of concentrated synthetic sewage at 5 C expressed as a function of time.

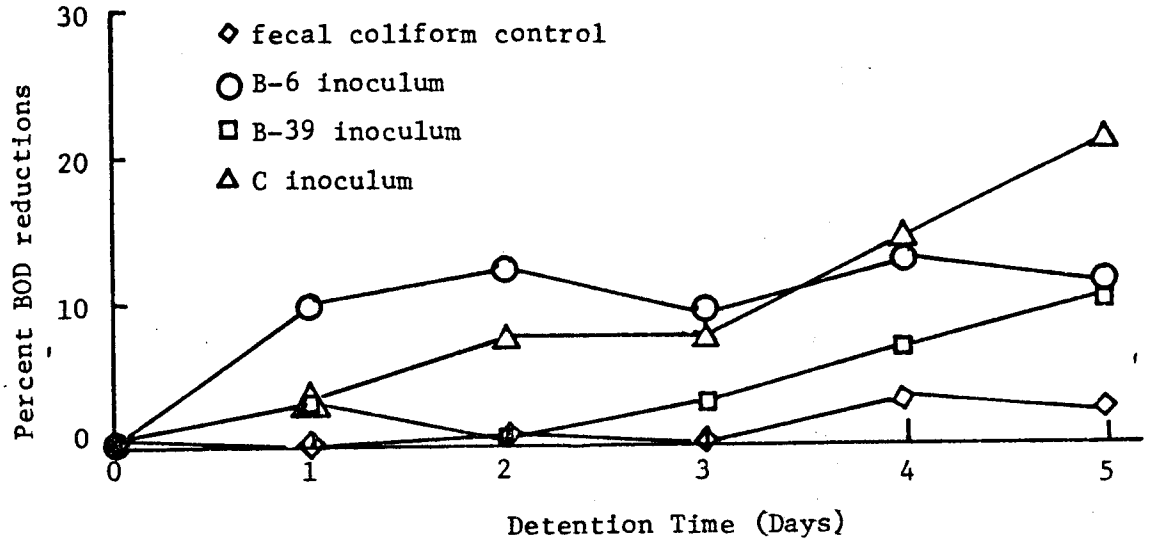


Figure 13. The effects of inoculated microorganisms on percent BOD reductions in concentrated synthetic sewage at 5 C expressed as a function of time.

III. Comparison of the ability of Alaskan psychrophilic isolates to stabilize a concentrated synthetic sewage, measured by BOD and percent BOD reduction, with the stabilization ability of a known mesophile (fecal coliform) in the same substrate.

The effects of inoculated psychrophiles on the BOD of a concentrated synthetic sewage as compared to effects exerted by a fecal coliform inoculated in like manner as a mesophilic control are presented in Figure 12.

Systems inoculated with organisms B-39 and C show trends toward decreasing BOD values. The system inoculated with organism B-6 exhibited a relatively rapid reduction of BOD within the first two days of holding after which very little appears to have been removed. The fecal coliform system demonstrated rather extreme increases in BOD at the second and third days of detention; however, after the third day of detention the BOD dropped back to slightly below initial values. As expected, the trend exhibited by the fecal coliform did not appear to be one of a decreasing BOD.

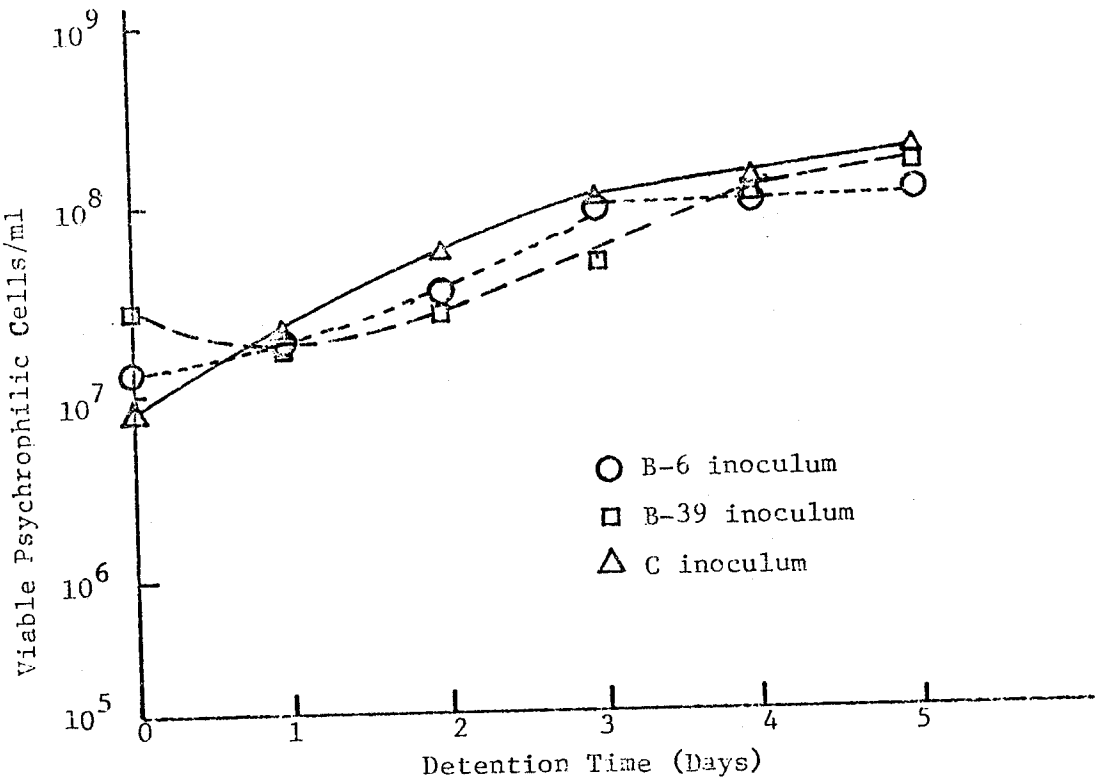


Figure 14. Viable psychrophilic cell number in treated and untreated concentrated synthetic sewage at 5 C expressed as a function of time.

Figure 13 gives percent BOD reduction in the psychrophilic and mesophilic systems. Data contained in this Figure indicates that proportionately less reductions in BOD were obtained in the concentrated synthetic sewage by each psychrophile than in the raw settled sewage substrate previously described, although more absolute BOD was removed by each organism in the synthetic system as shown in Figure 12. Percent BOD reductions in the mesophilic fecal coliform system were very low grade, never exceeding 3 percent.

Viable psychrophilic cell numbers in the concentrated substrate are given in Figure 14. These curves resemble the curves produced by each psychrophile in the raw settled sewage in that cell numbers increased only slightly more than one log unit over the five day

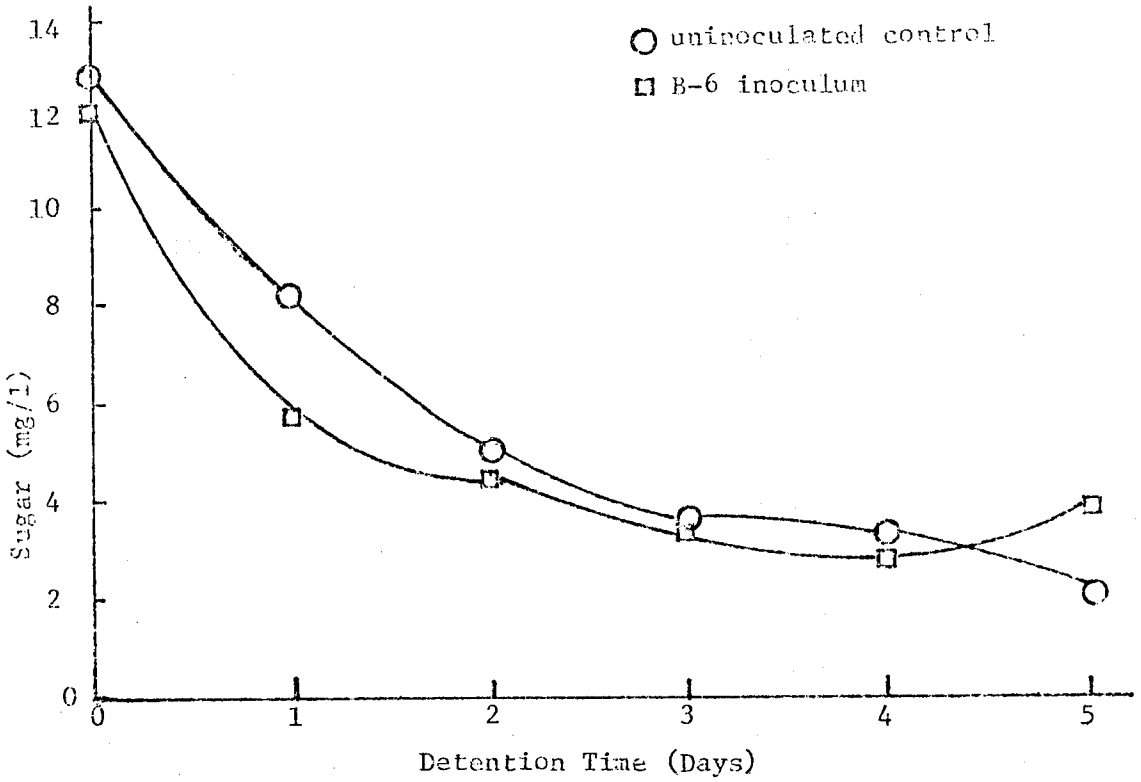
period of incubation and cell growth was very slow. However, organisms B-39 and C were still increasing in number at the end of the five day period as contrasted to their growth in raw settled sewage where they reached the stationary phase of growth in two days. Organism B-6 reached the stationary phase of growth in three days in the concentrated synthetic sewage as compared to two days required to attain the same stage of growth in the raw settled sewage. Since the fecal coliform was mesophilic in nature, no growth was obtained in the flasks containing this organism due to the low incubation temperature and for this reason Figure 14 contains no data for this organism.

IV. Comparison of the effects of inoculated Alaskan psychrophiles on the chemical and physical composition of the wastewater substrate with effects exhibited by the raw sewage's indigenous flora.

In order to determine what effects were being exerted upon the chemical and physical composition of the wastewater by inoculated psychrophilic microbes and psychrophiles naturally present in the raw sewage, analyses for carbohydrates in the form of sugar, nitrate-nitrite nitrogen, and orthophosphate were done in the incubation milieu at daily intervals over a five day period. Viscosity determinations were also made initially and at the end of the five day detention.

Uptake of sugar by psychrophilic organisms is indicated in Figures 15 and 16. These figures indicate that sugars are rapidly removed from the raw sewage by both inoculated and indigenous psychrophiles. Inoculated psychrophiles seem to remove the sugar faster than the natural psychrophilic flora of the raw sewage within the first one to two days detention; after that differences between inoculated and uninoculated systems become insignificant. The results expressed in these figures are averaged data from three separate analyses, as were the numerical values obtained in the nitrate-nitrite nitrogen analysis, the analysis for orthophosphate and viscosity determinations.

Table 22-A lists the results of the analysis for nitrate-nitrite nitrogen. The data indicates that very little variation occurred in each system over the five day detention period. Neither inoculated psychrophiles nor indigenous psychrophiles appear to have exerted


uninoculated control
B-6 inoculum
Sugar (mg/l)
Detention Time (Days)

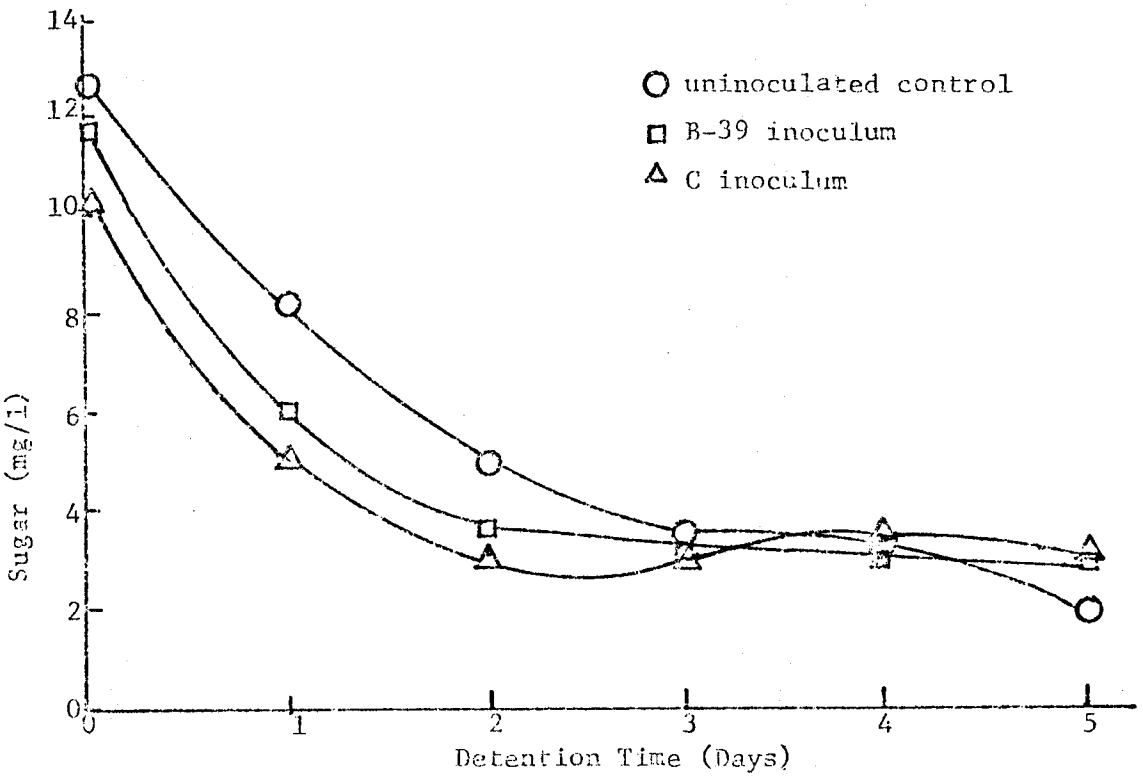

uninoculated control
B-39 inoculum
C inoculum
Sugar (mg/l)
Detention Time (Days)

Table 22. Analysis of treated sewage effluents

A. Nitrate & Nitrite Nitrogen (mg/l)

Detention Time (days)	Treatment			
	B-6	B-39	C	Raw Sewage Control
0	0.86	0.85	0.82	0.88
1	0.85	0.79	0.90	0.84
2	0.92	0.78	0.87	0.83
3	0.86	0.78	0.85	0.90
4	0.87	0.85	0.87	0.79
5	0.82	0.81	0.85	0.86

B. Orthophosphate (mg/l)

Detention Time (days)	Treatment			
	B-6	B-39	C	Raw Sewage Control
0	4.0	4.0	4.0	4.1
1	3.6	3.9	3.7	4.4
2	2.9	3.0	2.9	3.7
3	3.5	3.2	3.3	3.7
4	3.1	3.7	3.3	3.5
5	3.8	3.6	3.4	3.5

C. Viscosity (centipoise*)

Detention Time (days)	Treatment			
	B-6	B-39	C	Raw Sewage Control
0	0.98	1.00	1.00	0.99
5	1.02	1.01	1.02	1.02

*centipoise = 10^{-2} (gram mass)/(cm)(sec)

any effect upon the concentration of the nitrate-nitrite nitrogen present in the wastewater.

Concentrations of orthophosphate found in the systems treated by psychrophilic inoculation and in the uninoculated raw sewage control over a five day period are given in Table 22-B. There appears to be a slight uptake of orthophosphate by inoculated psychrophiles in the first two days of holding, but after the second day orthophosphate levels in the filtered effluent began to increase on the whole, over the given five day contact time, a substantial alteration of orthophosphate concentration apparently did not occur. Slight losses of orthophosphate in the uninoculated raw sewage were suggestive of a very low grade uptake by the sewage's natural flora, but once again the reduction in orthophosphate concentration was so small that it cannot be considered significant.

Changes in viscosity of the wastewater substrate are tabulated in Table 22-C. Slight increases in viscosity were observed in all systems studied. The viscosity in all systems studied, including sewage inoculated with treatment psychrophiles and uninoculated sewage maintained as a control, fell within the $\pm$ one percent error inherent in the instrument.

V. Comparison between growth rates and oxygen uptake rates of the three local psychrophilic isolates in heat sterilized sewage.

Figure 17 shows the Arrhenius plots of growth rates in sterilized sewage versus the inverse of temperature (^{o}K) for the three psychrophilic isolates. Isolates T28B and XVI-4 showed optimum growth rates between 15 and 25 C, but the optimum rate of growth for XXIV-1 could not be determined as there was no decline in growth rate, within the range plotted, as temperature increased. The figure shows that T28B was only slightly affected by decreasing the temperature from 10 to 5 C, but a corresponding temperature drop affected the other isolates greatly.

The Arrhenius plots of oxygen uptake rates in sterilized sewage by the three psychrophilic isolates grown in sterilized sewage supplemented with 1/5 m-PCB for less than 1 day are shown in Figure 18.

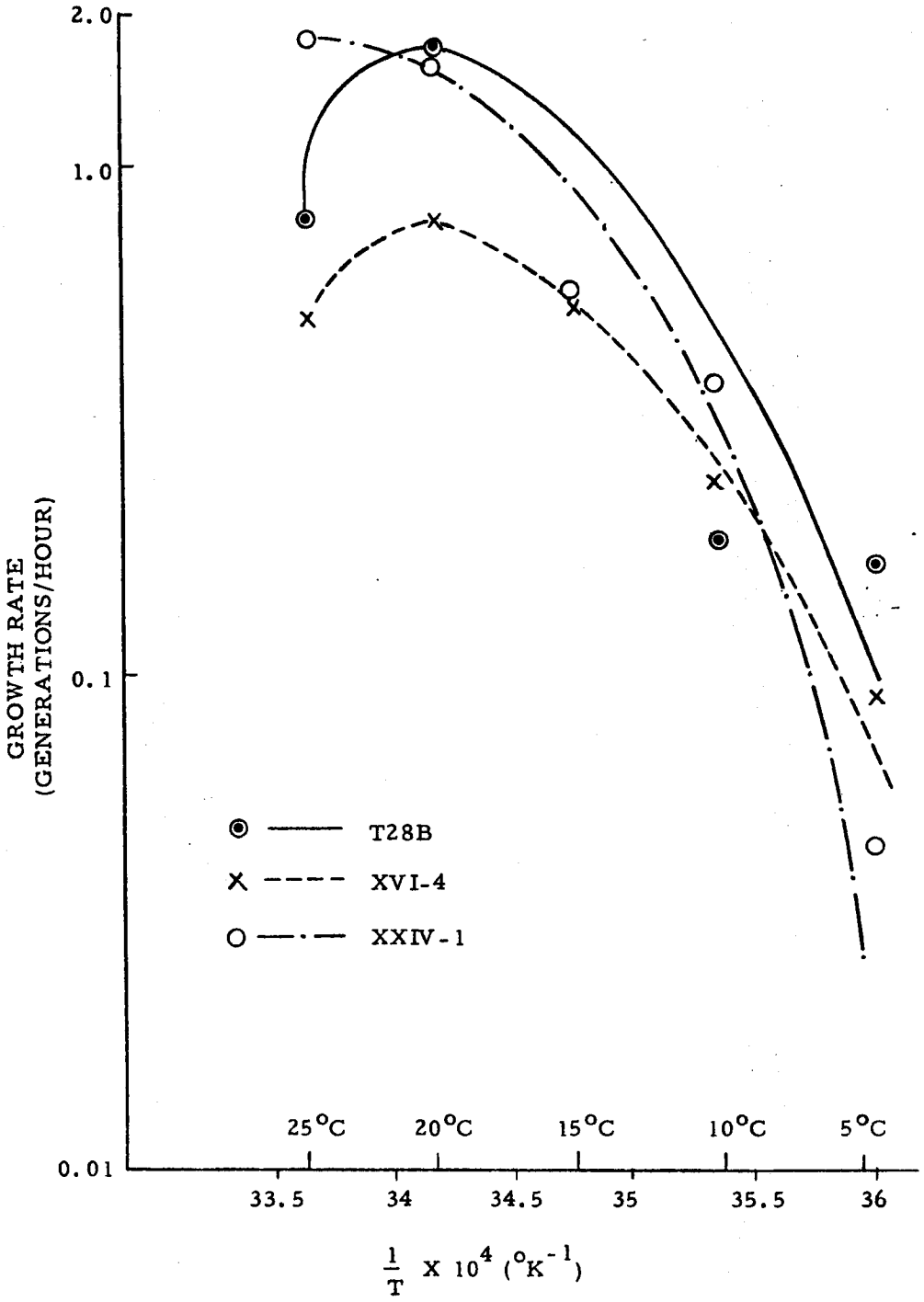


Figure 17. Arrhenius plots of growth rates (generations/hour) in sterilized sewage versus the inverse of absolute temperature for the three psychrophilic isolates.

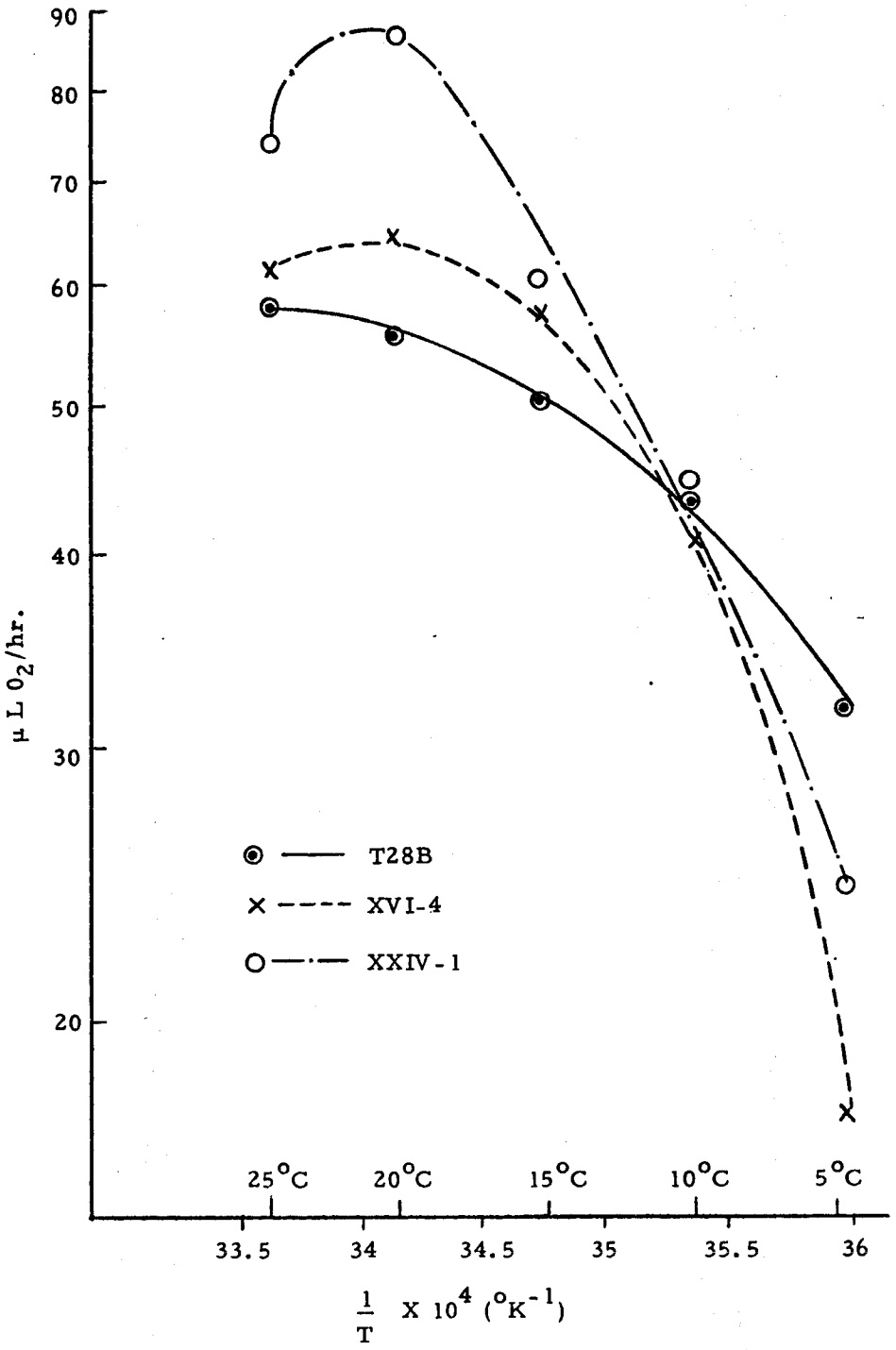


Figure 18. Arrhenius plot of oxygen uptake rates in sterilized sewage versus the inverse of absolute temperature for the three psychrophilic isolates grown for 1 day in sterilized sewage supplemented with 1/5 m-PCB(Difco).

The plot showed linearity only for isolate XXIV-1, and the plot was linear, with a negative slope, only between 5 and 20 C; at 25 C, the uptake rate was less than that at 20 C. Isolate T28B was the only isolate which showed no decline in uptake rate, within the range plotted, as temperature increased. Although T28B showed no decline in uptake rate at 25 C, its rate was still less than corresponding rates of XXIV-1 and XVI-4, but T28B had the greatest uptake rate at 5 C.

Table 23 shows that, with few exceptions, oxygen uptake rates by 1-day cultures were greater than uptake rates by 7-day cultures and that uptake rates by cultures grown in sterilized sewage supplemented with 1/5 m-PCB was greater than uptake by cultures grown in 1/2 m-PCB only.

Figure 19 shows oxygen uptake, at 5 and 20 C, versus time for 24-hr cultures of the three isolates grown in 1/2 m-PCB, and Figure 20 shows oxygen uptake, at 5 and 20 C, versus time for 7-day cultures of the isolates grown in sterilized sewage supplemented with 1/5 m-PCB. These figures were representative of oxygen uptake data in general.

VI. The effect of pH on the growth and oxidative ability of the Alaskan isolates in sterile sewage supplemented with 0.05 percent yeast extract.

The effects of pH on the growth of treatment organisms B-6, B-39, and C are given graphically in Figures 21, 23, and 25, respectively. Results given in these figures indicate that all of these organisms grow best in a pH range of approximately 6.8 to 8.6. The effects of pH on the oxidative ability of each psychrophile (given in Figures 22, 24, and 26) yielded largely the same results. A good correlation was observed between pH ranges at which maximal growth densities and substrate oxidation occurred for each psychrophile studied. The variation observed in different determinations of the same test for each bacterium was presumably due to fluctuations in the constituents of different sewage samples utilized by each organism as substrate. Three consecutive experimental determinations were plotted for Figures 21 through 26 with the exception of Figure 25 which contains the data obtained in only two consecutive experimental runs.

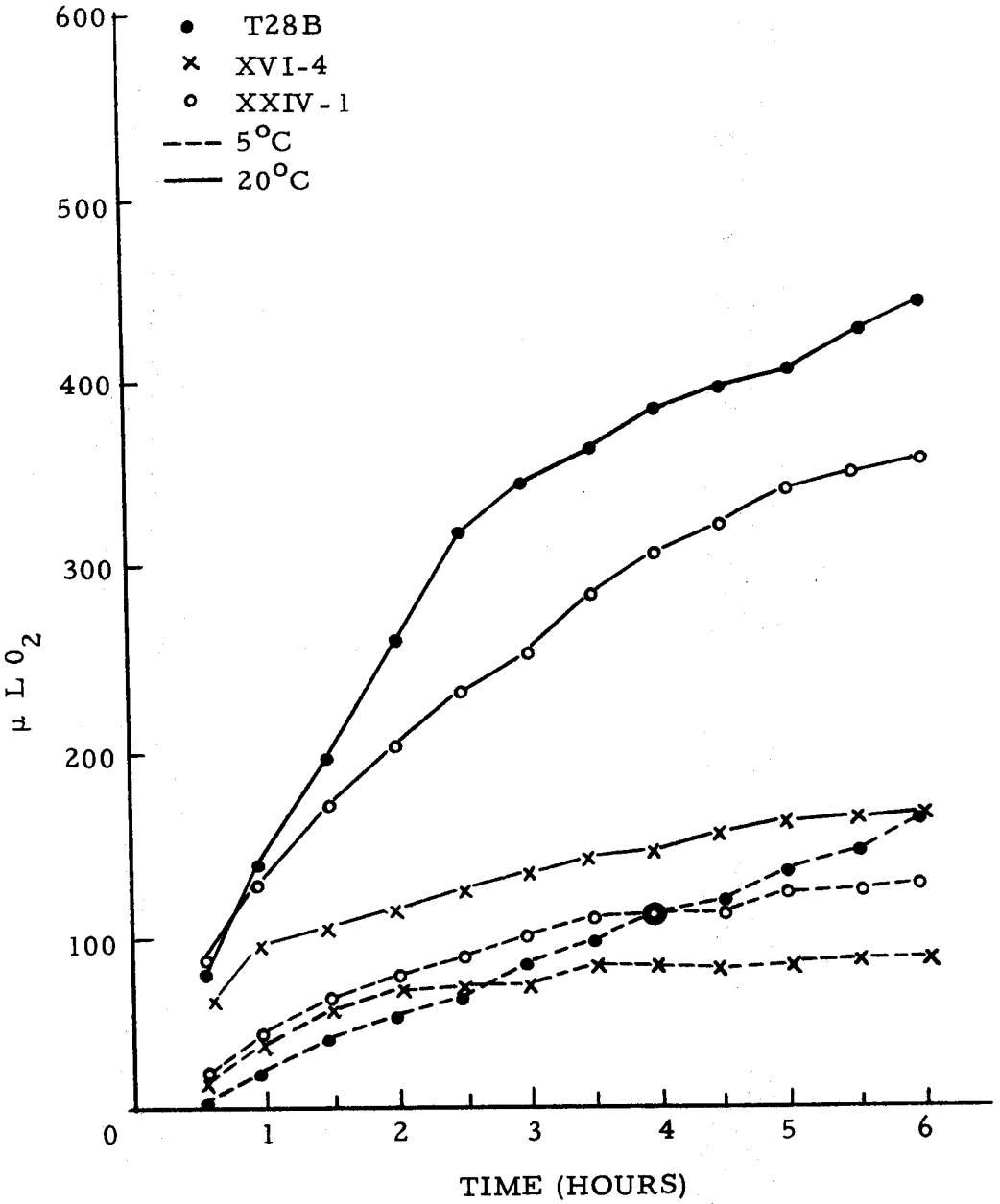


Figure 19. Oxygen uptake at 5 and 20 C in sterilized sewage by the psychrophilic isolates grown for 1 day in ½ m-PCB(Difco).

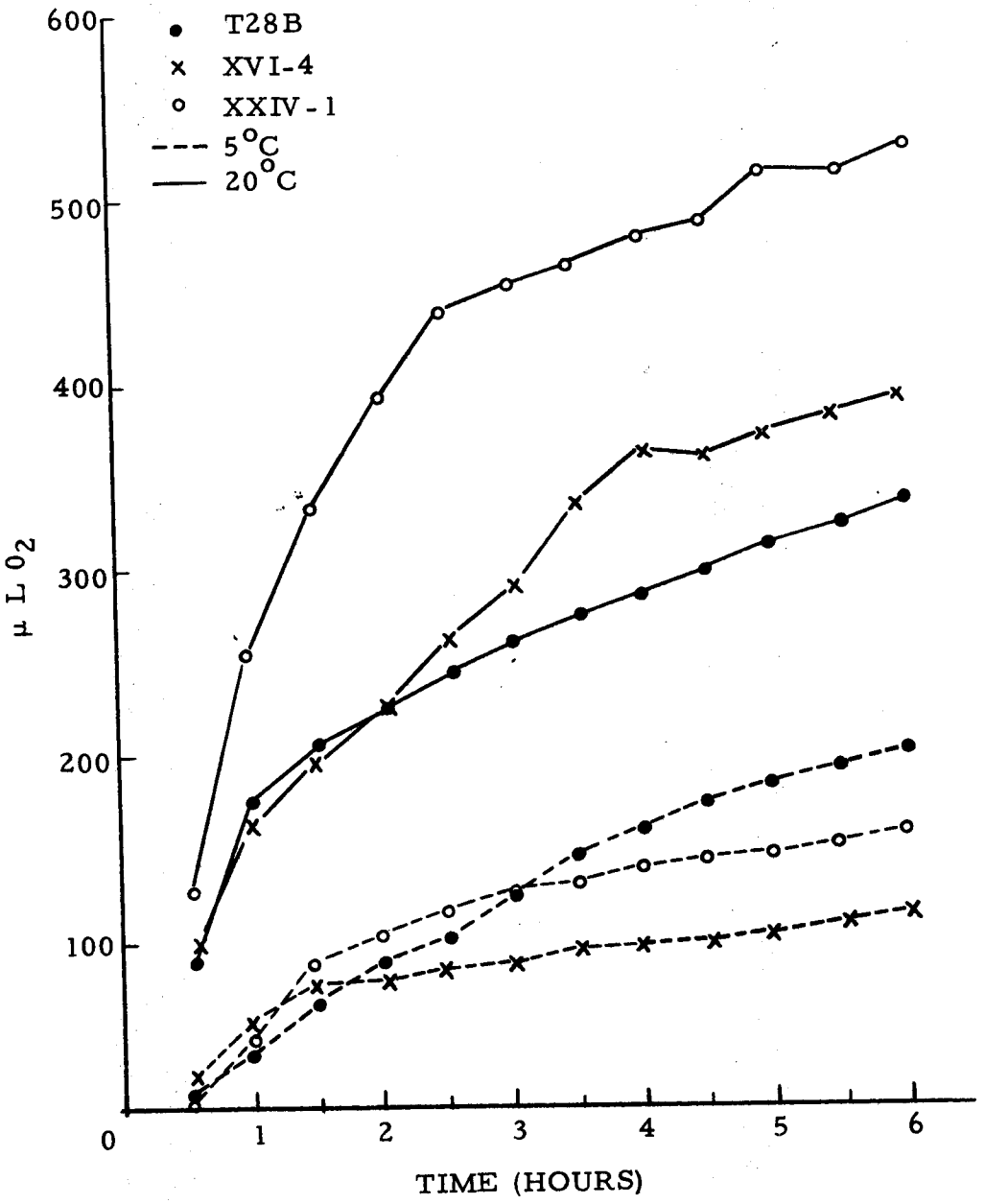
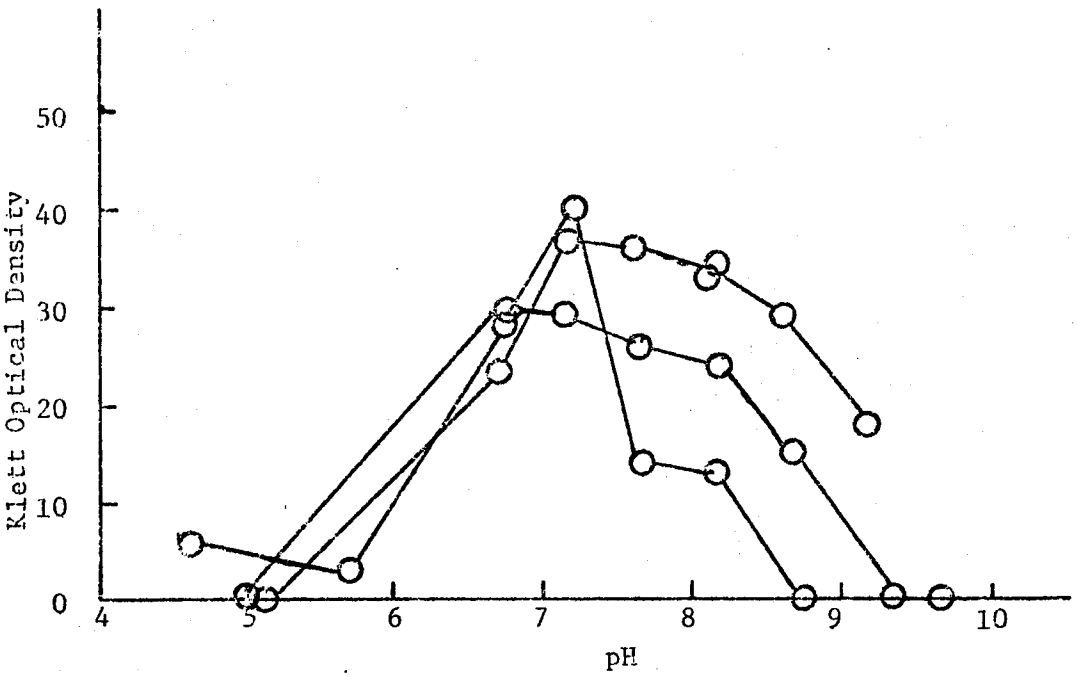
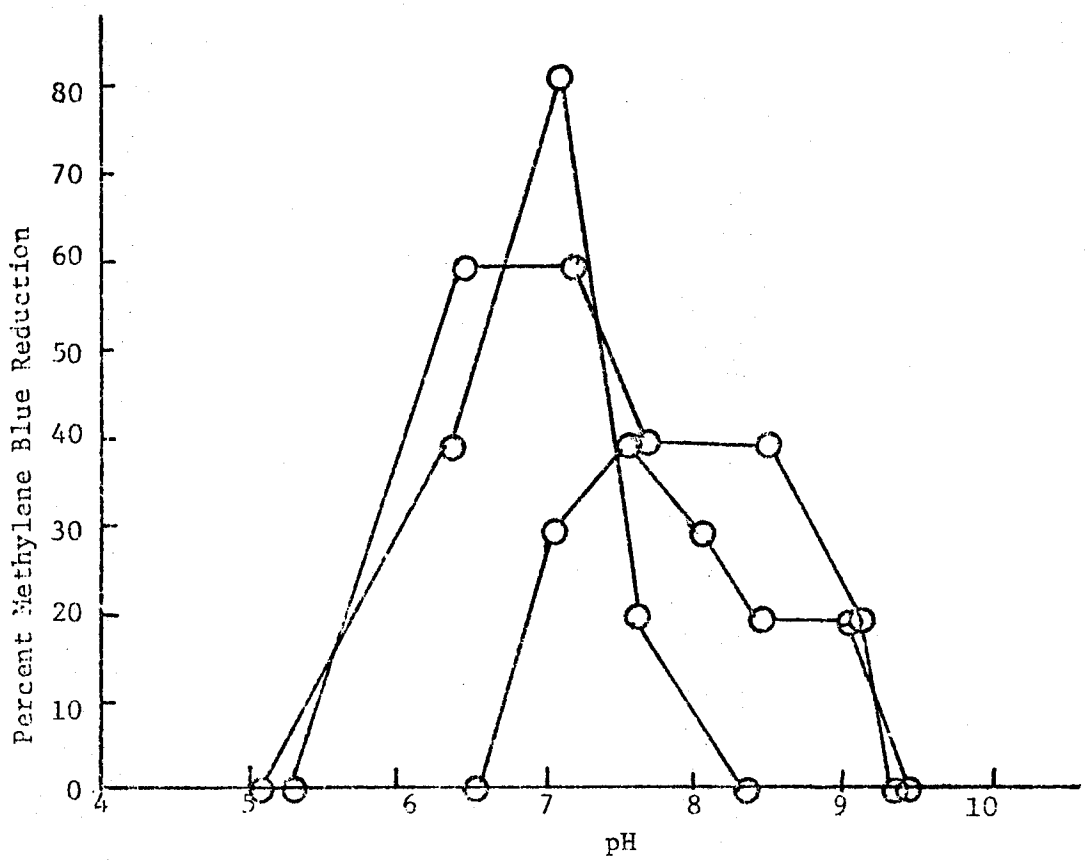


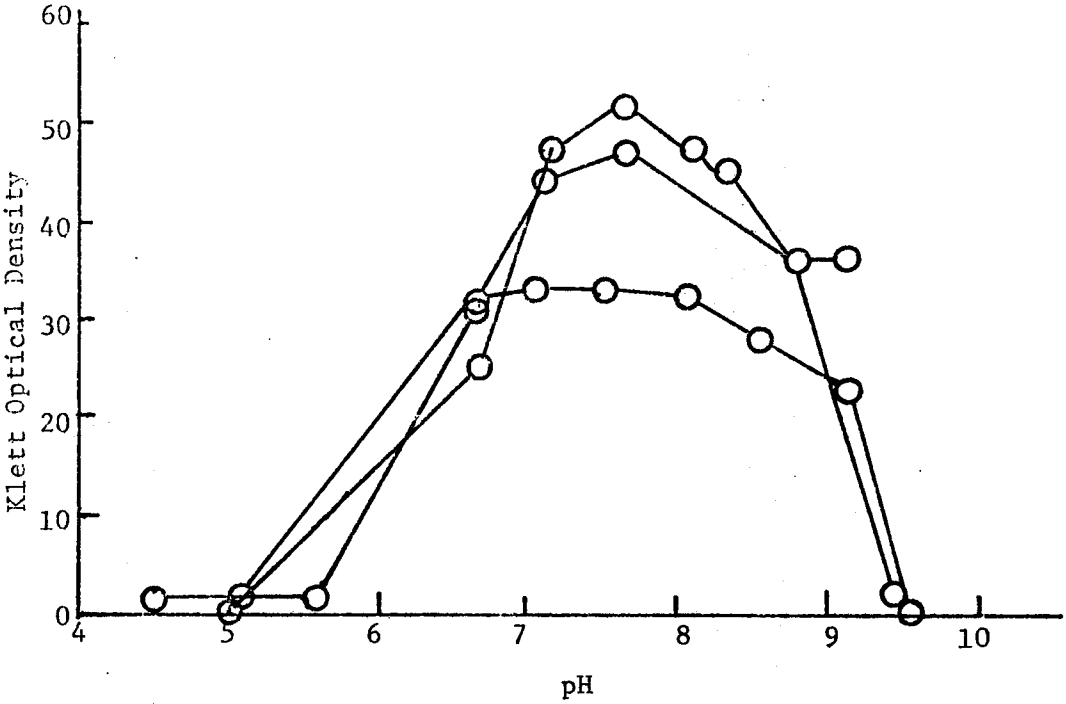
Figure 20. Oxygen uptake at 5 and 20 C in sterilized sewage by the psychrophilic isolates grown for 1 day in sterilized sewage supplemented with 1/5 m-PCB(Difco).

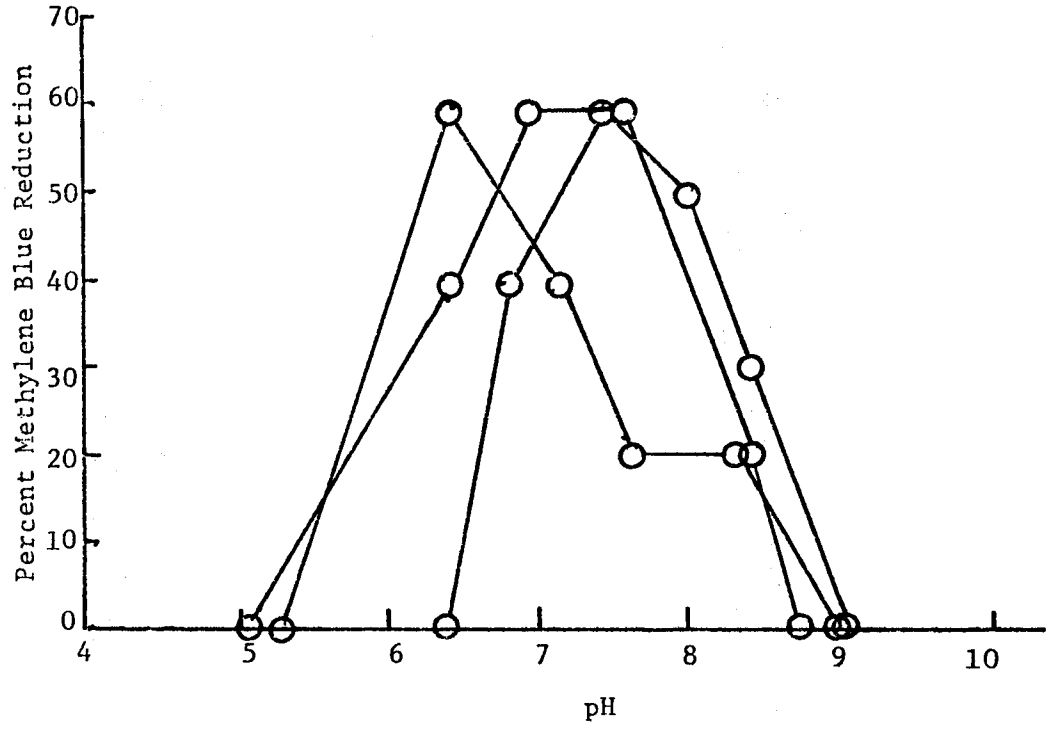
Table 23. Oxygen uptake at 10 C for XVI-4 cultured for 1 and 7 days in sterilized sewage supplemented with 1/5 m-PCB.

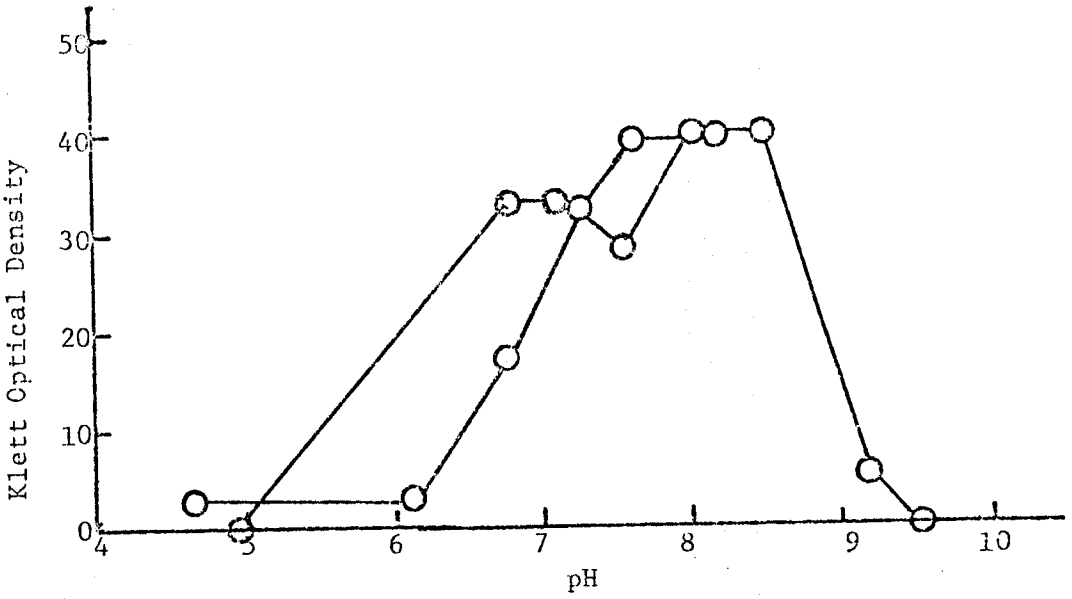
Uptake by 1-day Cultures ($\mu L\ O_2$)	Time (min)	Uptake by 7-day Cultures ($\mu L\ O_2$)
45.5	30	2.1
75.5	60	2.7
114.2	90	5.5
125.4	120	11.1
152.0	150	14.7
163.3	180	23.5
184.3	210	27.8
196.6	240	28.5
207.0	270	27.9
219.9	300	37.4
230.0	330	35.0
243.8	360	39.8


Klett Optical Density
0
10
20
30
40
50
4
5
6
7
8
9
10
pH


Percent Methylene Blue Reduction
0
10
20
30
40
50
60
70
80
4
5
6
7
8
9
10
pH


Klett Optical Density
60
50
40
30
20
10
0
4
5
6
7
8
9
10
pH


Percent Methylene Blue Reduction
70
60
50
40
30
20
10
0
4
5
6
7
8
9
10
pH


Klett Optical Density
50
40
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10
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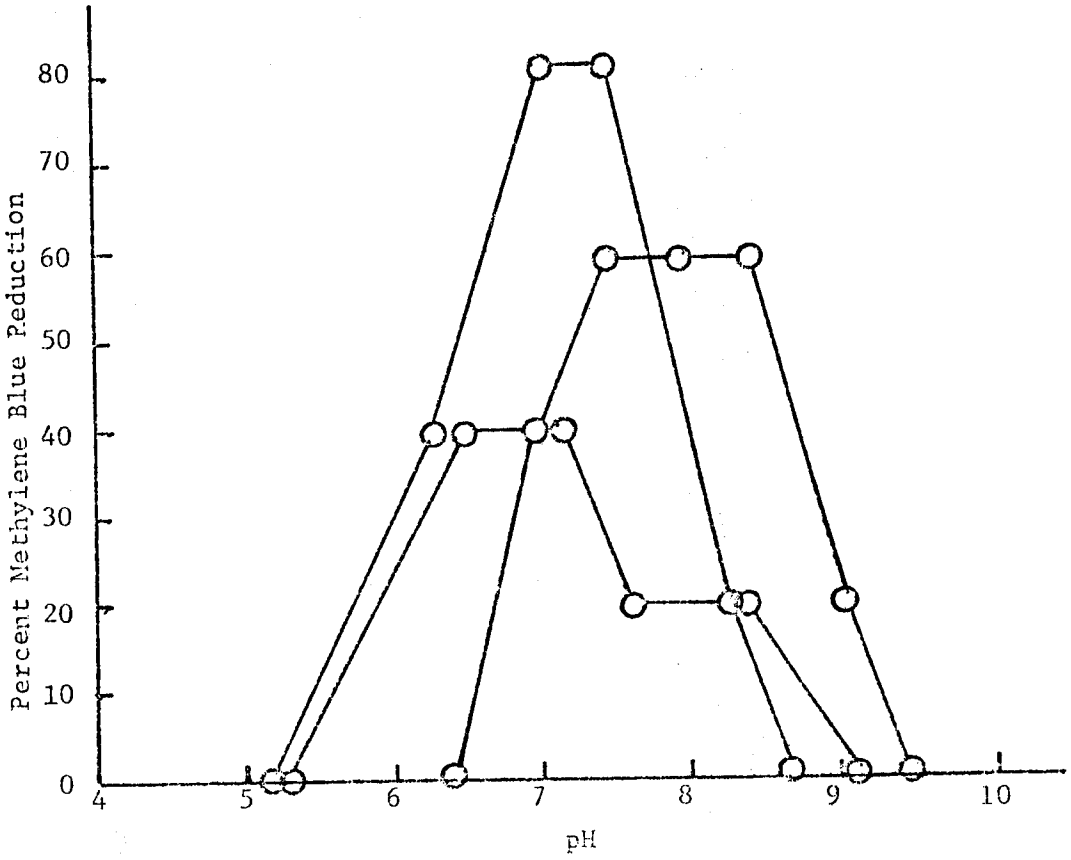
4
5
6
7
8
9
10
pH


Percent Methylene Blue Reduction
80
70
60
50
40
30
20
10
0
4
5
6
7
8
9
10
pH

DISCUSSION

Many contributions have been made in the field of pollution control, but one area which has been noticeably lacking in development is that of low temperature wastewater treatment. If population centers located in areas which commonly experience long periods of cold weather are to meet effluent standards imposed by governmental agencies, some method of adequate sewage treatment at low temperatures will have to be found. Although several approaches to the solution of this problem are available, the one investigated in this study was a biological approach, i.e., the use of inocula of psychrophilic bacteria to degrade raw domestic sewage. If a psychrophilic inoculum could be used to reduce oxygen demands to practical levels in a relatively short period of time on a laboratory scale, the potential for adapting similar organisms for use in conventional treatment systems in cold climates would be very good.

Although psychrophiles were used to remove BOD from sewage, they were initially selected on the basis of their relative growth rates in a nutrient medium rather than in sewage. Since the temperature characteristic of growth is independent of the growth medium (42), if all growth requirements were present in sewage, relative growth should be the same in sewage as it is in a nutrient medium. Isolates with relatively high growth rates in a nutrient medium should also have relatively high growth rates in sewage.

Many of the locally collected psychrophilic isolates were irradiated with UV in an attempt to increase growth rates of mutant strains at low temperature. Although conversions from psychrophily to mesophily (90, 91, 92), and from mesophily to psychrophily (5, 67) have been reported, the basis for such conversions have been changes in optimal or minimal growth temperatures rather than changes in growth or metabolic rates. Therefore, reported conversions from mesophily to psychrophily do not, necessarily, imply an accompanying increased rate of growth at a specific low temperature.

Whereas shifts in minimal and optimal temperatures of growth may result from minor changes in cell wall composition or in temperature sensitivity of an essential enzyme, changes in growth rates within

the range between growth optimum and minimum likely involve changes in more than one cellular system. Mutations could cause minor alterations resulting in shifts in optimal and minimal growth temperatures, but mutagenic treatment probably would not cause the many beneficial alterations necessary for increasing growth rates at low temperature without also causing a lethal mutation. The UV irradiation of the psychrophilic isolates did not yield any mutants with faster growth rates at low temperatures, possibly reflecting the difficulty of creating such a mutant. However, only preliminary efforts were expended toward obtaining mutants and more intensive work would have to be done before the phenomenon of increasing growth rates at low temperature could be definitely supported or refuted.

Following the results, the remaining discussion will be presented under the following headings:

I. Local isolates in sterilized sewage.
II. Raw sewage degradation.
    A. By local isolates.
    B. By Alaskan isolates.
III. Alaskan isolates in concentrated synthetic sewage.
IV. Chemical analysis of effluent after treatment with Alaskan isolates.

I. Local isolates in sterilized sewage.

Of the five local isolates which grew relatively well in sterilized sewage, two that were isolated from different environments were chosen to be used in these experiments and studies. A third isolate was chosen because it was a soil isolate selected for its relatively rapid oxygen uptake rate at 10 C. These three isolates were then used in the subsequent experiments and studies in raw and sterilized sewage.

Because the culture age of the inocula and the number of cells inoculated would be relatively easy to control and could influence BOD removals, the effects of these two treatments, as well as the effects of temperature and specific isolates used as inocula, were studied. Since each of the variables within the four treatments may have an influence on other treatments' variables, it was also necessary

to study the effects that the treatments had on each other. To minimize the effects of extraneous variables which were not measurable, heat sterilized sewage was the medium used in these preliminary experiments.

The complexity of determining the effects of treatments on each other made it necessary to utilize statistics to evaluate the significance of the treatments and treatment interactions. A treatment or treatment interaction was considered to influence BOD removals if it was statistically significant at the 0.5% level.

A significant effect required that the variables within the treatment, or treatment interaction, gave BOD removals which varied among the others, and, although the statistical approach defined which treatments affected BOD removals, it did not disclose why the variables within treatments differed. The BOD removal data with which the analysis was determined, and not just the analysis of variance, should, thus, be inspected for biologically explainable phenomena.

In experiments using inocula in heat sterilized sewage, the single treatment effects of organisms used as inocula, size of inocula, age of inocula and temperature were statistically significant (Table 3). The reasons for their significance, however, were not discovered in the analysis and conclusions drawn from the single treatment data (Tables 4-7) could not be made without also analyzing their interactions with the other treatments.

On the basis of the organism treatment data alone (Table 4), one would conclude that organism XXIV-1 was the best to use as an inoculum. A significant organism-temperature interaction (Table 8), however, indicated that the effect of temperature on BOD removals differed among organisms, reflecting differences in temperature characteristics of growth or oxygen uptake among the different organisms. Organism XXIV-1 may have removed the greatest overall percent BOD, but, because changes in temperature affected each organism differently, T28B removed more at 5 C.

Since the temperature of actual treatment plants does not remain constant, the selection of an organism to be used as the inoculum should be made considering the changes in BOD removals as temperature varies,

rather than BOD removals at a single low temperature. If more data were available, empirical equations relating BOD removals to temperature could be formulated, and such formulas could be used to determine which inoculum would result in the maximum BOD removals, if reasonable predictions of the waste's temperatures could be made.

The influence of temperature on percent BOD removals observed in these experiments, using single culture inocula, was different than the reported slight effect on actual treatment plants at temperatures above 10 C but serious effects below 10 C (39, 48, 79). Because of the diversity of the waste's natural biota, adaptation to changes in temperature is possible; thus, in a temperature range in which the biota can adapt, treatment efficiencies should be similar. In experiments using single inocula in sterilized sewage, the BOD removals should parallel the effects of temperature on growth or metabolism. Because growth and oxygen uptake rates of the isolates decreased as temperature decreased from 20 C (Figures 1 and 2), percent BOD removals observed in these experiments also decreased as temperature decreased.

It was also observed (Table 5) that, although removals decreased as temperature decreased from 20 to 10 C, the removals at 5 C were nearly the same as those at 10 C, despite the fact that Arrhenius plots of growth and oxygen uptake rates continued to decrease. The reasons for the unexpectedly high observed percent removals at 5 C are not known.

As expected, the largest difference in BOD removals came from the size treatment (Table 6). It is well recognized that the rates of biochemical enzyme reactions are proportional to the enzyme concentration, if substrate is in excess (21). Although there was a hundredfold difference between the number of cells in the large and small inocula, rather than a hundredfold difference in BOD removed, there were less than one and one-half times as much percent BOD removed by the large than by the small inocula; consequently, percent BOD removals must also be influenced by factors other than the number of cells inoculated.

The BOD removal data includes both the enzymatic phenomenon as well as some non-biochemical processes. The BOD removed biologically is related to the concentration of enzymes present. Although there was a hundredfold difference in initial cell numbers between the two

inocula sizes, the relative difference in cell numbers, and presumably the same relative proportion of active enzymes, after the 24-hr incubation may have been considerably less. Since growth rates decrease as cell concentrations increase, the large inoculum would grow slower than the small inoculum, resulting in less than a hundredfold difference between large and small inocula's final bacterial concentration and an accompanying relative increase in BOD removed by the small inoculum.

A relative increase in BOD removed by small inocula would be even greater if the large inocula did not grow at all. In the raw sewage studies, discussed later, it was observed that an inoculum of cells increased the initial BOD (Table 21). This increase in BOD can only be accounted for by assuming that cells add to the BOD by dying and subsequently becoming utilizable nutrients. Normal sewage contains about 2×10^7 cells per ml (57) and there were about 6×10^6 cells per ml inoculated into the raw sewage. Assuming McGauhey's estimate to be correct, the final viable bacterial population in inoculated raw sewage was probably less than 2.6×10^7 cells per ml, as some of the cells were contributing to the increased BOD. In experiments with heat sterilized sewage, when the large inoculum was used, the sterilized sewage initially was inoculated with 2×10^7 cells per ml; consequently, growth of the large inoculum would be limited.

The non-biochemical removal of BOD is due to settling, chemical oxidation, and volatilization. Since flocculation was not observed for either size of inoculum, the amount of BOD removed by settling should be constant and independent of inoculum content. The BOD removed by chemical oxidation and other non-biochemical methods should also be constant and independent of inocula size. By percentage, the amount of BOD removed by these non-enzymatic processes would be a larger portion of the total BOD removed when a small bacterial load was present. Therefore, it is reasoned that the experimentally unexpected slight differences in BOD removed between large and small inocula were caused by: 1) growth of the cells after small numbers inoculation while the large inoculum's cells only exhibit slight growth and 2) the effects of non-biochemical forms of BOD removal which have a proportionately greater influence on the observed percent BOD removed by the small than on that removed by the large inoculum.

The reasons for relatively slight differences in BOD removed by 1 and 7-day old cultures (Table 7) were not disclosed until the temperature-age (Table 12) and temperature-size-age (Table 14) interaction data were inspected. Whereas the removals by the 1-day averaged over all other treatments were slightly greater than removals by the 7-day old cultures, 1-day old cultures removed less BOD at 5 C than did the 7-day (Table 12). It was observed, however, that only the small inocula of 1-day old organisms removed less BOD at 5 C than corresponding 7-day old cultures; large inocula of 1-day old cultures at 5 C and both large and small inocula of 1-day seed at 10, 15, and 20 C removed more BOD than corresponding 7-day old cultures (Table 14). Since the organisms were grown at room temperature, the observed influence of temperature on BOD removals by different culture ages and sizes of inocula might be explained on the basis of cold shock.

Many authors reported that "physiologically young" cells from late lag or early exponential phases were sensitive to abrupt temperature changes (82, 89). Epthimion and Corpse (22) reported, however, that growth phase susceptibility to cold shock differed among strains. While some strains were sensitive if cells were old, others were sensitive if cells were young, and in other cases, cells of any age of growth phase were insensitive to cold shock. Although Epthimion and Corpse (22) reported that cold shock susceptibility could occur in any phase, depending on the organism, small inocula of 1-day old cultures of all isolates removed less BOD than corresponding small inocula of 7-day old cultures at 5 C (Table 2). It was also observed that the mixed, mesophilic population was more susceptible to cold shock than any of the three psychrophilic strains, a finding consistent with Farrel and Rose (24).

Although cold shock would affect cells of both large and small inocula of 1-day old cultures, only the 1-day old small inocula were observed to be adversely affected by cold shock (Table 14). Because a substantial part of the BOD removed by the small inocula is due to an increase in cell numbers with an accompanying, though not necessarily proportional, increase in oxidation rate, any physiological stress, such as cold shock, which decreases the viability and growth capability of the inoculated cells would adversely affect the inoculum's degradative

abilities. Cold shock, which affects growth of 1-day old cells, would have little influence on the large, non-growing, inoculum's oxidation of sewage.

Cold shock was not a factor influencing BOD removals when large inocula were used or when the experimental temperature was greater than 5 C. When cold shock did not influence waste degradation, the 7-day old cultures removed less BOD than 1-day old cultures, possibly because there were probably fewer viable cells in 7-day cultures and because the old cells would be partially depleted of necessary enzymes due to their prolonged starvation.

Averaged over all treatments, the 1-day old cultures were more efficient than 7-day old cultures (Table 7), but, since cultures were grown at room temperature, small inocula of 7-day old cultures were less efficient than corresponding 1-day old cultures at 5 C (Table 14), because of the adverse effects of cold shock on growth of the cells after inoculation. On the basis of these results, if inocula of psychrophiles were employed to increase treatment efficiency at low temperatures, stationary phase cultures, rather than exponential growth phase cultures, should be used as relatively fewer cells are capable of continued active growth in sewage. This is fortunate, since it would be more economical to use a small inoculum and easier to harvest cells not in exponential growth phase, as careful monitoring of the cultures to determine growth phase of cells would not be practical.

Because microbial interactions, such as synergism, competition or inhibition (30) could influence microbial activities, combinations of the three psychrophilic isolates were used and their percent BOD removals were compared to the percent BOD removed by single culture inocula (Table 17). Although there were no significant experimental differences found between removal abilities of single cultures and combinations of cultures, the phenomenon is worth additional study using a larger selection of organisms.

Since experiments thus far discussed were performed in heat sterilized sewage, extending the results to raw sewage is questionable, because sterilization alters the chemical composition of the sewage

and destroys it biota. Studies in sterilized sewage were necessary, however, to minimize the effects of extraneous variables resulting from the chemical and biological differences among separate raw sewage samples. Although gamma irradiated (46) or filter-sterilized sewage would probably be more similar, chemically, to raw sewage, heat sterilization was used because irradiation and filtration were not feasible. To decrease variation caused by fluctuations in the composition of the sewage samples, raw sewage, sampled and frozen as collected over a period of approximately 3 months, was pooled at the end of the 3 months, frozen to preserve its characteristics (28) and heat sterilized as needed.

II. Raw sewage degradation.

A. By local isolates.

Due to limitations involved with using heat sterilized sewage, it was necessary to perform experiments using inocula of locally isolated psychrophiles in raw sewage. In such studies 19% of the BOD was removed within 5 hours at 2 C by the psychrophile-inoculated sewage, but 15% was removed with no inoculum used. Although the absolute difference (19% - 15% = 4%) appears rather insignificant, the relative difference (19% - 15%/15% = 27%) is quite appreciable. If however, retention times were of sufficient duration to permit 80-90% removals, the absolute difference between BOD by inoculated or non-inoculated raw sewage would increase, but it is likely that the relative difference would decrease.

Although the psychrophilic inocula removed more percent BOD than the mesophilic or non-inoculated sewage, with the 5-hr retention times used for this study, the final BOD concentrations were greater for the inoculated sewages than for the non-inoculated ones, because the inoculation of a cell mass added more BOD to the system than could be compensated for by the increased removal ability of a psychrophilic inoculum. If retention times were longer than 8.5 hr and the removal rates were linear with respect to time, the activities of the psychrophiles at low temperatures would more than compensate for the added BOD. Even if rates were non-linear, increasingly longer retention times should result in an increasingly

better effluent BOD for the psychrophile-inoculated sewage relative to the non-inoculated raw sewage. If sufficiently long retention times were used, the final BOD concentrations for psychrophile-inoculated raw sewage would be appreciably less than the final concentrations in non-inoculated raw sewage, and the "inocula" method of improving treatment efficiency at low temperature would appear practical.

Because the selection of the isolates to be used as inocula could be accomplished on the basis of either growth or oxygen uptake rates, studies were performed to determine which method of selection corresponded better with observed BOD removals. A comparison of Arrhenius plots of growth and oxygen uptake rates for the three isolates used (Figures 1-2) with the BOD removed by the isolates at different temperatures (Table 8), revealed that oxygen uptake rates closely corresponded to BOD removals, but growth rates corresponded poorly. Although two of the isolates used in this study were selected because they showed rapid growth at low temperatures, the evidence from these studies indicate that it is more valid to select isolates on the basis of oxygen uptake rather than on growth.

Cells used as inocula for oxygen uptake studies were grown in either ½ strength m-PCB or in sterilized sewage supplemented with 1/5 strength m-PCB, because it was thought that cells grown in a sewage medium would physiologically adapt to its nutrients and oxidize sewage faster than cells grown in a nutrient medium only. The sewage medium was supplemented with only 1/5 strength m-PCB to supply adequate growth factors, with minimal stimulatory additions of utilizable carbonaceous compounds, to initiate growth and ensure that the cells would physiologically adapt to sewage nutrients. Results of this study indicate that adaptation to sewage nutrients does occur at all temperatures, as cells grown in the sewage medium nearly always had greater oxygen uptake rates than had cells grown in the nutrient medium.

B. By Alaskan isolates.

Raw settled sewage inoculated with each of the three selected Alaskan-psychrophiles and incubated at 5 C over a five day period

indicated that BOD values were being reduced at a very slow rate (Figures 3, 5 & 7); however, there was very little variation between the BODs in the inoculated sewage and the uninoculated controls. Percent BOD reductions in Figures 4, 6 and 8 present a mirror image of the BOD values. All reductions were very low grade never exceeding approximately 30 percent. Paired t tests used to evaluate differences between treatments and controls in both BOD and percent BOD reductions indicated that, statistically, differences did not exist. Viable psychrophilic cell counts, made concurrently with BOD determinations (Figures 9, 10 & 11), indicated that a psychrophilic population of approximately 10^4 cells per ml was present in uninoculated sewage controls, a concentration equivalent to that reported to have been found in rivers (32). Flasks seeded with treatment cell suspensions initially contained about 1000 times more viable psychrophiles per ml than the uninoculated substrate. Accordingly, since both treatments and their respective controls contained psychrophilic populations, both would be expected to reduce BOD, but because seeded systems contained much denser populations, a more rapid rate of BOD reduction was expected in these flasks. This did not prove to be the case within the five day detention. Dias and Bhat (19) theorized that a single bacterial species would likely be limited in its ability to utilize organic substances that occur in sewage. This may be part of the reason that substantially greater BOD reductions in inoculated sewage were not observed over the uninoculated controls.

If the treatment organisms were able to utilize only a small portion of the organic constituents present in the sewage, a heavy inoculum would only result in a relatively rapid depletion of these materials, leaving most of the remaining carbonaceous compounds to be degraded by the varied sewage psychrophile population at their own rate. When the compounds utilized as substrate became limiting, the treatment cell numbers would become stationary, but because degradation would still be occurring by indigenous cells, the BOD would be expected to continue to drop. This is largely what was observed. Inoculated cell numbers became stationary within 2 days; yet the BOD continued to drop to the end of the five day incubation

at approximately the same rate as that observed in the uninoculated control. However, with the exception of the B-6 control, indigenous cell numbers did not become stationary within the same period. The rapid reduction expected in the growth-limiting nutrient during the two days that the cells were actively metabolizing and growing may not have been reflected in the BOD due to the natural sewage psychrophile population degrading a much wider range of carbon compounds.

III. Alaskan isolates in concentrated synthetic sewage.

Support for the limited-growth concept comes from a series of experiments using a synthetic sewage to determine the effects of substrate concentration at 5 C. Concentrating the sewage would increase the amounts of the nutrient components available to the inoculated cell mass and cell numbers would be expected to either not become stationary at all or become stationary at a later period. B-6 cell numbers became stationary one day later than in the raw settled sewage, and B-39 and C cell numbers never became stationary at all in the five day test period (Figure 14).

It is also interesting to note that bacterial activity in the concentrate generally resulted in a greater absolute BOD reduction (Figure 12) than in the natural sewage substrate, but proportionately less BOD was removed in the synthetic medium (Figure 13). The fecal coliform inoculum utilized as a control exhibited a behavior quite different from what was expected. The BOD of the medium containing the mesophile increased to a rather high level at three days detention, then rapidly dropped to initial BOD levels. Because fecal coliforms do not survive low temperatures well, the release of protoplasm into the medium by cell death and lysis could explain the BOD increase. However, the most penetrating question to answer is not,"Why did the BOD increase?",but rather,"Why did the BOD decrease?". There is no good apparent answer to this question. The reason could not be metabolic activity, so the only alternative is some unknown physical phenomenon.

The greatest difficulty in evaluating the results of the concentrated synthetic sewage experiments lies in the fact that the sewage

was artificial, and therefore, bacterial responses are almost certain to vary from those obtained in raw domestic sewage to which the cells were originally adapted.

IV. Chemical analysis of effluent after treatment with Alaskan isolates.

The analysis for sugar in the raw settled sewage, depicted in Figures 15 and 16, also lends support to the limited-growth hypothesis. This type of carbohydrate was utilized very rapidly by both the Alaskan psychrophilic isolates and those naturally present in the sewage. The greatest differences between sugar utilized by inoculated systems and their uninoculated controls occurred within the first two days of incubation at 5 C. Slightly over half of the existing sugar was depleted in inoculated systems in the first day of detention as compared to a little over one-third being removed by the uninoculated control. Although greater amounts of sugar were likewise removed in inoculated flasks during the second day, differences between treatments and controls were not as pronounced. Beyond two days of detention, differences between treatments and control were virtually nil. If the inocula cells became limited at the end of the two day period due to the lack of availability of essential nutrients, differences between their respective abilities to remove sugars and the ability of the uninoculated control to do the same would be expected to disappear. It is conceivable, but by no means certain, that the inocula psychrophiles may be capable of degrading only sugars as a carbon source.

The results of an analysis for nitrate-nitrite nitrogen (Table 22-A) indicate that no significant change or alteration occurred in amounts present during the five day contact period in the filtered effluent of either inoculated or control flasks. This conforms to the findings of earlier workers that psychrophiles are unable to either denitrify (12) or nitrify (14, 83) at low temperatures. The nitrogen source utilized by these organisms must obviously be in some form other than nitrate or nitrite, possibly ammonia.

Table 22-B lists concentrations of orthophosphate observed in the fluid filtrates of psychrophile inoculated and control flasks. The data obtained suggested that a low grade phosphate uptake at 5 C was occurring in the inoculated systems for the first two days of detention

but increases occurred in phosphate concentration beyond two days. The control exhibited what appeared to be a gradual uptake in phosphate at a much lower rate than observed in the inoculated systems during the first two days of incubation. The high phosphate value obtained in the control flask at the second day of detention is likely due to experimental error. Although the reason that phosphate concentrations increased in treated systems in the last three days of detention is uncertain, MacKelvie, Campbell and Gronlund (55) found that carbon limited Pseudomonas aeruginosa excreted both ammonia and ultraviolet absorbing materials into incubation medium. It is possible that nutrient limited cells may also release certain amounts of phosphate as well.

Many psychrophiles are characterized by their ability to produce storage polymers which greatly increase the viscosity of the medium in which the organism is being grown. An increased viscosity in raw sewage would produce the undesirable effect of holding matter in suspension and thereby rendering primary settling difficult at best. For this reason, viscosity measurements of the filtered wastewater in both inoculated and uninoculated sewage were made to determine whether or not the organisms being used would exert such a deleterious effect (Table 22-C). Initial as well as final values of viscosities in treatments and control all fell within the ± 1 percent error of the viscosimeter. Most findings regarding the psychrophilic production of these viscous polymers indicate that the molecule produced is often a polysaccharide such as dextran (25, 29); if such a substance were being produced it should have been detected in the analysis for sugar. The fact that increases in sugar content were not observed tends to support the finding of the viscosity data that significant increases did not occur in any of the systems examined.

The effects of pH on treatment cell growth and substrate oxidation are given in Figures 21-26. As expected, the data obtained suggests a definite correlation between oxidation and cell growth. Ranges of pH which resulted in the greatest amount of oxidation also provided for maximal rate of growth. All three treatment organisms appeared to function well at 5 C in the pH range of 7-8, well within values found in the raw sewage substrate. The variation observed between each of the three curves obtained for each test can be accounted for as being due to

differences in each sewage sample used as a substrate. The chemical composition of raw sewage varies greatly from sample to sample. Lamanna and Mallette (49) point out that for any given exzyme an optimal pH exists for its activity; this optima is not fixed, but changes with ". . . the nature and concentration of salts present." Undoubtedly, bacterial enzyme systems undergo the same fluctuation in optima with changes in surrounding chemical composition that individual enzymes do.

SUMMARY AND CONCLUSIONS

Bacterial isolates capable of growing near 0 C were used as inocula for experiments in heat sterilized, raw and concentrated synthetic sewage to determine whether BOD removals could be enhanced at low temperatures. Growth and oxygen uptake rates in sterilized sewage of these isolates were also determined as was a chemical analysis of raw sewage before and after treatment.

The percent BOD removals from heat sterilized sewage at temperatures ranging from 5 to 20 C by two culture ages (1 and 7 days) and two quantities of inocula (10^{10} and 10^{8} cells/200 ml) of the three psychrophilic isolates and of a mixed mesophilic population were analyzed by 4 X 4 X 2 X 2 factorial analysis of variance (Table 3). Culture age, quantity of cells inoculated, organisms inoculated, temperature, and many of the interactions among single treatments affected BOD removals.

One-day old cultures generally removed more BOD than 7-day old cultures (Table 4). Further inspection of the data revealed that 7-day cultures were more efficient only when the small inoculum was used (Table 14) or when at 5 C (Table 12). It was thought that these interactions of culture age with inoculum size and temperature were caused by the effects of cold shock on the inoculated cells.

The effect of number of cells inoculated on percent BOD removed was very significant, as seen by the large F value found in Table 3. The large (10^{10} cells) always removed more percent BOD than the small inoculum (10^{8} cells). Although the number of cells in the large inoculum was a hundredfold greater than the smaller, they removed only about one and a half times as much BOD as the small inoculum (Table 6). This relatively slight, though statistically significant, difference was thought to be caused by active growth of the cells of the small inoculum after inoculation while the cells of the large inoculum did not grow.

The differences in BOD removals among locally isolated organisms used as inocula were relatively slight, although statistically significant, probably because the organisms were psychrophiles having similar oxidative abilities. The isolates were affected differently

by changes in temperature (Table 8). As expected, the mesophilic population was more adversely affected by a decrease in temperature than the psychrophiles.

A decrease in temperature brought a general decrease in percent removals. It was observed, however, that the overall removals at 5 C were not much less than those at 10 C, although growth or oxygen uptake rates were much less at 5 C (Figures 17 and 18). It was suspected that, at the lower temperatures, non-biological removals of BOD (such as settling) were more significant than the oxidation of the organic wastes; thus, the adverse effect of low temperatures on biological oxidation rates were masked.

A comparison between BOD removed by single inocula and combinations of the single organisms revealed that combining the isolates did not increase oxidative abilities. Although this study did not show increased efficiency by using combinations of organisms as inocula, it is possible that synergisms and successive substrate utilizations by combinations of the appropriate bacteria could result in the oxidation of more substrate.

Because heat sterilized sewage is distinctly different from raw sewage, experiments were performed to determine whether inocula of psychrophiles could also improve BOD removal efficiency in raw sewage at low temperatures. The experiments showed that sewage inoculated with pure cultures of psychrophiles had approximately 19% of the BOD removed within 5 hr at 2 C, whereas the sewage inoculated with a mixed population of mesophiles had 15% removed and the raw sewage with no inoculum had 14% of the BOD removed. Although the inoculated sewages had the greatest percent BOD removed, they also had a higher final BOD, because BOD was added with the inoculum. Increased retention times might allow the more efficient psychrophile inoculated sewage to remove enough BOD and result in a lower absolute BOD concentration.

Low temperature stabilization of raw domestic wastewater already containing a heterogenous psychrophilic population is not substantially affected by the addition of a pure culture psychrophilic inoculum. Viable psychrophilic cell counts suggest that treatment psychrophiles became growth limited in two days at 5 C. Since the substrate apparently never became limited with respect to either nitrogen or phosphorus,

it seems likely that these organisms became carbon limited because they were only able to degrade a small portion of the non-carbohydrate organic matter present. Although the BOD itself is a carbon limited concept, its usefulness as a parameter of wastewater treatment efficiency is dependent upon a microbial population capable of oxidizing a wide range of organic compounds. Hydrogen ion concentrations apparently did not present a problem in the growth of the psychrophiles. Future studies into the psychrophilic degradation of wastewater might prove more fruitful if emphasis were placed on mixed culture inocula utilizing psychrophiles screened not only for rapid growth, but growth on a wide variety of carbon sources.

Oxygen uptakes in heat sterilized sewage were generally higher when cells were grown in a culture medium containing heat sterilized sewage rather than in ½ m-PCB. Uptakes by 1-day old cultures were greater than 7-day old cultures. Oxygen uptake rates by 1-day old cultures grown in a medium containing sewage more closely corresponded to BOD removals than did growth rates.

On the basis of these experiments it was concluded that:

1. Psychrophiles increased BOD removals at low temperatures, but it was also found that an inoculum increased the initial BOD. Long retention times will be needed to overcome the higher initial BOD and result in an acceptable effluent BOD.
2. Cultures in stationary growth phase were not susceptible to the effects of cold shock. Older cells would, therefore, probably make a better inoculum when added to a low temperature wastewater.
3. Because BOD removals more closely corresponded to oxygen uptake rates than to growth rates, the selection of psychrophiles to be used as inocula would probably be best if based on oxygen uptake rates at low temperatures rather than on growth rates.
4. The stabilization of wastewater by psychrophilic microorganisms is not effectively enhanced by concentrating the sewage substrate.

5. Future studies on psychrophilic degradation of domestic sewage should emphasize mixed culture inocula of organisms capable of growth on a wide variety of carbon sources.

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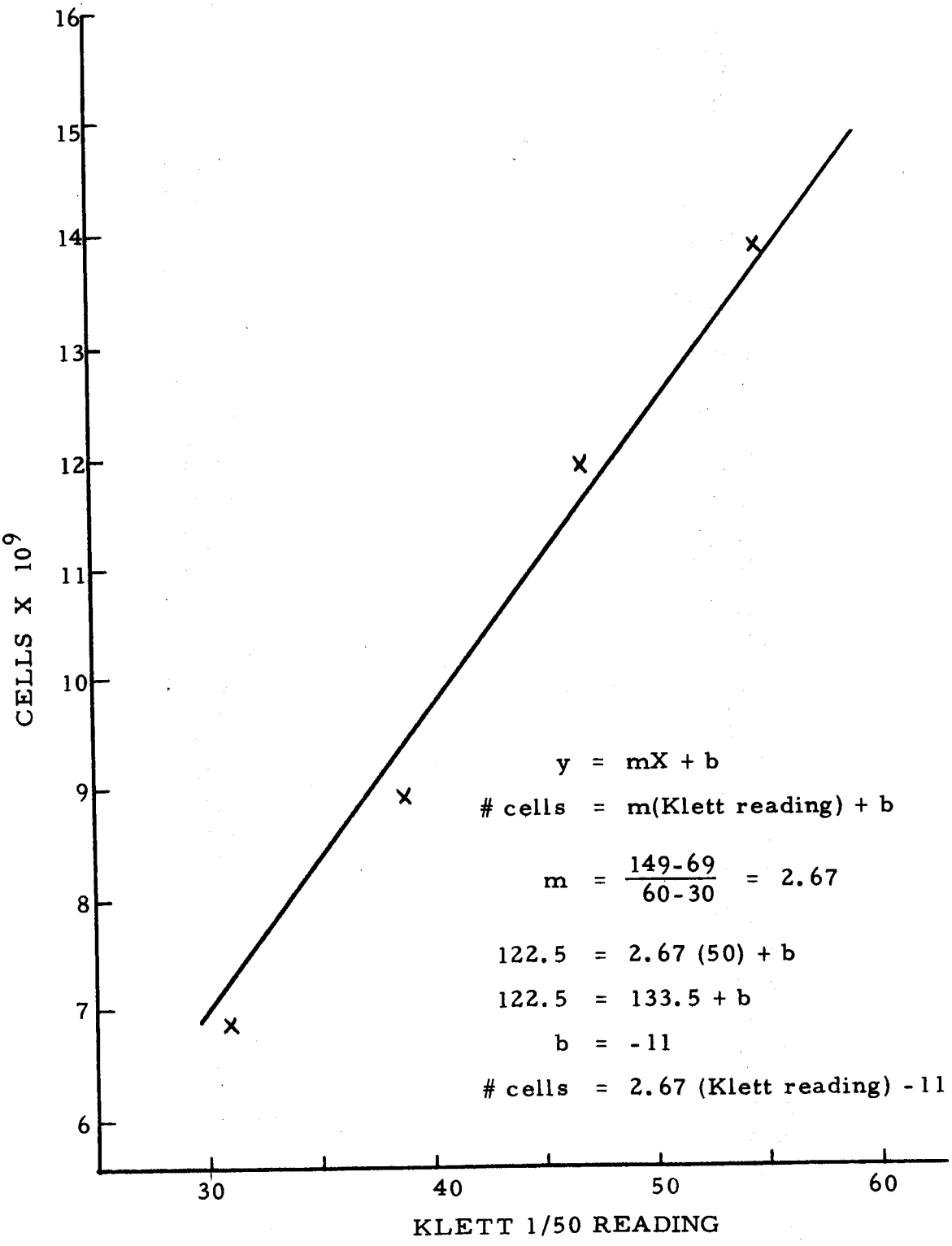
Appendix 1. Relation between number of cells/ml and Klett 1/50 reading.



THE MECHANISM OF WASTE TREATMENT AT LOW TEMPERATURE

PART B: SANITARY ENGINEERING

by

John C. Ward, John S. Hunter,
Richard P. Johansen

ENVIRONMENTAL RESOURCES CENTER

Colorado State University
Fort Collins, Colorado

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No. 34

THE MECHANISM OF WASTE TREATMENT AT LOW TEMPERATURE

PART B: SANITARY ENGINEERING

Partial Completion Report

OWRR Project No. A-007-COLO

by

John C. Ward
John S. Hunter
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Environmental Engineering Program
Department of Civil Engineering
Colorado State University

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Colorado Water Resources Research Institute
Colorado State University
Fort Collins, Colorado

Norman A. Evans, Director

PREFACE

This report is part B of the overall project entitled, "The Mechanism of Waste Treatment at Low Temperature". Part A of this report was published separately by S. M. Morrison (director of the overall project), G. C. Newton, G. D. Boone, and K. L. Martin, Microbiology Department.

This report itself is composed of 2 parts: Part 1 entitled, "The Effects of Water Temperature and Elevation upon Aeration," by John S. Hunter and John C. Ward; Part 2 entitled, "BOD and COD Removal from Wastewater by Aeration with Air and Oxygen," by John C. Ward and Richard P. Johansen.

This report covers the work performed during the period of this part of the project, namely July 1, 1970, through June 30, 1972 (2 years). The total amount of support received from the Office of Water Resources Research was $4,700 per year.

ACKNOWLEDGMENT

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SYMBOLS AND ABBREVIATIONS

PART 1 - THE EFFECTS OF WATER TEMPERATURE AND ELEVATION UPON AERATION

a	air-water interfacial surface area per unit volume of water (ℓ^{-1})
atm	atmosphere ($m\ell^{-1}t^{-2}$)
A	altitude (ℓ) or area (ℓ^2)
cal	calorie
cm	centimeter (ℓ)
C	DO concentration (mg/ℓ)
C_1	saturation DO concentration in pure water at a barometric pressure of 1 atm, (mg/ℓ)
C_s	DO concentration in pure water at stated barometric pressure (mg/ℓ)
^oC	degrees centigrade (Celsius)
$CoCl_3$	cobalt chloride
D	coefficient of molecular diffusion (ℓ^2t^{-1})
DO	dissolved oxygen concentration (mg/ℓ)
E	efficiency
E_a	activation energy (cal/mole)
Eq.	equation
ft	foot (ℓ)
^oF	degrees Fahrenheit
g	gram (m) or acceleration of gravity (981 cm/sec^2)
gal	gallon (ℓ^3)
hp	horsepower ($m\ell^2t^{-3}$)
hr	hour (t)
in.	inch (ℓ)
k	chemical reaction rate (t^{-1})
^oK	degrees Kelvin (^oC + 273)
K_L	over-all mass transfer coefficient (ℓt^{-1})
K_La	over-all volumetric mass transfer coefficient, liquid-phase base (t^{-1})
ℓ	length
lb	pound (mt^{-2})
ln	natural logarithm
log	common logarithm
m	mass
M	Molecular weight of air (29 g/mole)
min	minute (t)
mg/l	milligram per liter ($m\ell^{-3}$)
ml	milliliter (ℓ^3)
mm Hg	millimeter of mercury
Na_2SO_3	sodium sulfite
Na_2SO_4	sodium sulfate

No.	number
O_2	molecular oxygen
p	vapor pressure of water at the stated temperature (atm)
psi	pounds per square inch $(m\ell^{-1}t^{-2})$
P	atmospheric pressure $(m\ell^{-1}t^{-2})$
rpm	revolutions per minute
R	universal gas constant [1.99 cal/(mole)(^{o}K)]
SCFH	standard cubic feet of gas per hour at 70^{o}F and 1 atm $(\ell^{3}t^{-1})$
t	time
T	temperature (^{o}C or ^{o}K as specified)
V	volume (ℓ^{3})
ρ	density $(m\ell^{-3})$
θ	temperature correction coefficient for $K_L a$ (dimensionless)

PART 2 - BOD AND COD REMOVAL FROM WASTEWATER BY AERATION WITH AIR AND OXYGEN

atm	atmospheres
BOD	biochemical oxygen demand
COD	chemical oxygen demand
^{o}	degree
^{o}C	degrees centigrade
^{o}F	degrees Fahrenheit
DO	dissolved oxygen
mg	milligram
mg/ℓ	milligram per liter
OD	oxygen demand
%	percent
t	time in days
$t_{1/2}$	time required for removal of 50% of the initial oxygen demand, days
$t/t_{1/2}$	ratio of aeration time to time required for removal of 50% of the initial oxygen demand

PART 1 - THE EFFECTS OF WATER TEMPERATURE AND ELEVATION UPON AERATION

by

John S. Hunter and John C. Ward

I. Introduction

The central thrust of this project has been the study of wastewater treatment under conditions of high altitude and low water temperature. Aeration is a fundamental operation used in many wastewater treatment facilities designed to function under these conditions. The study of how aeration is affected by such conditions, then, has become an integral part of this project.

Changes in elevation are accompanied by changes in barometric pressure and atmospheric temperature. Changes in barametric pressure affect aeration directly through changes in the dissolved oxygen saturation concentration because this value determines the available driving force for transferring oxygen from the atmosphere into the water. The dissolved oxygen saturation concentration is directly proportional to atmospheric pressure in accordance with Henry's law. Henry's law is valid over a wide temperature range and can be applied whenever the proportionality constant for the particular wastewater being considered is known (Eckenfelder, 1966, page 52). The proportionality constant for domestic sewage is approximately 95 percent that of clean water (Fair, Geyer, and Okun, 1968, page 23-10).

Changes in atmospheric temperature which accompany changes in elevation do not have as direct an effect on aeration as do changes in atmospheric pressure. The degree to which atmospheric temperatures influence wastewater temperatures is a function of each individual wastewater collection and treatment system. In general, however, warmer wastewater temperatures can be expected in warmer climates and colder wastewater temperatures can be expected in colder climates. It is found through the examination of atmospheric tables that atmospheric temperatures fall with increasing elevation. Because this project was oriented about the effects of high altitude it was necessary to critically examine existing methods for predicting the effects of low water temperature upon aeration.

A review of the literature indicates that most studies which have been performed to date (for determining oxygen mass transfer rates into water at various water temperatures) have typically been carried out in systems where the water temperature was varied between 10 and 30°C. Field studies carried out under this project revealed that many wastewater treatment facilities located in the mountainous regions of Colorado aerate wastewaters having temperatures below 10°C during significant portions of the year. A few plants were found to be aerating wastewaters with temperatures below 1°C. Some doubt exists as to the appropriateness of extrapolating existing aeration data down

into the 0 to 10°C range, especially for the design of facilities that are to treat wastewaters having a temperature below 1°C. As a result, the main emphasis of the aeration studies portion of this project has been placed on finding a reliable method for predicting the effect of water temperature upon aeration with particular attention being paid to water temperature between 0 and 10°C. Such a method has been found. It is accurate over the temperature range 0 to 40°C. Henry's law has been incorporated into this method to show in graphical form the combined effect of elevation and wastewater temperature upon aeration. A range in elevation of -3,000 to +18,000 feet above mean sea level and a temperature range of 0 to 40°C have been represented.

II. Experiment Design

The laboratory technique used in this research effort to determine overall oxygen transfer coefficients at various water temperatures closely parallels that used in the testing of full-scale aerators. The underlying method employed is commonly referred to as the clean water, nonsteady state, test technique. Large samples of distilled water were deaerated and then each was subjected to the same mixing and submerged air discharge conditions. The temperature of each sample was rigidly controlled and the barametric pressure was measured for each test run. The absolute value of the dissolved oxygen (DO) concentration of every sample was continuously monitored during each test run with a dissolved oxygen probe. This data was logged by a strip chart recorder so that the DO values were obtained as a graphical display of DO versus time for the entire duration of each test.

A. Test Apparatus

The aeration tank and aerator used in these experiments consisted of a large Pyrex glass jar and a small turbine. The jar was cylindrical in shape with a nearly flat bottom. It had an inside diameter of 11 7/16 in. (29.05 cm) and was 17 5/8 in. (44.77 cm) deep. A flat blade impeller with 6 blades was mounted coaxially within the test cylinder at a distance of 1 7/8 in. (4.76 cm) from the bottom. The impeller diameter was 4 in. (10.16 cm) and each blade was 1/2 in. (1.27 cm) wide. An air sparger was mounted directly under the impeller. It consisted of a small, contoured, plastic, reagent dropper bottle which had been placed on one of its flat sides and then glued to a 5/16 by 2 in. (0.79 by 5.08 cm) brass bar which extended across the full width of the aeration tank bottom. The bottle had a 1/4 in. (0.64 cm) hole which was bored at the center of the flat side facing the impeller. There was approximately a 1/4 in. (0.64 cm) clearance between the sparger and the impeller. Air was supplied to the sparger through a flexible plastic tube which was positioned along the aeration tank bottom and then up the tank wall immediately behind a baffle with respect to the liquid flow induced by the impeller. Baffles were installed in the tank to prevent the formation of a vortex thereby providing better mixing conditions. Four baffles, each 1/8 in. (0.32 cm) thick, 1 in. (2.54 cm) wide, and 17 in. (43 cm) long were positioned at equal intervals around the tank. These baffles were mounted perpendicularly with respect to the tank walls and floor. Each baffle was

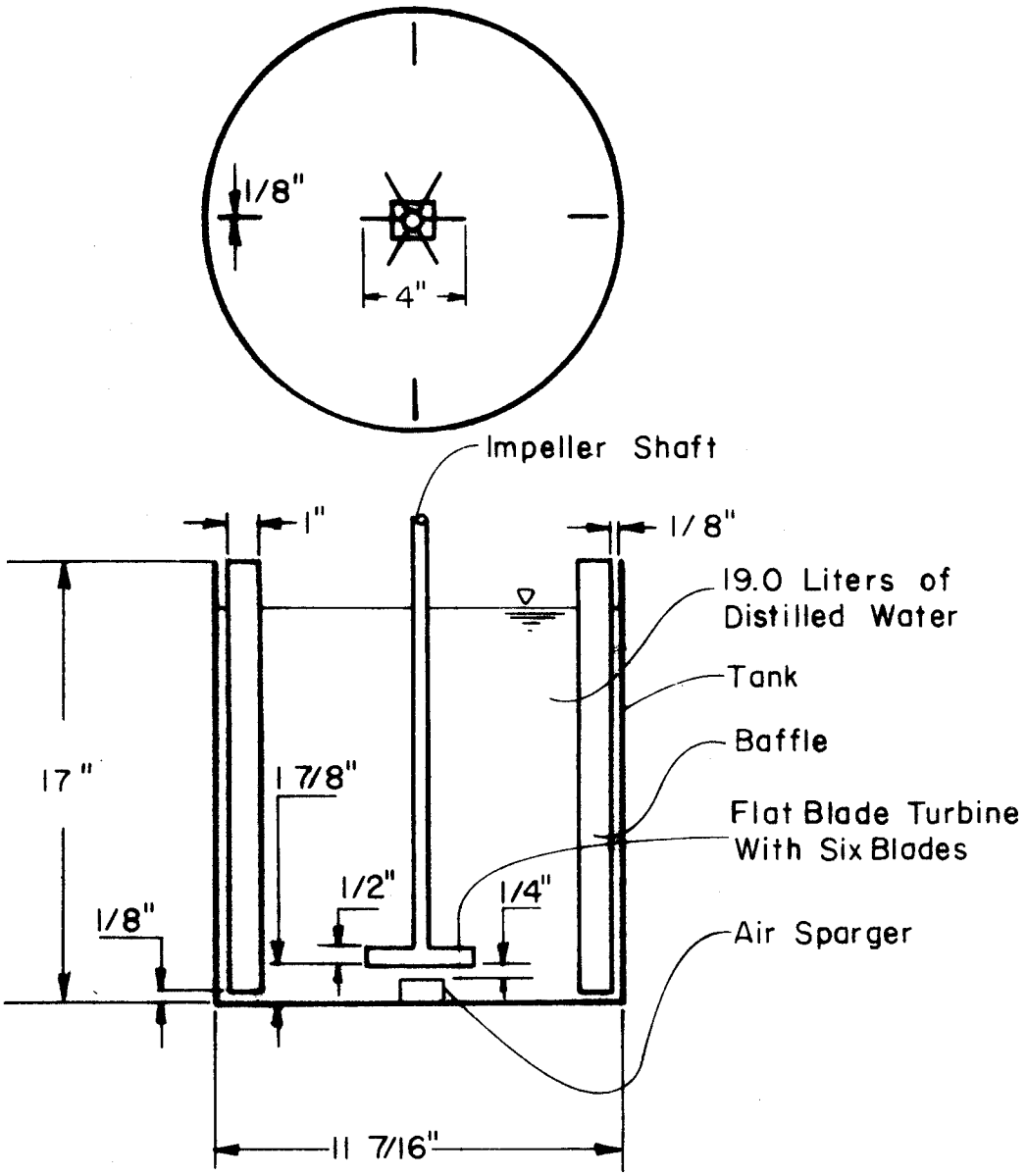

Fig. 1 Experimental Aeration Vessel Configuration

positioned 1/8 in. (0.32 cm) from the tank floor and walls to prevent the formation of any areas of stagnant flow. The above aeration test apparatus geometry was chosen as it meets the pertinent requirements of geometric similarity necessary for comparing results of this study with those of other liquid mixing studies. (Further details on this subject are available from: Bates, Fondy, and Fenic, 1966, pages 111-178; Sterbacek and Tausk, 1965, pages 73-124; and Holland and Chapman, 1966, pages 1-18.)

The compressed air supplied to the sparger was first passed through a laboratory air filter to remove any foreign materials (such as oil) that might affect the oxygen mass transfer process. The air was then passed through bubblers containing distilled water at a temperature approximately that of the water sample in the aeration vessel. This was done to reduce any effects on the data which could arise from heat or water mass exchange between the air bubbles and the water test sample. The air flow rate was controlled by means of a rotometer.

A Model ELB Experimental Agitator power drive and stand manufactured by Chemineer, Inc., of Dayton, Ohio, were used to turn the impeller shaft and hold the shaft in position. This unit could be controlled to provide any constant speed desired for the impeller from 0 to 1100 rpm.

One of the most important tasks involved in carrying out this study was the bringing of water samples to the temperatures of interest and then maintaining them at these temperatures within very small tolerances throughout the duration of the experiment. This was accomplished by supporting the aeration vessel within a slightly larger vessel which had a heat exchange fluid constantly circulating through it from a temperature regulated reservoir. A 7 cubic foot (198 liter) capacity reservoir constructed of stainless steel was used. Cooling was carried out by means of a Model Number PCC-13A Constant Flow Portable Cooling Unit manufactured by the Blue M Electric Company. Heating was accomplished by means of a hot water jacket built into the wall of the reservoir. Hot water was obtained from a laboratory hot water tap. Steam was used instead of hot water in some instances. Uniform temperature of the sample was maintained by adjusting the flow of heat exchange fluid (water containing methyl alcohol as an anti-freeze) between the reservoir and the vessel containing the aeration tank.

Instrumentation included a Cole-Parmer "touchless", portable, solid state, photo-reflective, electronic tachometer which was used for measuring the turbine shaft rotational speed; a dynamometer used to measure power input to the aeration vessel which was supplied by Chemineer, Inc., for use with its impeller drive assembly; and a Yellow Springs Instrument Company Model 54 DO meter which was connected to a Model SR-15 (S72180-15) Sargent strip chart recorder in order to provide continuous time referenced DO data.

B. Test Procedure

1. Place 5.02 gallons (19.00 liters) of distilled water in the aeration tank.

2. Turn on the impeller drive assembly and adjust impeller rotational speed to 600 rpm.

3. Turn on the air supply and adjust flow rate to 1.0 SCFH (see Appendix C for procedure).

4. Add the proper amount of cobalt chloride ($CoCl_3$) solution to the water sample (see Appendix D for dosage calculation).

5. Adjust the water sample temperature to the desired value $\pm$ 0.1°C. It will normally require 1 to 4 hours to attain a stable water sample temperature. When a stable water sample temperature is attained, make any necessary adjustments to the impeller rotational velocity and air flow rate to ensure that they are 600 rpm and 1 SCFH, respectively.

6. Calibrate the dissolved oxygen meter and strip chart recorder with the probe of the DO meter located within the aeration tank and fixed in its monitoring position (it was found that the smoothest, most consistent data output could be obtained by positioning the probe just under the water sample surface on the front side of a baffle with respect to the water flow pattern induced by the impeller. The probe membrane surface should be tilted approximately 10 to 15° from horizontal to permit constant flow of the test sample water across it). Calibrate the monitoring and recording system to the saturation DO concentration in pure water at the water test sample temperature and the existing barometric pressure (see Appendix A for calibration procedure). After the system is calibrated, leave it on line -- do not place on standby or turn if off.

7. Add the proper amount of sodium sulfite (Na_2SO_3) solution (see Appendix D for dosage calculation).

8. Measure torque being applied to the impeller shaft.

9. Continue the test run until the agitated water sample is nearly saturated with dissolved oxygen (this varies between 15 and 30 minutes, depending upon the sample temperature). Constantly monitor and make any necessary adjustments in water sample temperature and air discharge rate during all test runs.

10. Repeat steps 7, 8, and 9 two times in order to obtain 3 test runs at each desired water temperature.

11. Dispose of the water sample.

III. Data Evaluation

A. Raw Data

The above test procedure was followed in obtaining oxygen mass transfer data in water samples maintained at the following temperatures: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, and 40°C. A summary of the raw data appears in Table 1. The values recorded in this table are DO concentrations in the samples (C_t) as read from the continuous curves of data produced during each test run. The values were read at one-minute intervals with an arbitrary zero reference time chosen such that $C_{1\ min.}$ occurred within the first portion of what appeared to be a first order reaction rate response for the recovery of DO in the sample. Normally, $C_{1\ min.}$ occurred

TABLE 1--RAW DATA

Test No.	Run No.	1 min. (mg/ℓ)	2 min. (mg/ℓ)	3 min. (mg/ℓ)	4 min. (mg/ℓ)	5 min. (mg/ℓ)	6 min. (mg/ℓ)	7 min. (mg/ℓ)	8 min. (mg/ℓ)	9 min. (mg/ℓ)	10 min. (mg/ℓ)	11 min. (mg/ℓ)	12 min. (mg/ℓ)	13 min. (mg/ℓ)	14 min. (mg/ℓ)	15 min. (mg/ℓ)	16 min. (mg/ℓ)	17 min. (mg/ℓ)	T (°C)
	Data I.D.									C_t									
1	1	1.95	3.25	4.25	5.00	5.60	6.00	6.35	6.65	6.85	7.00	7.10	7.17	7.25	--	--	--	--	20.0
	2	1.95	3.25	4.25	5.00	5.65	6.10	6.50	6.70	6.87	7.02	7.13	7.20	7.25	--	--	--	--	
	3	1.95	3.30	4.35	5.15	5.75	6.15	6.48	6.72	6.88	7.02	7.13	7.20	7.25	--	--	--	--	
2	1	2.35	3.55	4.57	5.40	6.03	6.55	6.93	7.25	7.50	7.69	7.84	7.93	8.02	8.08	8.14	8.17	8.20	15.0
	2	2.12	3.49	4.55	5.43	6.07	6.60	6.99	7.30	7.55	7.73	7.87	7.98	8.06	8.13	8.18	8.20	8.25	
	3	2.27	3.68	4.68	5.55	6.17	6.65	7.05	7.38	7.58	7.75	7.89	8.01	8.10	8.15	8.22	8.25	--	
3	1	2.17	3.85	5.26	6.16	6.87	7.43	7.87	8.23	8.50	8.69	8.85	8.95	9.06	9.13	9.22	--		10.0
	2	2.45	3.81	4.90	5.80	6.53	7.15	7.65	8.04	8.31	8.54	8.73	8.88	8.98	--	--	--	--	
	3	3.20	4.47	5.50	6.26	6.97	7.47	7.90	8.21	8.45	8.67	8.84	8.96	--	--	--	--	--	
4	1	2.10	3.60	4.65	5.65	6.45	7.05	7.60	8.00	8.32	8.56	8.76	8.90	9.05	9.15	9.23	9.30	--	9.0
	2	1.30	3.04	4.30	5.33	6.22	6.86	7.44	7.85	8.21	8.49	8.70	8.87	9.00	9.12	9.20	9.26	--	
	3	1.61	3.08	4.35	5.26	6.10	6.78	7.30	7.73	8.10	8.35	8.57	8.80	8.92	9.05	9.14	9.21	9.30	
	4	0.90	2.17	3.93	4.90	5.80	6.48	7.10	7.58	7.95	8.25	8.50	8.69	8.85	8.97	9.08	9.15	9.20	
5	1	1.23	2.73	4.13	5.17	6.05	6.77	7.36	7.82	8.21	8.54	8.77	8.95	9.13	9.26	9.37	9.46	9.54	8.0
	2	1.58	3.13	4.44	5.47	6.32	7.03	7.67	8.02	8.38	8.64	8.88	9.06	9.20	9.31	9.42	9.50	9.56	
	3	0.95	2.50	3.92	5.07	6.02	6.78	7.37	7.86	8.25	8.55	8.80	8.99	9.18	9.30	9.40	9.51	9.55	
6	1	2.75	4.00	5.05	5.90	6.63	7.21	7.70	8.10	8.43	8.70	8.92	9.11	9.24	9.35	9.42	9.52	9.62	7.0
	2	2.51	3.88	5.02	5.92	6.64	7.30	7.77	8.18	8.52	8.79	8.02	9.17	9.30	9.42	9.50	9.60	9.67	
	3	2.83	4.18	5.25	6.19	6.88	7.47	7.93	8.34	8.61	8.68	9.08	9.24	9.38	9.46	9.57	9.63	9.68	
7	1	2.67	4.02	5.11	6.08	6.82	7.50	8.04	8.47	8.81	9.10	9.32	9.50	9.68	9.80	9.90	9.97	10.00	6.0
	2	2.70	4.02	5.18	6.17	6.93	7.58	8.10	8.49	8.84	9.10	9.31	9.53	9.70	9.84	9.93	9.99	10.06	
	3	2.29	3.74	4.96	5.97	6.79	7.46	8.02	8.44	8.79	9.09	9.30	9.49	9.66	9.76	9.89	9.99	10.02	
8	1	2.74	4.12	5.28	6.22	7.00	7.63	8.19	8.60	8.93	9.23	9.44	9.65	9.80	9.93	10.01	10.11	10.19	5.0
	2	2.48	3.91	5.09	6.08	6.88	7.59	8.13	8.55	8.91	9.20	9.43	9.62	9.82	9.96	10.04	10.15	10.19	
	3	2.53	4.00	5.17	6.17	6.97	7.60	8.17	8.60	8.95	9.23	9.45	9.67	9.81	9.97	10.07	10.13	10.20	
9	1	1.60	3.19	4.48	5.61	6.53	7.30	7.91	8.40	8.84	9.18	9.47	9.71	9.91	10.08	10.20	10.30	10.40	4.0
	2	1.20	2.81	4.17	5.30	6.29	7.08	7.77	8.32	8.74	9.10	9.39	9.66	9.89	10.08	10.20	10.31	10.40	
	3	1.24	2.80	4.19	5.30	6.29	7.13	7.79	8.33	8.77	9.17	9.46	9.71	9.93	10.08	10.22	10.34	10.39	
10	1	1.69	3.15	4.50	5.67	6.60	7.40	8.06	8.58	9.02	9.42	9.73	10.01	10.21	10.39	10.52	10.65	10.77	3.0
	2	1.45	2.96	4.33	5.50	6.47	7.31	7.99	8.55	8.99	9.36	9.70	9.97	10.20	10.38	10.52	10.68	10.75	
	3	1.41	2.93	4.30	5.49	6.47	7.34	8.00	8.60	9.02	9.40	9.77	10.03	10.25	10.42	10.56	10.69	10.80	
11	1	1.30	2.79	4.17	5.38	6.39	7.23	7.93	8.57	9.02	9.43	9.79	10.09	10.30	10.50	10.68	10.79	10.90	2.0
	2	1.75	3.14	4.40	5.55	6.54	7.35	8.04	8.60	9.07	9.47	9.80	10.09	10.33	10.50	10.65	10.80	10.90	
	3	1.70	2.95	4.20	5.30	6.30	7.14	7.88	8.50	8.96	9.37	9.73	10.05	10.26	10.48	10.62	10.78	10.89	
12	1	1.90	3.50	4.87	6.00	6.98	7.75	8.44	9.02	9.50	9.88	10.20	10.48	10.70	10.89	11.03	11.21	11.30	1.0
	2	0.99	2.58	4.10	5.33	6.43	7.34	8.10	8.73	9.22	9.67	10.03	10.34	10.58	10.78	10.96	11.11	11.23	
	3	1.83	3.06	4.47	5.65	6.63	7.48	8.20	8.79	9.27	9.70	10.04	10.34	10.60	10.80	10.97	11.10	11.25	
13	1	1.60	3.10	4.50	5.65	6.73	7.55	8.30	8.91	9.40	9.83	10.23	10.51	10.77	11.00	11.15	11.30	11.45	0.5
	2	1.95	3.40	4.73	5.98	6.90	7.78	8.50	9.06	9.53	9.98	10.35	10.63	10.84	11.07	11.23	11.38	11.50	
	3	1.60	3.02	4.30	5.57	6.61	7.51	8.30	8.90	9.40	9.87	10.23	10.54	10.78	11.02	11.19	11.34	11.48	
14	1	1.70	3.40	4.80	6.05	7.05	7.90	8.63	9.18	9.63	10.07	10.37	10.64	10.83	10.99	11.12	11.30	11.40	1.0
	2	2.69	4.18	5.43	6.53	7.47	8.23	8.80	9.32	9.75	10.08	10.38	10.67	10.84	11.00	11.13	11.23	11.35	
	3	2.59	4.08	5.37	6.47	7.39	8.14	8.78	9.28	9.75	10.11	10.40	10.67	10.87	11.01	11.16	11.30	11.40	
15	1	1.47	2.54	3.77	4.65	5.43	6.03	6.49	6.84	7.14	7.38	7.56	7.69	7.79	7.87	7.94	7.99	8.03	15
	2	1.74	3.12	4.23	5.07	5.75	6.26	6.68	7.01	7.26	7.47	7.63	7.75	7.85	7.91	7.97	8.02	8.05	
	3	1.42	2.85	4.01	4.92	5.62	6.16	6.59	6.93	7.20	7.42	7.57	7.69	7.78	7.86	7.92	7.96	8.00	
16	1	1.73	2.83	3.87	4.68	5.32	5.83	6.19	6.48	6.72	6.88	7.02	7.13	7.20	7.26	7.32	7.37	7.40	20
	2	1.83	3.03	4.06	4.83	5.46	5.92	6.28	6.58	6.77	6.92	7.06	7.15	7.22	7.27	7.32	7.35	7.39	
	3	1.82	3.07	4.15	4.89	5.48	5.91	6.26	6.52	6.75	6.91	7.04	7.12	7.19	7.25	7.30	7.35	7.37	
17	1	1.48	2.54	3.43	4.10	4.51	4.83	5.17	5.52	5.80	6.03	6.21	6.33	6.42	6.48	6.53	6.58	6.62	25
	2	1.15	2.44	3.52	4.33	4.93	5.39	5.72	5.98	6.17	6.31	6.40	6.47	6.53	6.58	6.62	6.63	6.65	
	3	1.31	2.55	3.61	4.42	5.00	5.44	5.75	5.98	6.18	6.32	6.42	6.49	6.55	6.59	6.63	6.65	6.68	
18	1	1.52	2.78	3.68	4.38	4.83	5.18	5.43	5.63	5.76	5.84	5.90	5.96	6.00	6.00	6.02	6.05	6.07	30
	2	1.83	3.01	3.86	4.49	4.94	5.27	5.48	5.65	5.76	5.86	5.92	5.95	6.02	6.05	6.06	6.07	6.08	
	3	1.57	2.88	3.75	4.42	4.88	5.23	5.45	5.63	5.75	5.85	5.92	5.97	6.01	6.03	6.06	6.07	6.09	
19	1	1.45	2.50	3.41	3.99	4.42	4.69	4.92	5.07	5.17	5.24	5.30	5.33	5.35	5.38	5.40	5.42	5.43	35
	2	1.10	2.38	3.27	3.92	4.37	4.67	4.91	5.08	5.17	5.24	5.31	5.35	5.38	5.40	5.42	5.43	5.45	
	3	1.48	2.63	3.48	4.08	4.51	4.78	4.99	5.12	5.23	5.32	5.37	5.40	5.43	5.45	5.45	5.47	5.49	
20	1	1.64	2.52	3.22	3.72	4.08	4.32	4.52	4.62	4.69	4.75	4.80	4.82	4.85	4.87	4.87	4.88	4.89	40
	2	1.12	2.27	3.07	3.63	4.03	4.28	4.48	4.62	4.69	4.75	4.80	4.83	4.85	4.87	4.88	4.88	4.89	
	3	1.49	2.50	3.27	3.78	4.15	4.38	4.54	4.64	4.72	4.78	4.82	4.85	4.87	4.88	4.89	4.90	4.90	
21	1	1.57	2.57	3.50	4.30	4.98	5.52	5.98	6.35	6.69	6.95	7.21	7.43	7.62	7.74	7.88	7.98	8.07	10
	2	1.43	2.27	3.07	3.78	4.45	4.94	5.43	5.83	6.18	6.47	6.73	6.94	7.15	7.32	7.46	7.59	7.68	
	3	1.72	2.43	3.14	3.77	4.34	4.81	5.22	5.57	5.90	6.17	6.43	6.64	6.80	6.95	7.12	7.27	7.37	
22	1	3.57	4.63	5.53	6.33	6.97	7.48	7.92	8.31	8.64	8.91	9.11	9.27	9.37	9.44	9.50	9.60	9.64	7
	2	3.11	4.37	5.33	6.23	6.92	7.48	7.91	8.30	8.65	8.90	9.09	9.23	9.37	9.45	9.50	9.60	9.67	
	3	3.55	4.70	5.65	6.43	7.07	7.60	8.00	8.38	8.62	8.85	9.09	9.25	9.37	9.44	9.52	9.58	9.61	
23	1	1.35	2.78	4.06	5.08	5.97	6.66	7.18	7.62	7.94	8.21	8.42	8.57	8.76	8.86	8.94	9.01	9.08	10
	2	1.34	2.79	4.03	5.05	5.87	6.53	7.04	7.50	7.85	8.14	8.32	8.47	8.73	8.85	8.93	9.01	9.05	
	3	1.42	2.82	4.07	5.08	5.87	6.52	7.08	7.52	7.85	8.14	8.37	8.56	8.70	8.82	8.91	8.98	9.05	
24	1	1.42	2.46	3.23	3.75	4.11	4.35	4.53	4.66	4.73	4.78	4.81	4.84	4.86	4.88	4.90	4.90	4.90	40
	2	0.91	2.12	2.98	3.58	3.98	4.26	4.46	4.59	4.68	4.74	4.79	4.83	4.85	4.86	4.89	4.89	4.90	
	3	1.76	2.71	3.37	3.84	4.17	4.40	4.55	4.64	4.73	4.78	4.80	4.83	4.85	4.86	4.88	4.89	4.90	

approximately 1 minute after the sample DO started to rise. The DO values increased from 10 to 80 percent of saturation within 12 to 17 minutes for all test runs. Kalinske (1969, page 2) reports that the most meaningful data for aerator tests is obtained if the DO in the liquid volume increases over this range somewhere within 10 to 30 minutes.

B. Theoretical Considerations

In the technique utilized for these experiments, the sodium sulfite, catalyzed by means of cobalt chloride, reacts with the oxygen present in the sample to form sodium sulfate, removing the dissolved oxygen from the water sample by chemical reaction. Oxygen is constantly being added to the water by the aerator so the finite amount of sodium sulfite added to the sample becomes exhausted with time, and then the DO rises towards its saturation value. An equilibrium DO value of approximately 0.5 mg/l was usually noted during the period when the sodium sulfite was present in significant quantity. A point in time was reached, however, when the quantity of sodium sulfite present was reduced so much as not to be able to maintain this equilibrium DO value and, in accordance with Le Chatelier's principle, the DO level would rise. If the oxygen mass transfer process is a diffusion only process with no chemical reaction involved, the mass transfer of oxygen into the sample should follow first order kinetics as described by Fick's first law of diffusion. It was noted in these experiments that there was a short transition period between the time when the DO value first started to increase from its low equilibrium value and the time when the reaeration of the sample could be described by first order kinetics. As previously noted, this period was approximately 1 minute in duration. This transition period can be interpreted as being the time necessary for the incoming oxygen to eliminate the last fraction of sodium sulfite remaining in the sample.

Assuming that the reaeration process observed was a diffusion only process with no chemical reaction involved and that this process can be adequately described by Fick's first law of diffusion (assumptions made in all aerator tests), the rate of oxygen mass transfer, $\partial m/\partial t$, into the water sample is

$$\frac{\partial m}{\partial t} = - D\left(\frac{\partial C}{\partial x}\right) dy\ dz \qquad (1)$$

where $\partial m/\partial t$ is the rate of mass transfer, mg/hr
D is the coefficient of molecular diffusion, cm^2/hr
$\partial C/\partial x$ is the concentration gradient, mg/cm^4
and $dy\ dz$ is the area through which the oxygen molecules pass.
Fick's law is written in the form of a partial differential equation because the concentration gradient, $\partial C/\partial x$, at any fixed location in the X direction changes with time (Fair, Geyer, and Okun, 1968, page 23-5). For computational convenience the above equation can be reduced to the one-dimensional form

$$\frac{dm}{dt} = - DA \frac{dC}{dx} \qquad (2)$$

where A is the surface area (in cm^2) normal to the X axis across which the mass transfer occurs and x is the distance in the X direction. Expressed in terms of the Whitman two-film theory (Lewis and Whitman, 1924, pages 1215-1220) Eq. 2 takes the form

$$\frac{dm}{dt} = DA \frac{(C_s - C)}{x_L} \tag{3}$$

where C_s is the saturation DO concentration, C is the DO concentration in the water, and x_L is the thickness of the water film across which the oxygen mass transfer takes place. Because there is no way to measure the liquid film thickness, x_L, directly, it and the diffusivity term, D, are normally combined into one term designated K_L. By substituting K_L for D/x_L in Eq. 3 and then dividing both sides by the aeration tank volume, V, in cm^3, the following expression is obtained:

$$\frac{dm/dt}{V} = \frac{dC}{dt} = \frac{K_L A}{V} (C_s - C) . \tag{4}$$

The interfacial area, A, encountered in aeration practice is not easily measured. To overcome this difficulty, a term representing the interfacial surface area per unit volume of liquid, a, defined as

$$a \equiv A/V \tag{5}$$

is used to form the product $K_L a$. This product has the dimensions of reciprocal time and is usually expressed in hr^{-1}. $K_L a$ is the overall volumetric mass transfer coefficient, liquid phase base. It is commonly used to calculate oxygen mass transfer rates for aeration systems through use of the following equation which is obtained by combining Eq. 4 and Eq. 5:

$$\frac{dC}{dt} = K_L a (C_s - C) \tag{6}$$

where C_s and C are normally expressed in terms of mg/l and $K_L a$ in terms of hr^{-1}.

In addition to the assumptions already mentioned, it is important to note that certain other assumptions have been made in developing the discussion presented up to this point. These include:

1. Perfect mixing is attained and is maintained throughout the entire test procedure,

2. The overall mass transfer coefficient, $K_L a$ is constant regardless of time or DO concentration, and

3. The Whitman two-film theory (Lewis and Whitman, 1924, pages 1215-1220) describes the oxygen mass transfer process and the resistance of the gas film to oxygen transfer is negligible compared to the resistance of the liquid film.

Perfect mixing is nearly impossible to attain. Deviations from this condition, however, were considered to be slight for the test apparatus used in these experiments. Also, any deviation from perfect conditions, i.e., the development of DO cencentration gradients within the aeration tank, were compensated for, in part, by placing the DO probe in the same monitoring position for all test runs. The second assumption is apparently correct, but test conditions seem to influence the range of DO concentrations over which $K_L a$ can be observed to be constant during a test run. According to Landberg, Graulich, and Kipple (1969, page 447), $K_L a$ in full-scale surface aerator tests is constant only when the water sample DO is in the approximate range of 30 to 80% of saturation. Apparently, above 80% of saturation, the errors in the values of C and C_s are compounded by taking their difference. In addition, the value of C is not likely to be precisely the same over the whole aeration volume. Consequently, test results may depend upon such test conditions as size of the aeration tank and aerator characteristics. For example, in this particular study, $K_L a$ appeared to be constant between DO concentrations of approximately 5 to 80 percent of saturation. Discussion by Landberg, Groulich, and Kipple (1969, pages 447-448) indicates that the expansion of this range over which a constant $K_L a$ applies (which was noted in this study) was due, in part, by the attainment of more perfect mixing conditions than those which occur in full-scale aerator tests. The third assumption that the Whitman two-film theory is applicable, although not necessarily correct in the physical sense, provides a mathematical model which seems to provide very reasonable results (Rich, 1961, page 172).

C. Data Analysis

Solving Eq. 6 by integration yields

$$\ell n\left(\frac{C_s - C_o}{C_s - C}\right) = K_L a(t - t_o) \qquad (7)$$

where C_o is the value of C at time t_o. Equation 7 can be rewritten as

$$\ell og\ (C_s - C) = \ell og(C_s - C_o) + \frac{K_L a\ t_o}{2.3} - \frac{K_L a\ t}{2.3} \quad . \qquad (8)$$

From Eq. 8, it can be seen that a graphical plot of $\log(C_s - C)$ versus t will be a straight line with

$$\text{slope} = -K_L a/2.3 \tag{9}$$

and ordinate intercept at $t = 0$ of

$$\text{ordinate intercept} = \log(C_s - C_0) + \frac{K_L a\, t_o}{2.3} . \tag{10}$$

Values of $(C_s - C)$ were calculated at 1-minute intervals for each test run and then plotted as described above. The $K_L a$ for each test run was then determined by calculating the slope of the straight line of best fit using Eq. 7. The $K_L a$ values found are listed in Table 2 and are displayed graphically in Figure 2. A linear relationship between $K_L a$ and water temperature, T, was apparent from visual inspection. The observation was verified mathematically as the data produced a correlation coefficient for a straight line of 0.989 which shows that the regression explains 97.8 percent of the observed variance. A least squares analysis produced the following equation of the straight line, including 95 percent confidence limits for the slope and intercepts:

$$K_L a = 10.3812\ (\pm\ 0.2000) + 0.2935\ (\pm\ 0.0110)T \tag{11}$$

where $K_L a$ is expressed in terms of hr^{-1} and T in ^{o}C.

A close examination of the semi-log plots of $(C_s - C_t)$ versus time, t, revealed that several curved away from the straight line of best fit. Some of these plots curved up and some curved down with increasing t. The general shape of these curves was similar to the shape of the curves shown in Figure 3. The curves shown in this figure were generated by calculating $(C_s - C_t)$ values from an idealized, hypothetical set of data using C_s values which were equal to C_s, $C_s \pm 0.1$ mg/l, $C_s \pm 0.2$ mg/l, and $C_s \pm 0.5$ mg/l. If the raw data from any test run is accurate and is representative of first-order kinetics, it should plot as a straight line if the proper value of C_s is used in calculating $(C_s - C)$. This case is represented by the straight line in Figure 3 which lies in the center of the group of curves which have been generated. It can be seen from Figure 3 that if the C_s value used in calculating $(C_s - C)$ is in error, the data will plot as a curve, not as a straight line as theoretically predicted. More significantly, it can be seen that the slope of the straight line of best fit is influenced by the assumed value of C_s. The $K_L a$

TABLE 2--OBSERVED VALUES OF C_s AND K_La

Temp. (°C)	Data Identification: Test Number	Data Identification: Run Number	C_s: Calculated (mg/ℓ)	C_s: True (mg/ℓ)	K_La: Using Calculated C_s (hr^{-1})	K_La: Using True C_s (hr^{-1})	Note
20	1	1	7.52	7.50	15.8	15.7	
		2	--	--	--	--	1
		3	--	--	--	--	1
15	2	1	8.22	8.32	14.9	14.9	
		2	8.22	8.36	15.6	15.2	
		3	8.22	8.38	16.0	15.3	
10	3	1	9.34	9.34	(16.0)	(16.0)	2
		2	9.34	9.52	13.3	13.1	
		3	9.34	9.46	14.6	13.8	
9	4	1	9.57	9.57	13.3	13.3	
		2	9.57	9.57	13.4	13.4	
		3	9.57	9.57	12.6	12.6	
		4	9.58	9.50	12.3	13.0	
8	5	1	9.83	9.83	12.6	12.6	
		2	9.83	9.83	12.9	12.9	
		3	9.83	9.84	13.1	13.0	
7	6	1	10.03	9.91	11.4	11.9	
		2	10.03	9.93	12.1	12.5	
		3	10.03	9.93	12.4	12.7	
6	7	1	10.33	10.38	11.7	12.0	
		2	10.33	10.36	12.1	12.2	
		3	10.33	10.33	12.5	12.5	
5	8	1	10.53	10.53	11.9	11.9	
		2	10.53	10.57	12.1	11.7	
		3	10.53	10.53	12.2	12.2	
4	9	1	10.87	10.85	11.4	11.3	
		2	10.87	10.87	11.6	11.6	
		3	10.87	10.87	11.7	11.7	
3	10	1	11.24	11.24	11.2	11.2	
		2	11.24	11.25	11.4	11.2	
		3	11.24	11.31	11.7	11.2	
2	11	1	11.48	11.48	11.0	11.0	
		2	11.48	11.48	10.8	10.8	
		3	11.48	11.48	10.9	10.9	
1	12	1	11.78	11.88	10.7	10.7	
		2	11.78	11.85	11.0	10.8	
		3	11.78	11.85	10.6	10.8	
0.5	13	1	12.12	12.12	10.4	10.4	
		2	12.12	12.12	10.6	10.6	
		3	12.12	12.12	10.6	10.6	
1	14	1	11.82	11.90	11.5	11.5	
		2	11.82	11.82	11.1	11.1	
		3	11.82	11.90	11.1	11.0	
15	15	1	8.23	8.20	14.2	14.3	
		2	8.23	8.19	14.3	14.5	
		3	8.23	8.13	14.5	14.9	
20	16	1	7.44	7.47	15.3	15.5	
		2	7.44	7.46	15.7	15.9	
		3	7.44	7.43	15.6	16.0	
25	17	1	6.73	--	--	--	3
		2	6.73	6.71	17.4	17.4	
		3	6.73	6.71	18.2	17.7	
30	18	1	6.08	6.08	19.8	19.8	
		2	6.08	6.08	19.7	19.7	
		3	6.08	6.09	19.5	19.7	
35	19	1	5.42	5.41	20.6	21.3	
		2	5.42	5.43	21.2	21.1	
		3	5.42	5.50	22.2	20.1	
40	20	1	4.90	4.89	20.8	21.2	
		2	4.90	4.90	22.1	22.1	
		3	4.90	4.88	22.9	23.2	
10	21	1	9.19	--	--	--	4
		2	9.19	--	--	--	4
		3	9.19	--	--	--	4
7	22	1	10.03	9.93	11.5	12.0	
		2	10.03	9.93	11.9	12.3	
		3	10.03	9.88	11.8	12.3	
10	23	1	9.35	9.30	13.2	13.5	
		2	9.35	9.35	12.7	12.7	
		3	9.35	9.30	12.6	13.1	
40	24	1	4.93	4.91	22.1	22.4	
		2	4.93	4.86	21.6	22.4	
		3	4.93	4.89	21.2	21.9	

NOTES:

1. Control of the sample temperature was lost.
2. This K_La value was discarded as it was 4.7 standard deviations from the estimated K_La based upon all other K_La values calculated through the use of C_s values determined from published tables and application of Henry's law (referred to in this report as "calculated" C_s values) and 7.0 standard deviations from the estimated K_La based upon all other K_La values calculated through the use of C_s values determined by a new graphical procedure described later in this report (referred to as "true" C_s values).
3. It was not possible to calculate a K_La from the data obtained during this test run as the data did not fit a first-order pattern. The raw data tends to indicate that a zone of stagnation developed in the water sample at the DO probe membrane.
4. Some problem developed with the instrumentation which resulted in the loss of calibration. This is apparent from the fact that the recovery in DO towards saturation after an extended aeration time was only about 90 percent while in all other test runs DO recoveries of approximately 97 percent were attained within roughly the same time period. Values of K_La calculated with the data obtained gave results from 8 to as many as 17 standard deviations from the expected value.

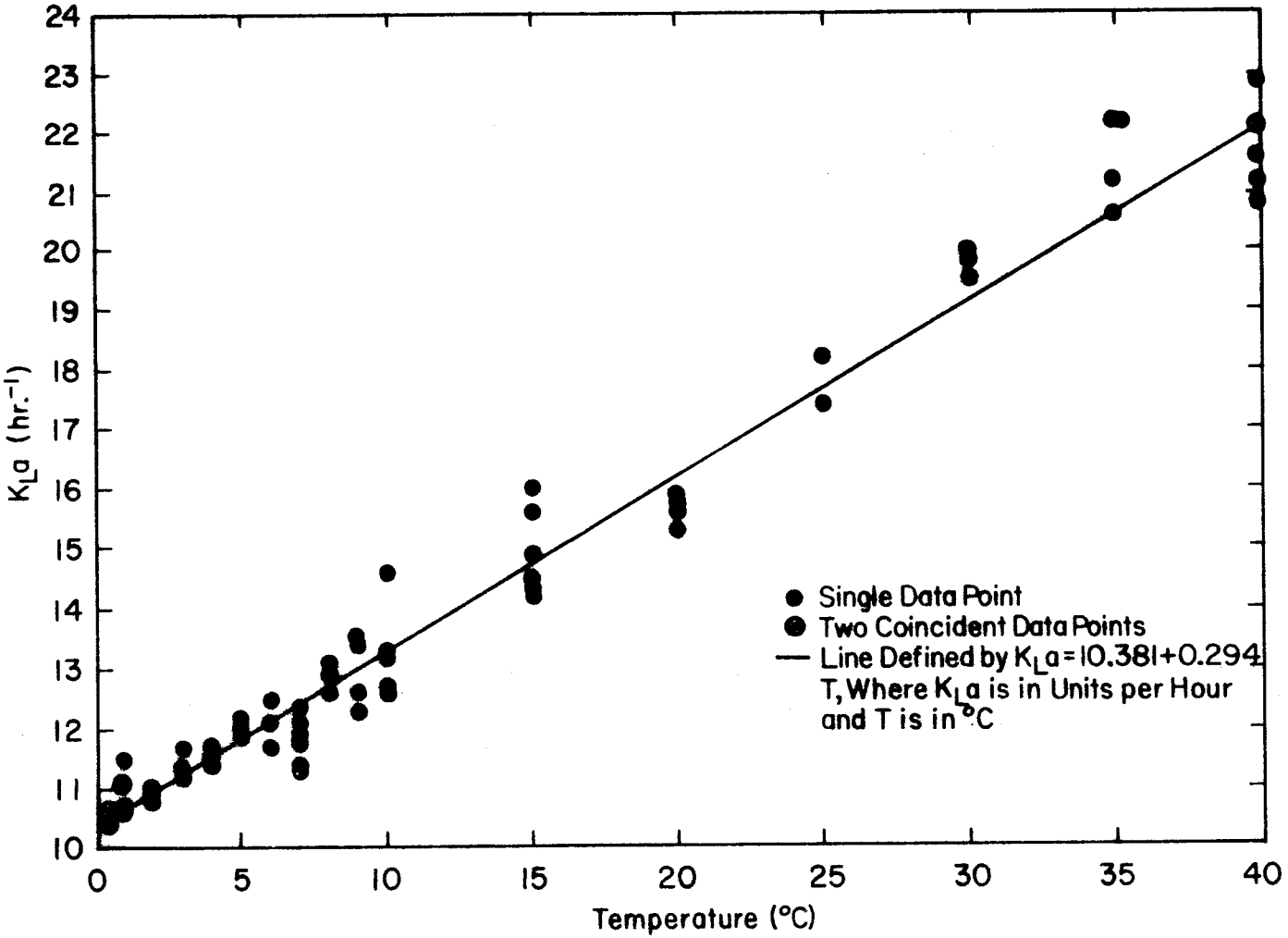

Fig. 2 Values of K_La Based on Calculated C_s

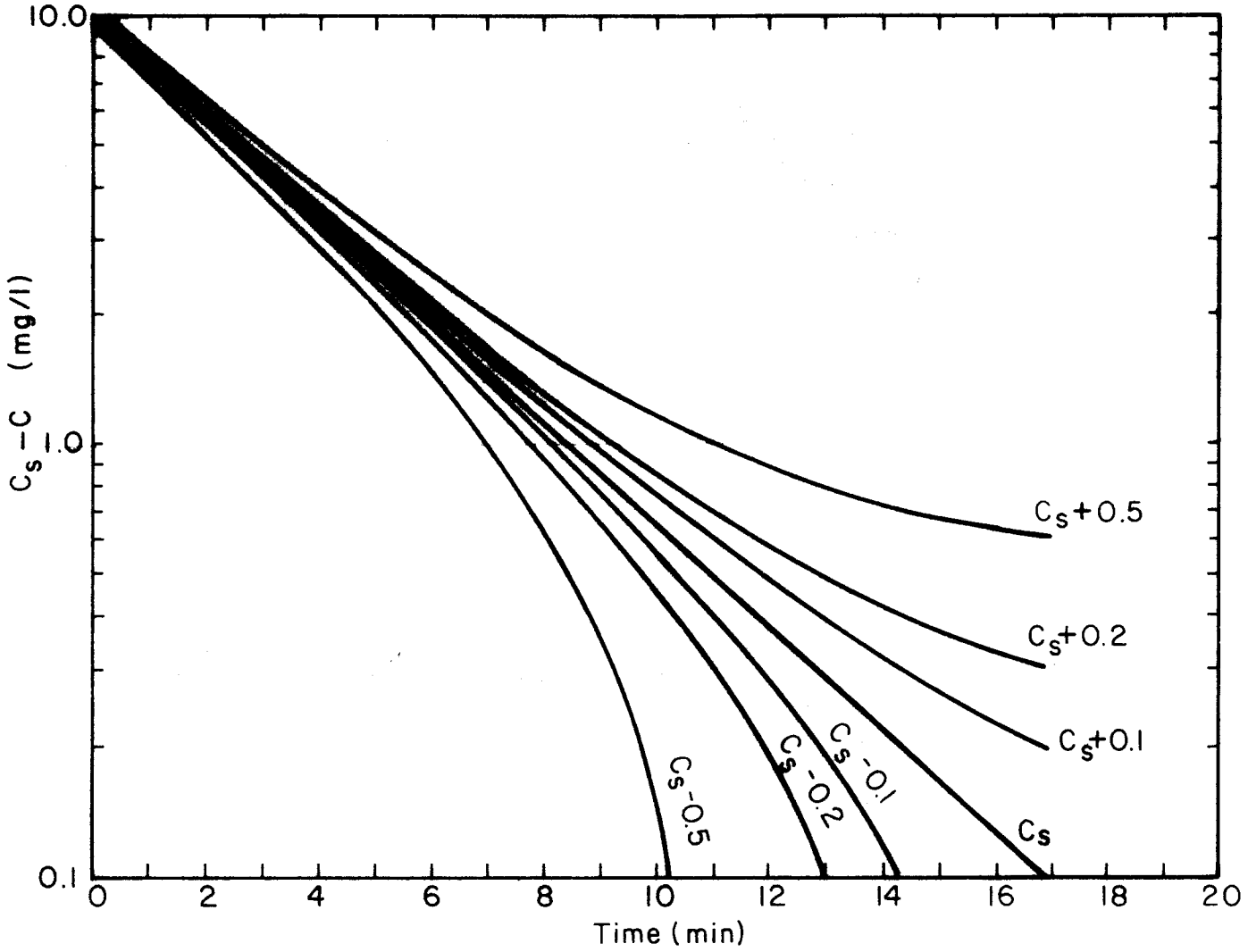

Fig. 3 Deviation From Linearity Due to Assumed Value of C_s

calculated will be too low if the C_s used in the calculations is above the true value for C_s and will be too high if the C_s used in the calculations is below the true C_s value.

After studying Figure 3, it was decided that the semi-log plots used to calculate K_La which were not linear may have been caused by the use of incorrect values of C_s when calculating $(C_s - C_t)$. A trial-and-error procedure was applied to each set of curved data plots to determine if a C_s value could be found which would make the plot of $(C_s - C_t)$ versus t linear. This proved to be a successful approach to the data for, without exception, C_s values were found which could generate linear plots. An example is shown in Figure 4. Data from Test Number 2, Run Number 3 is shown using both the calculated[1] and the true[2] value of C_s. This technique for finding the true value of C_s is analogous to one suggested by Dobbins (1964, page 60) for determining constants in modified versions of the Streeter-Phelps DO sag equation which is used in the analysis of natural stream assimilative capacity.

A tabulation of the true C_s values and respective K_La values calculated by means of the above trial-and-error procedure appears in Table 2. A graph of these K_La values versus water temperature, T, appears in Figure 5. A linear relationship between K_La and temperature was even more apparent than with the same plot using K_La values determined through the use of calculated rather than true C_s values. The data produced a correlation coefficient for a straight line of 0.995 which demonstrated that the regression explains 99.0 percent of the observed variance. A least squares analysis yielded the following equation for this straight line, including 95 percent confidence limits for the slope and intercept:

$$K_La = 10.3878\ (\pm\ 0.1363) + 0.2953\ (\pm\ 0.0075)T \qquad (12)$$

where K_La is expressed in terms of hr^{-1} and T in ^oC. A comparison of the results obtained from the two different methods of determining K_La appears in Table 3. It can be seen that the straight-line equation

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1. C_s values which have been determined from published oxygen saturation concentration tables and the application of Henry's law are referred to herein as "calculated" C_s values.

2. C_s values which have been determined by means of the trial-and-error procedure described above are referred to herein as "true" C_s values.

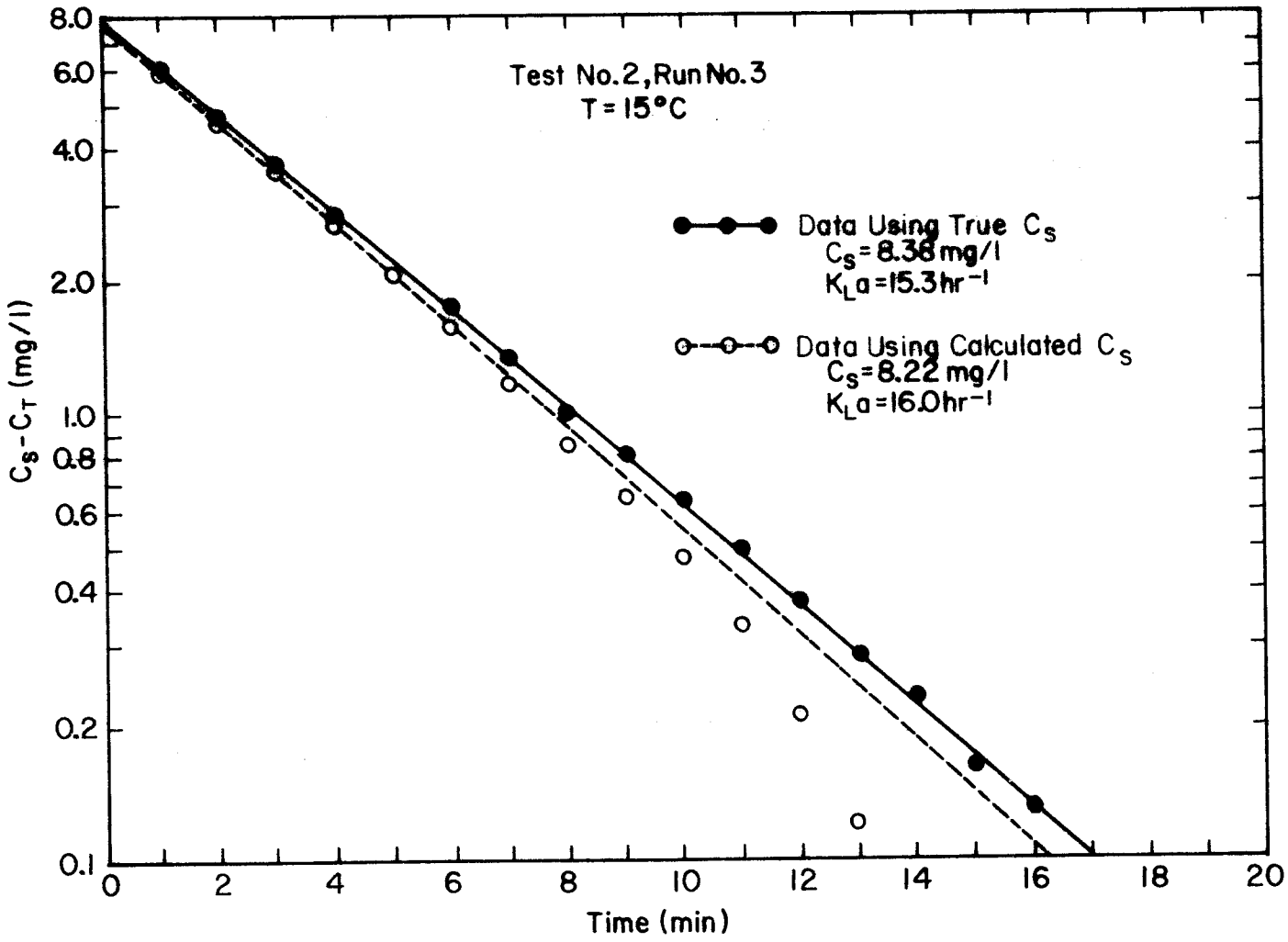

Fig. 4 Plots of $(C_s - C_t)$ Using Calculated and True C_s Values

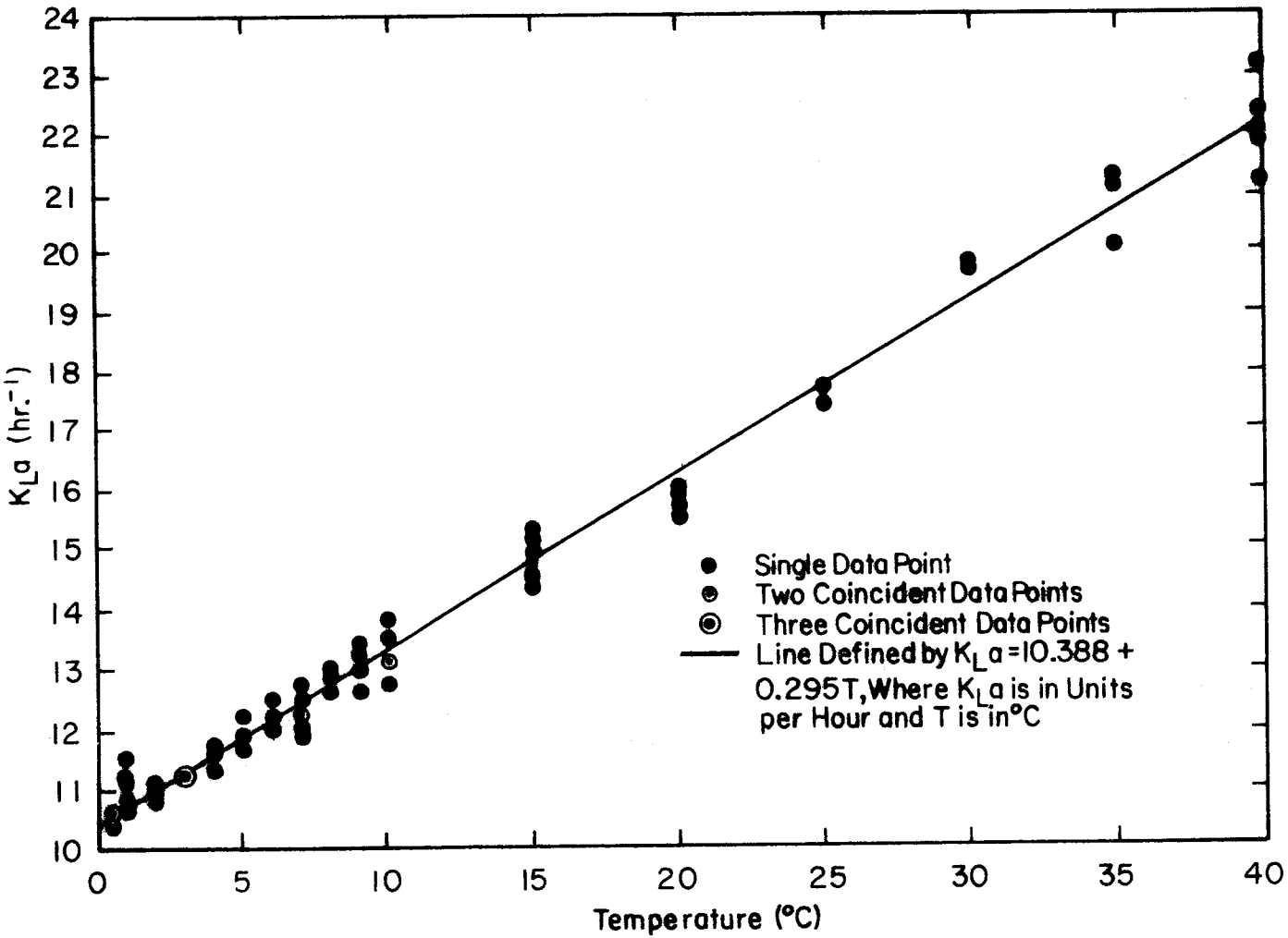

Fig. 5 Values of K_La Based on True C_s

Table 3

Statistical Analysis Summary for Both Methods of Determining $K_L a$

Computational Technique for Determining $K_L a$	Straight Line Correlation Coefficient	Estimate of Standard Error of Estimate (hr^{-1})	Intercept (hr^{-1})	95% Confidence Interval on (hr^{-1})	Slope ($hr^{-1}/^oC$)	95% Confidence Interval on Slope ($hr^{-1}/^oC$)
$K_L a$ from Calculated C_s	0.989	0.557	10.3812	$\pm$0.2000	0.2935	$\pm$0.0110
$K_L a$ from True C_s	0.995	0.379	10.3878	$\pm$0.1363	0.2953	$\pm$0.0075

describing the variation of $K_L a$ with water temperature is essentially the same, no matter which method is used for determining $K_L a$ but that, statistically speaking, the method which utilizes the true value of C_s provides a more precise analysis. This is also apparent from a visual comparison of Figure 2 with Figure 5 as there is less scatter of the data about the line of best fit in Figure 5.

A partial sensitivity analysis was performed to obtain information about how critical the assumed value of C_s is in determining $K_L a$. Actual data from 0.5, 20, and 40°C test runs were used to plot $(C_s - C_t)$ on semi-log paper for true C_s, true $C_s + 0.10$, and true $C_s - 0.10$ mg/l. $K_L a$ was calculated for each plot. The results appear in Table 4. As expected, the $K_L a$ determination at higher temperature is more sensitive to the value assumed for C_s. This is due to the greater relative effect equal changes in C_s have on $(C_s - C_t)$ because such small values of C_s are encountered at high water temperatures as compared to low water temperatures. It is interesting to note that an error of $\pm$ 6 percent in the value of $K_L a$ can be expected at a water temperature of 40°C if the assumed value of C_s is 0.10 mg/l from the true value. Even at 0.5°C an error of either -4 or +2 percent in $K_L a$ can be expected if C_s is either + 0.10 or - 0.10 mg/l, respectively, from the true value.

From Table 2 it can be seen that calculated values of C_s differed from the true C_s by as much as + 0.18 to - 0.15 mg/l. A summary of the differences appears in Table 5. Approximately 32 percent of the calculated C_s values were found to be the same as the true C_s values, 37 percent differed by less than $\pm$ 0.05 mg/l, 9 percent differed from $\pm$ 0.05 up to $\pm$ 0.10 mg/l, and 22 percent differed by $\pm$ 0.10 mg/l or more. The distribution of these differences with respect to temperature appears to be random indicating that the methods used for calculating C_s were correct but that the test conditions influenced the C_s values which actually existed. For example, even though C_s values were calculated for the temperature specified for each test, the water temperature actually maintained during any experiment could vary within the range of $\pm$ 0.1°C according to the tolerances established for these investigations. At a water temperature of 20.0°C, a variation in temperature of $\pm$ 0.10°C changes the C_s value by 0.02 mg/l and at 0.0°C the same change in water temperature affects the value of C_s by 0.04 mg/l. Other assumed "constant" conditions of the experiments could also have varied slightly. In addition, factors

Table 4

Sensitivity Analysis -
Effect of $K_L a$ of 0.10 mg/l Change in C_s

	T 0.5°C Test No. 13 Run No. 1	T 20°C Test No. 16 Run No. 2	T 40°C Test No. 24 Run No. 2
$K_L a$ from True C_s	10.4	16.0	22.4
$K_L a$ from C_s + 0.10	10.0	15.2	21.0
Variation from True $K_L a$	-0.4	-0.8	-1.4
% Variation from True $K_L a$	-4	-5	-6
$K_L a$ from C_s - 0.10	10.6	16.6	23.7
Variation from True $K_L a$	+0.2	+0.6	+1.3
% Variation from True $K_L a$	+2	+4	+6

which were not considered or which were assumed to not have any effect on the test results may, in fact, have influenced the data in some unexplained way. The most likely factors involved are the temperature and humidity of the atmosphere in the laboratory which was in contact with the free water surface of the test sample at the top of the aeration tank. Although the effects of these factors cannot be predicted, their influence on the test results can be eliminated in the data analysis step by the procedure outlined for determining the true C_s of the actual test conditions which existed. The number and magnitude of the differences between calculated and true C_s values encountered in this study were great enough to require that the trial-and-error procedure presented for identifying the true value of C_s be used if individual K_La values were to be determined in the most precise manner. It is important to note, however, that the standard

Table 5

Differences Between Calculated C_s and True C_s

Difference (mg/ℓ)	Number Greater than True C_s	Number Less than True C_s	Number Equal to True C_s
0.000			21
0.001 - 0.049	8	17	
0.050 - 0.099	6	0	
0.100 - 0.149	6	6	
$\geq$ 0.150	2	1	

approach of calculating C_s from tables and the application of Henry's law gave essentially the same result in terms of the relationship between K_La and temperature. The overall conclusions drawn from this study, then, are not dependent upon the method utilized to compute C_s. (This further substantiates the interrelated conclusions drawn earlier that variations in the true C_s values encountered were random, these variations were due to unbiased differences between assumed and actual test conditions, and the standard approach to calculating C_s is correct.)

IV. Results

A. Effect of Water Temperature Upon Power Requirements

The rotational speed of the experimental aeration vessel impeller was maintained at 600 rpm in order to ensure consistent and idential mixing conditions throughout all test runs. The torque on the

impeller shaft was measured during each test run to determine if water temperature had any effect upon power requirements. The applied torque, a measure of the power input to the aeration vessel, was found to be a constant 0.308 foot-pounds for all water temperatures tested (0 - 40°C). It was concluded, therefore, that water temperature has no effect upon the power required to achieve the same degree of mixing in aeration operations. The power required (3.08 foot-pounds per second) was applied to a volume of 0.669 cubic feet, so that the power per unit volume was 4.61 pounds/(second)(ft^2). The mean velocity gradient is the square root of the ratio of the power per unit volume divided by the absolute viscosity. For the range of water temperatures involved in this study, the average velocity gradient varied from 351 sec^{-1} at 0°C to 581 sec^{-1} at 40°C.

B. Effect of Water Temperature Upon Aeration

The nameplate ratings of aerators are usually referenced to an operating water temperature of 20°C. To find the aeration capacity when the expected operating water temperatures are different from 20°C, it has become standard engineering practice to make corrections by adjusting the overall oxygen mass transfer coefficient, K_La . The calculation employed is

$$K_La_{(T)} = K_La_{(20^oC)}\ F \tag{13}$$

where F is the 20°C reference correction factor which is defined by

$$F = \frac{K_La_{(T)}}{K_La_{(20^oC)}} \tag{14}$$

and T is the water temperature in °C. When a temperature, T , is specified, it has been assumed in the literature that the 20°C reference correction factor, F , is a constant for all aeration configurations. If this assumption is correct, F can be expressed in terms of this study's results as

$$F = \frac{10.3878 + 0.2953\ T}{16.2938} \tag{15}$$

or,

$$F = 0.6375 + 0.0181\ T\ . \tag{16}$$

Therefore, the effect of water temperature upon aeration is given by the expression

$$K_La_{(T)} = K_La_{(20^oC)}\ (0.6375 + 0.0181\ T)\ . \tag{17}$$

The 1.81 percent per ^{o}C variation in the $20^{o}C$ reference correction factor, F, measured in these experiments agrees reasonably well with values found by other investigators. In four sets of experiments, Downing and Truesdale (1955) observed variations of 2.02, 2.38, 1.73, and 2.06 percent per ^{o}C. Truesdale and Vandyke (1958) obtained results of 1.8, 1.5, 0.8, and 1.8 percent per ^{o}C. The average of these 8 observations is 1.76 percent per ^{o}C, a value which differs little from the results of this study.

It is more meaningful to reference corrections in $K_L a$ to a temperature of $0^{o}C$ because in this way it is possible to show the actual rate of change in $K_L a$ with temperature rather than the variation in the $20^{o}C$ correction factor. The results of this study referenced to $0^{o}C$ are expressed as (from equation 12)

$$\frac{K_L a}{K_L a_o} = 1 + 0.0284\ T \qquad (18)$$

where $K_L a_o$ is the value of $K_L a$ at $T = 0^{o}C$. From Eq. 18 it can be seen that the actual rate of change in $K_L a$ is 2.84 percent per ^{o}C. Plots of variations in $K_L a$ with water temperature, T, for a cross section of correction factors which have been reported in the literature are presented in Figure 6. All have been referenced to $0^{o}C$. As will be explained later, the correction factor has been reported in both the exponential and linear form. In Figure 6, those correction factors that are exponential are denoted by θ_e and those that are linear are denoted by θ_ℓ, θ being the coefficient which is either raised to the T power or multiplied by T for the exponential and linear forms, respectively. From Figure 6, it appears that the correction factor identified in this study is approximately the mean of the other correction factors which have been reported in the literature, be they exponential or linear in form.

It can be shown that for the values of θ reported and the water temperatures involved, $\theta_\ell \doteq \theta_e - 1$, so that values of θ_ℓ and θ_e can be compared directly.

C. Combined Effect of Elevation and Water Temperature Upon Aeration

Equation 6 can be used to construct diagrams which show the dependence of aerator capacity upon both elevation and water temperature. A different diagram is required for each operating DO concentration, C, of interest. Only one of these diagrams will be presented herein. The full process of its development will be given as an example of how other such diagrams can be prepared. The operating DO concentration, C, selected for this example was 2.0 mg/l because the use of this

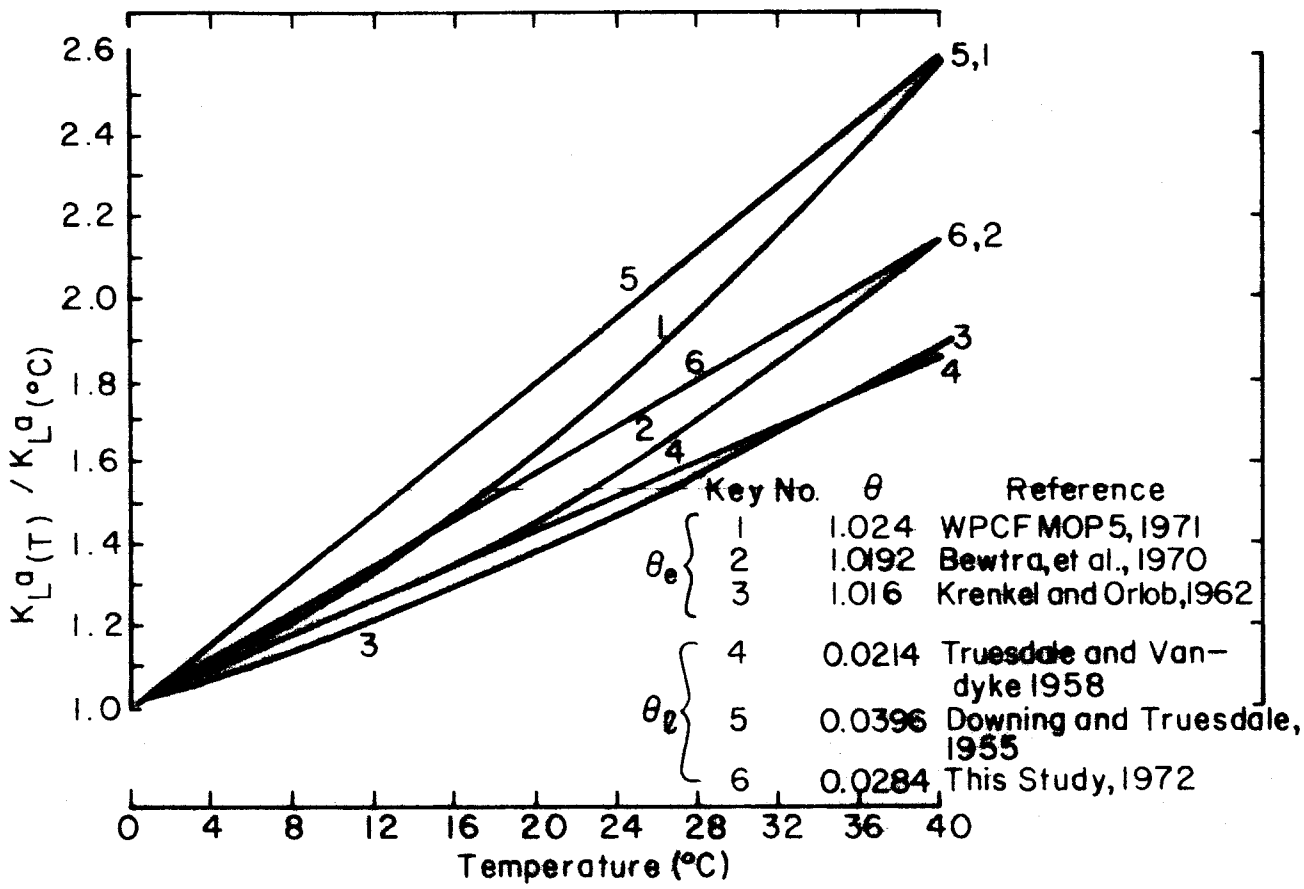

Fig. 6 Variation of $K_L a$ with Temperature

value seems to be common practice within the sanitary engineering profession. (It is stated in Water Pollution Control Federation MOP 5 (1971, page 15) that it is usually necessary to maintain this DO concentration in the aeration tank effluent in order to avoid the development of septic conditions in final settling tanks. In addition, Eckenfelder (1970, page 137) states that this DO concentration should be maintained if nitrification of the wastes being treated is to be achieved.)

The development of a diagram which can be used to determine the dependence of aerator capacity upon both elevation and water temperature follows these seven basic steps:

1. Calculate C_s for a wide range of water temperatures and elevation (see Table 6).
2. Calculate $(C_s - C)$ for all the above values of C_s (see Table 7).
3. Calculate values of K_La for all the water temperatures using Eq. 12 (see Table 8).
4. Multiply the corresponding values of $(C_s - C)$ and K_La (see Table 9).
5. Calculate the value $K_La(C_s - C)$ for water at 20^oC located at sea level.
6. Calculate values of operating efficiencies relative to the nameplate rating conditions specified in Step 5 for all water temperatures and elevations by means of the following relationship which has been developed from Eq. 6 (see Table 10):

$$\text{relative operating eff.} = \frac{K_La_{(T)}\ (C_s - C)}{K_La_{(20^oC)}\ (C_{s_{\text{sea level}}} - C)} \quad (19)$$

7. Enter all the values of Eq. 19 on a piece of rectilinear graph paper with elevation as ordinate and water temperature as abscissa. Draw isoaeration lines connecting all points of equal relative aeration efficiencies at desired intervals (see Figure 7).

It can be seen from Figure 7 that an aerator maintaining a constant aeration tank DO of 2.0 mg/l which is located at any combination of elevation and water temperature represented by the 1.00 isoaeration line will be transferring the same amount of oxygen into the water as the same aerator could if it was maintaining the same operating DO in the same aeration tank located at mean sea level and containing water having a temperature of 20^oC. Likewise, the same aerator operating in water with a DO concentration of 2.0 mg/l and located at any combination of elevation and water temperature represented by the 0.50 isoaeration line can only produce 50 percent of its 20^oC, constant 2.0 mg/l DO concentration, mean sea level output.

Charts for other operating DO concentrations would have the same general configuration as represented in Figure 7 although the isoaeration lines would not be located in idential positions.

Table 6

DO at Saturation for Various Elevations and Water Temperatures

Elevation (ft)	Atmospheric Pressure (atm.)	C_s(mg/ℓ) for								
		0°C	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
+ 18,000	0.499	7.28	6.35	5.60	4.98	4.48	4.06	3.70	3.35	3.01
+ 15,000	0.565	8.24	7.19	6.33	5.64	5.07	4.60	4.18	3.79	3.40
+ 12,000	0.637	9.30	8.12	7.15	6.37	5.72	5.19	4.72	4.28	3.84
+ 9,000	0.715	10.43	9.12	8.03	7.15	6.43	5.83	5.30	4.81	4.32
+ 6,000	0.802	11.74	10.25	9.04	8.05	7.24	6.56	5.97	5.42	4.85
+ 3,000	0.897	13.11	11.44	10.08	8.98	8.08	7.32	6.66	6.05	5.42
sea level	1.000	14.65	12.79	11.27	10.03	9.02	8.18	7.44	6.75	6.05
- 3,000	1.114	16.33	14.27	12.57	11.19	10.06	9.12	8.30	7.53	6.75

Table 7

$C_s - C$ ($C = 2.0$ mg/ℓ)

Elevation (ft)	$C_s - C$ (mg/ℓ) for								
	0°C	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
+ 18,000	5.28	4.35	3.60	2.98	2.48	2.06	1.70	1.35	1.01
+ 15,000	6.24	5.19	4.33	3.64	3.07	2.60	2.18	1.79	1.40
+ 12,000	7.30	6.12	5.15	4.37	3.72	3.19	2.72	2.28	1.84
+ 9,000	8.43	7.12	6.03	5.15	4.43	3.83	3.30	2.81	2.32
+ 6,000	9.74	8.25	7.04	6.05	5.24	4.56	3.97	3.42	2.85
+ 3,000	11.11	9.44	8.08	6.98	6.08	5.32	4.66	4.05	3.42
sea level	12.65	10.79	9.27	8.03	7.02	6.18	5.44	4.75	4.05
- 3,000	14.33	12.27	10.57	9.19	8.06	7.12	6.30	5.53	4.75

Table 8

$K_L a$ (hr^{-1})

0°C	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
10.39	11.86	13.34	14.81	16.29	17.76	19.24	20.70	22.18

Table 9

$K_L a\ (C_s - 2.)$

Elevation (ft)	$K_L a(C_s - 2.)$ mg/(ℓ)(hr) for								
	0°C	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C
+ 18,000	54.8	51.5	48.0	44.2	40.4	36.6	32.7	28.0	22.4
+ 15,000	64.8	61.5	57.8	53.9	50.0	46.2	41.9	37.0	31.1
+ 12,000	75.8	72.5	68.7	64.7	60.6	56.6	52.3	47.2	40.8
+ 9,000	87.6	84.4	80.5	76.2	72.2	68.0	63.5	58.2	51.5
+ 6,000	101.1	97.7	93.9	89.5	85.3	81.0	76.4	70.8	63.2
+ 3,000	115.5	111.8	107.9	103.3	99.0	94.4	89.6	83.8	75.8
sea level	131.4	127.8	123.8	118.9	114.3	109.7	104.7	98.8	89.8
- 3,000	149.0	145.3	141.0	136.0	131.2	126.3	121.2	114.4	105.2

Table 10

$$\frac{K_La_{(T)}(C_s - 2.0)}{K_La_{(20^oC)}(C_{s_{sea\ level}} - 2.0)}$$

Elevation (ft)	$K_La_{(T)}(C_s - 2.0)/K_La_{(20^oC)}(C_{s_{sea\ level}} - 2.0)$								
	0^oC	5^oC	10^oC	15^oC	20^oC	25^oC	30^oC	35^oC	40^oC
+ 18,000	0.47	0.45	0.41	0.38	0.35	0.32	0.28	0.24	0.19
+ 15,000	0.56	0.53	0.50	0.47	0.43	0.40	0.36	0.32	0.27
+ 12,000	0.66	0.63	0.60	0.56	0.53	0.49	0.45	0.41	0.35
+ 9,000	0.76	0.73	0.70	0.66	0.63	0.59	0.55	0.50	0.45
+ 6,000	0.88	0.85	0.82	0.78	0.74	0.70	0.66	0.61	0.55
+ 3,000	1.01	0.97	0.94	0.90	0.86	0.82	0.78	0.73	0.66
sea level	1.14	1.11	1.08	1.04	1.00	0.95	0.91	0.86	0.78
- 3,000	1.30	1.27	1.23	1.18	1.14	1.10	1.06	1.00	0.92

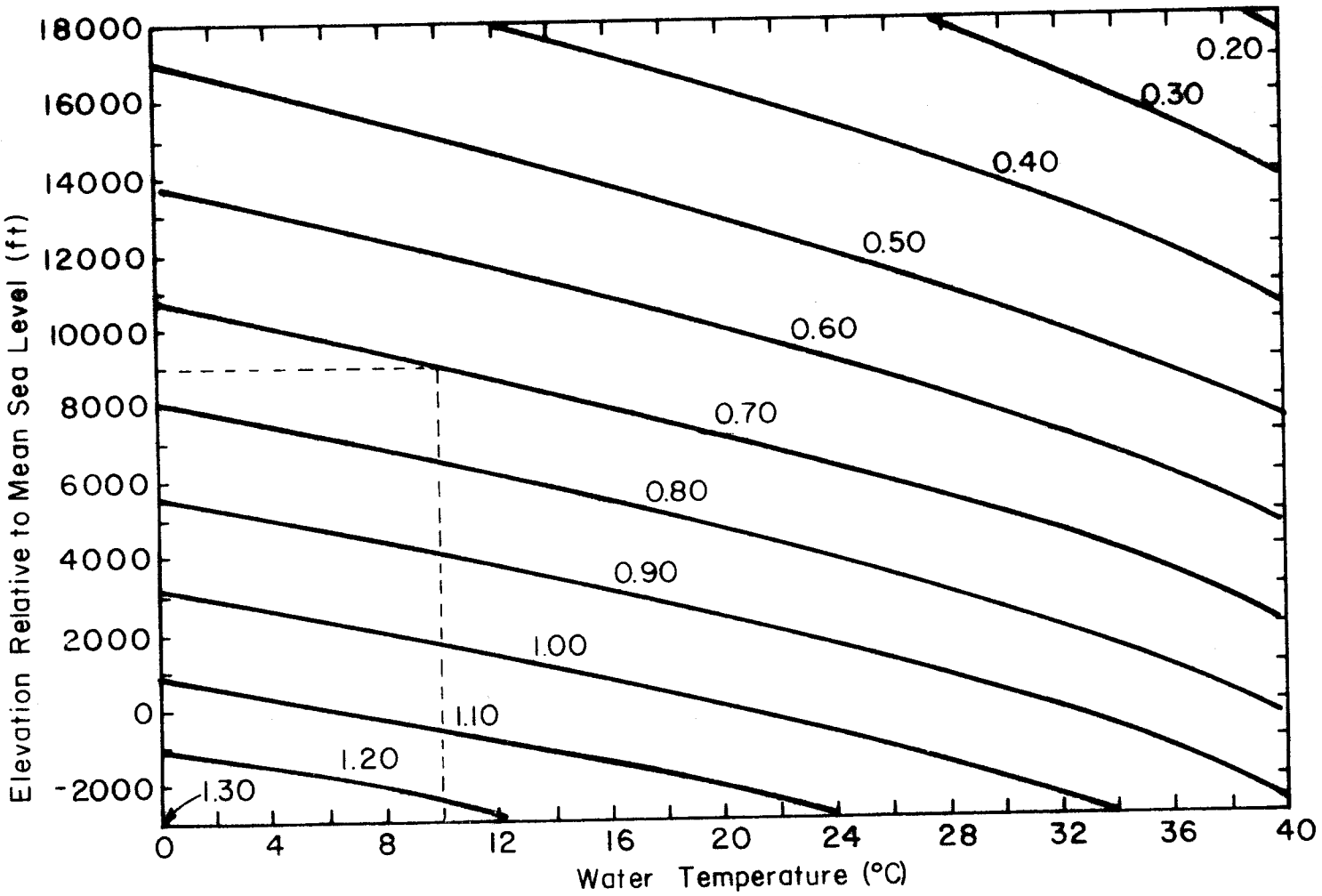

Fig. 7 The Combined Effect of Elevation and Water Temperature Upon Aeration (Operating DO= 2.0mg/l)

In order to use Figure 7 in aeration design it is only necessary to calculate aerator capacity at mean sea level in water having a temperature of 20°C and a DO concentration of 2.0 mg/l by means of Eq. 6. This value is then multiplied by the relative operating efficiency factor found in Figure 7 at the point represented by the operating elevation and water temperature. This procedure is illustrated in the following example:

A 50-hp aerator is rated at 2.5 lb O_2/(hp)(hr) in a 10^6 gal aeration tank. What will its output be in a 10^6 gal aeration tank located at an elevation of 9,000 ft. The water temperature of concern is 10°C and the DO level to be maintained is 2.0 mg/l.

1. Calculate aerator capacity in a 10^6gal aeration tank located at an elevation of mean sea level and containing water at a temperature of 20°C and a DO level of 0.0 mg/l.

$$(50 \text{ hp})(2.5 \text{ lb } O_2/(\text{hp})(\text{hr}) = 125 \text{ lb } O_2/\text{hr} .$$

2. Apply Eq. 6 to find $K_L a$ of the aerator

$$K_L a = \frac{125 \text{ lb/hr}}{(10^6 \text{ gal})(9.02 \text{ mg/l})} \times \frac{\text{mg/l}}{(8.34 \text{ lb}/10^6 \text{ gal})} = 1.66 \text{ hr}^{-1}.$$

3. Apply Eq. 6 to find aerator capacity at mean sea level, a water temperature of 20°C, and an operating DO concentration of 2.0 mg/l .

$$\frac{\Delta C}{\Delta t} = (1.66)(9.02 - 2) = 11.63 \frac{\text{mg}/\ell}{\text{hr}} .$$

4. Apply the correction factor obtained from Figure 7:

$$(0.7)(11.63)(8.34) = 67.8 \text{ lb } O_2/\text{hr}.$$

The above answer can be adjusted to any other design DO concentration, C , through Eq. 6. For design purposes, however, a chart based on a 0.0 mg/l DO design concentration instead of 2.0 mg/l would be more convenient as it would eliminate one calculation step if the design DO was different than 2.0 mg/l. The 2.0 mg/l DO example chart was produced in this report in order to more adequately show actual relative operating efficiencies commonly encountered in standard design practice.

Although the procedure outlined for developing the chart in Figure 7 appears to be dependent upon the $K_L a$ values characteristic of the aerator used in these experiments, it, in fact, was not. This can be seen by rewriting Eq. 19 of Step 6, the basis for constructing this chart, in the following form:

$$\text{rel. op. eff.} = \left[\frac{K_L a_{(T)}}{K_L a_{(20^\circ C)}}\right]\left[\frac{(C_s - C)}{(C_{s_{\text{sea level}}} - C)}\right]. \qquad (20)$$

The ratio $K_La_{(T)}/K_La_{(20^oC)}$, as mentioned earlier, is independent of the aerator being considered. So are the terms $(C_s - C)$ and $(C_{s_{\text{sea level}}} - C)$. Figure 7 and any other charts like it which are produced for other operating DO concentrations, therefore, are general in nature and are applicable for the design or evaluation of any aeration device.

One basic question has been of special interest in this investigation. That question is whether or not the tendency toward decreased aerator output due to the decreasing C_s and K_La values resulting from decreasing atmospheric pressure and water temperature, respectively, which are associated with increasing elevation can be overcome by the tendency toward increased aerator output due to increasing C_s values resulting from expected decreases in water temperature with increasing elevation. This question can be answered from the relationships shown in Figure 7. Most of the isoaeration lines are very nearly linear within the temperature range of 0 to 15°C, a range which includes water temperatures commonly encountered in domestic wastewater treatment facilities located in the mountainous regions of Colorado and, presumably, in many other similar regions of the United States. The slopes of those portions of the isoaeration lines located in this water temperature range vary from approximately 140 ft/°C for the 1.10 isoaeration line to 200 ft/°C for the 0.50 isoaeration line. By visually matching these values with their respective isoaeration lines in Figure 7 it is seen that a 1°C increase in water temperature is equivalent to an increase in elevation of between 140 and 200 ft as far as aerator performance is concerned, the actual value depending upon the particular altitude of reference. In order to determine whether the change in altitude or whether the change in water temperature predominates in influencing aerator performance it is necessary to find out if the change in elevation associated with a 1°C change in water temperature in actual wastewater treatment plants falls above or below the 140 to 200 ft range. Water temperatures encountered in wastewater aeration units are not solely a function of elevation. Many other factors are involved, the most important being detention time and ambient air temperature. Two extreme cases are encountered: (1) a completely housed activated sludge facility and (2) shallow aerated ponds. Intuitively, there are only moderate changes in water temperature with changes in elevation for an enclosed activated sludge plant. There should be little doubt that it would require a change in elevation greater than 200 ft to realize a 1°C change in water temperature. The remaining critical consideration is whether or not the case on the other extreme, the shallow aerated pond, would also require a change in elevation greater than 200 ft to realize a 1°C change in water temperature. In this case, the water temperature will be strongly influenced by ambient air temperature due to the long detention times involved. According to standard atmospheric tables, for ground elevations existing in the United States (or in the world for that matter), there is a 1°C decrease in atmospheric temperature for approximately every

500 foot increase in elevation. Even if the change in aeration pond temperature with elevation was twice that experienced in the atmosphere, it would still mean that a change in elevation greater than 200 ft would be required to experience a $1^{o}C$ change in the water temperature within the aeration pond. Because this is unlikely, it indicates that for all types of treatment facilities, ranging from enclosed activated sludge plants to shallow aeration ponds, those factors associated with increasing altitude which tend to reduce aerator output predominate over those factors which tend to enhance aerator output. This same conclusion can be drawn by examining Figure 7 by itself. There is absolutely no way to obtain a relative operating efficiency of 1.00 at elevations greater than 3,200 ft, no matter what the operating water temperature. If the effects of increasing elevation could be overcome by reduced water temperatures, it would be possible to find water temperatures at any elevation where a relative operating efficiency of 1.00 or greater could be attained.

It is interesting to derive and examine the mathematical relationship between elevation, water temperature, and efficiency, a specific case being represented in Figure 7. The saturation DO concentrations used in this study were calculated from the following empirical equation (ASCE Committee on Sanitary Engineering Research, 1960, page 48)

$$C_s = 14.652 - 0.41022\ T + 7.991\ X\ 10^{-3}\ T^2 - 7.7774\ X\ 10^{-5}\ T^3 \qquad (21)$$

where C_s is in mg/ℓ and T is ^{o}C. Equation 21 becomes quite inaccurate at water temperatures above $40^{o}C$.

From Eq. 6, the value of dC/dt at $0^{o}C$, zero elevation, and for $C = 0$ mg/ℓ is

$$\left(\frac{dC}{dt}\right)_0 = 14.652\ K_L a_0 \quad . \qquad (22)$$

For an atmospheric pressure P (in atm), Eq. 6 can be written

$$\frac{dC}{dt} = K_L a(PC_s - C) \quad . \qquad (23)$$

If one divides Eq. 23 by Eq. 22, one obtains

$$\frac{dC/dt}{(dC/dt)_0} = \left(\frac{K_L a}{K_L a_0}\right) \frac{(PC_s - C)}{14.652} \equiv E \qquad (24)$$

where E is the relative aeration efficiency. Setting $C = 0$ mg/ℓ in Eq. 24, gives

$$E_0 = \left(\frac{K_L a}{K_L a_0}\right) \frac{PC_s}{14.652} \quad . \qquad (25)$$

For water temperatures between 0 and 100°C, the following empirical equation is reasonably accurate:

$$\ell n \left(\frac{-dC_s}{dT}\right) = \alpha + \beta T \qquad (26)$$

where the numerical values of α and β are -0.92 and -0.0398 respectively. Integration of Eq. 26 gives

$$C_s = C_{s0} - \frac{e^{\alpha}}{\beta}(e^{\beta T} - 1) \qquad (27)$$

where C_{s0} is the value of C_s at $T = 0^{o}C$. Substitution of the appropriate values of α and β into Eq. 27 gives

$$C_s = C_{s0} - 10 + 10 \exp(-0.04\ T)\ . \qquad (28)$$

Clearly a plot of $\log[(C_s + 10 - C_{s0})/10]$ versus T should be a straight line. Defining $\beta \equiv -\gamma$, for $\gamma T < 1$,

$$e^{-\gamma T} - 1 \cong -\gamma T + \frac{\gamma^2 T^2}{2} - \frac{\gamma^3 T^3}{6} + \ldots \qquad (29)$$

so that Eq. 27 can be approximated by (for $T < 25^{o}C$)

$$C_s = C_{s0} - 0.4\ T + 8 \times 10^{-3} T^2 - 1.07 \times 10^{-4}\ T^3 + \ldots \qquad (30)$$

It is worth noting that the coefficients in Eq. 30 are practically identical to those of Eq. 21.

Equations 18, 25, and 28 can be combined to obtain

$$\frac{E_o}{P} = 0.317 + 0.009\ T + \frac{(0.683 + 0.0194\ T)}{e^{0.04\ T}}\ . \qquad (31)$$

Values of E_o/P calculated by Eq. 31 are listed in Table 11.

Table 11

Maximum Possible Efficiencies as a Function of Water Temperature

Water Temperature, ^{o}C	Maximum Possible Relative Efficiency, E_o/P, atm^{-1}
0	1.000
10	0.994
20	0.979
30	0.968

Table 11 (continued)

Maximum Possible Efficiencies as a Function of Water Temperature

Water Temperature ^oC	Maximum Possible Relative Efficiency, E_o/P, atm^{-1}
40	0.972
50	0.991
60	1.025
70	1.072
80	1.128
90	1.194
100	1.266

From Table 11, it is clear that $E_o/P \doteq 1$ for the water temperature range of 0 to 60^oC with a maximum error of only 3.2% (at 30^oC), which is less than the error in reported dissolved oxygen saturation concentrations (5% at 30^oC).

Equations 24 and 25 can be combined to obtain

$$E = E_o - \left(\frac{K_L a}{K_L a_o}\right) \frac{C}{14.65} \quad . \tag{32}$$

In addition, it is true that

$$E_o = (E_o/P)P \quad . \tag{33}$$

Making use of the fact that $E_o \doteq P$ for $0 \leq T \leq 60^oC$, Eq. 32 can be written

$$E = P - \left(\frac{K_L a}{K_L a_o}\right) \left(\frac{C}{14.65}\right) \quad . \tag{34}$$

The variation of P with elevation is given by the following equation which is derived in Appendix E

$$P = (1 - 6.87 \times 10^{-6} A)^{5.29} \tag{35}$$

where A is the altitude in feet and P is in atm. Equations 18, 34, and 35 can be combined to obtain

$$E = (1 - 6.87 \times 10^{-6} A)^{5.29} - (1 + 0.0284 T)(C/14.65) \quad . \tag{36}$$

Equation 36 is plotted in Figures 8 and 9.

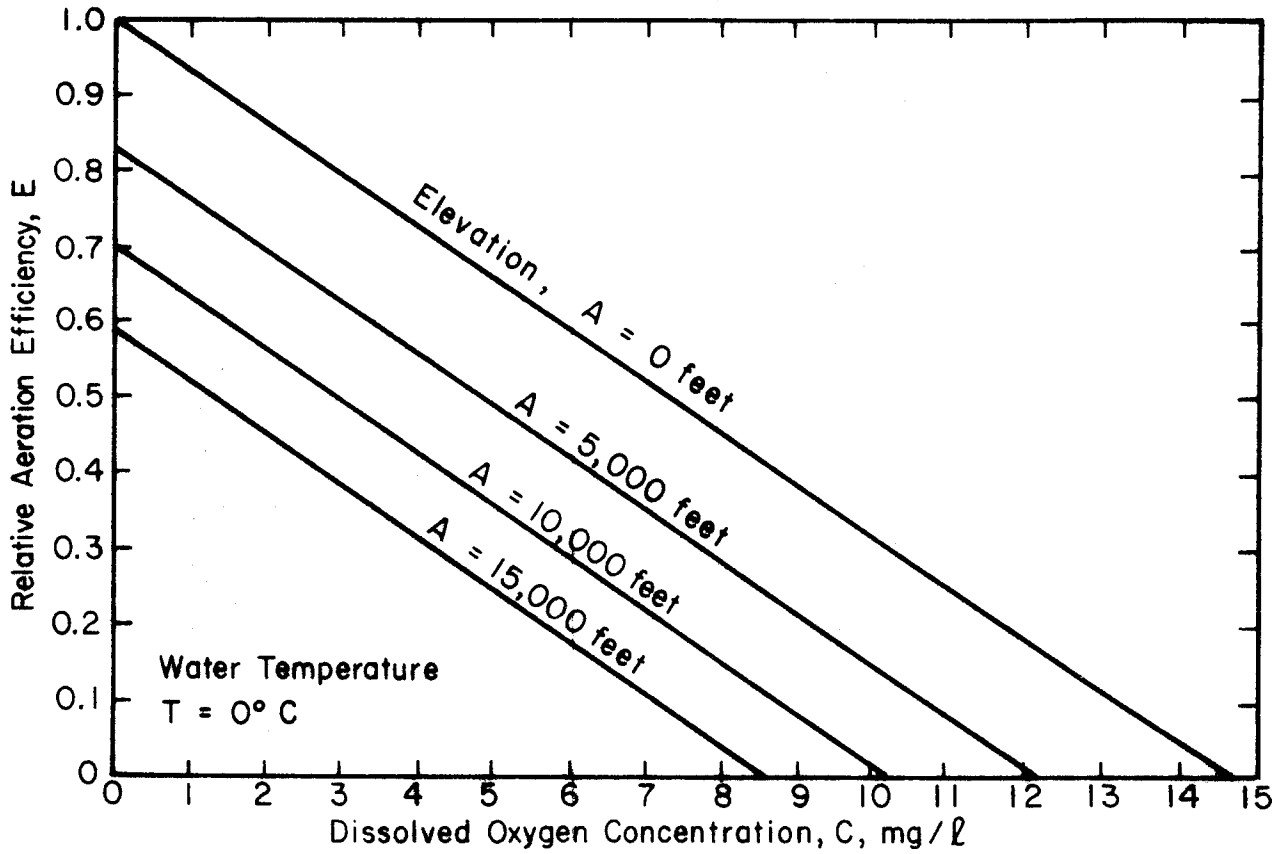

Fig. 8 - Effect of Elevation on Relative Aeration Efficiency.

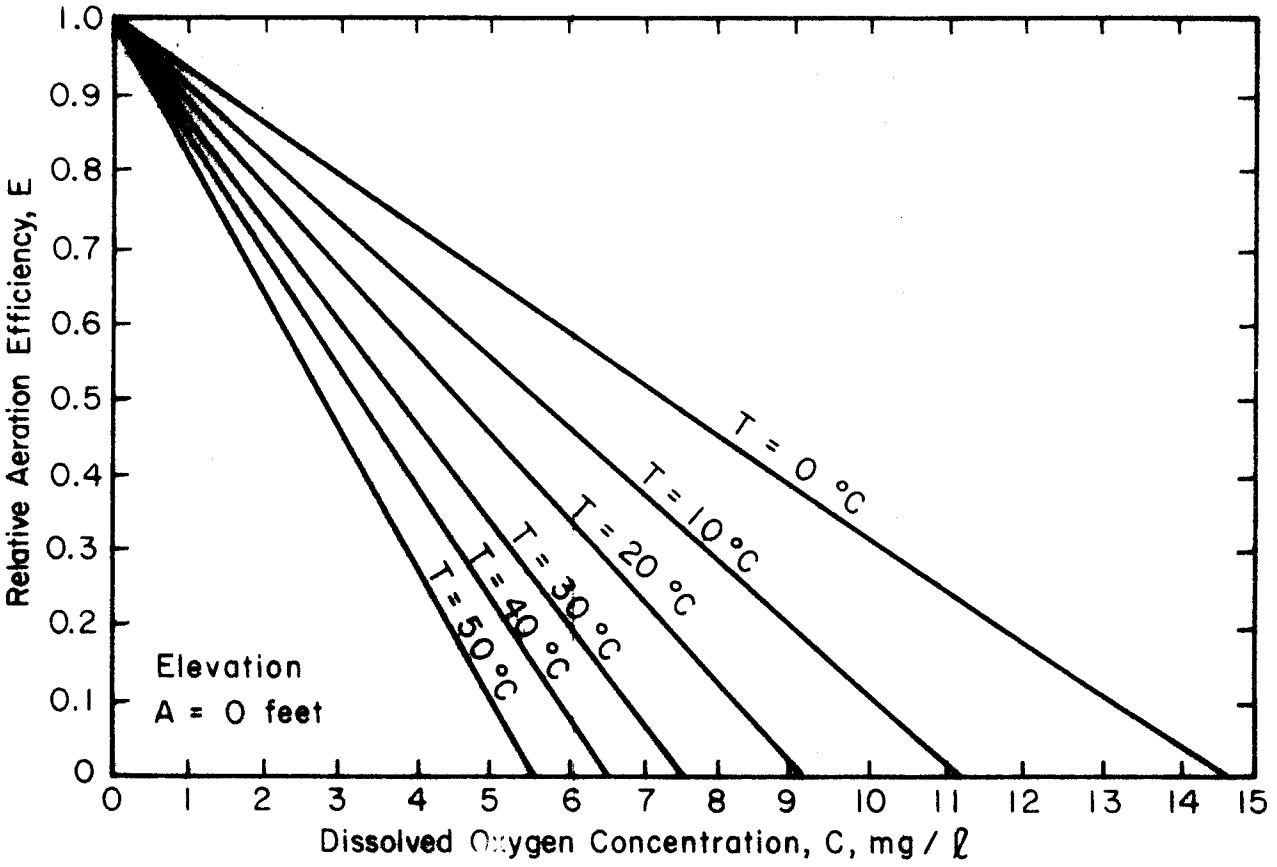

Fig. 9 - Effect of Water Temperature on Relative Aeration Efficiency.

V. Discussion

A. Arrhenius Equation

The rates of all chemical reactions are influenced by temperature. The fundamental relationship involved was first set down by Arrhenius. Working from the van't Hoff equation describing the dependence of the chemical equilibrium constant upon temperature he formulated the following equation which is commonly referred to as the van't Hoff-Arrhenius equation:

$$\frac{d(\ell n\ k)}{dT} = \frac{E_a}{RT^2} \tag{37}$$

where k is the reaction rate, T is temperature in degrees Kelvin ($^oC + 273$), E_a is the activation energy in cal/mole, and R is the universal gas constant, 1.99 cal/(mole)(^oK). Arrhenius assumed that the activation energy, E_a, was independent of temperature. Using this assumption and solving Eq. 37 by integration yields

$$\ell n \frac{k_2}{k_1} = \frac{E_a}{R} \left(\frac{1}{T_2} - \frac{1}{T_1}\right) . \tag{38}$$

If one defines

$$\ell n\ B \equiv \frac{E_a}{RT_1} + \ell n\ k_1 \tag{39}$$

then, Eq. 38 can be expressed in the commonly encountered exponential form of the van't Hoff-Arrhenius equation, i.e., (Andrews, 1970, pages 320-322)

$$k = B \exp(-E_a/RT) . \tag{40}$$

From Eq. 40 it can be seen that when experimental measurements are made of the variation in rate constant, k, with temperature, the ideal rate theory predicts that a plot of $R(\ell n\ k)$ versus $1/T$ should produce a straight line having a slope equal to $-E_a$. Likewise, a semi-log plot with k on the log ordinate scale and $1/T$ on the abscissa should produce a straight line with slope $-E_a/2.3R$.

It has been suggested by Krenkel and Orlob (1962, pages 58-59) that it is reasonable to consider the aeration process as an energy controlled kinetic rate process and, as such, its rate dependence upon temperature should be described by the van't Hoff-Arrhenius equation. They suggest that the activation energy, E_a, should be approximately 5 kcal/mole. A semi-log plot of K_La versus $1/T$ for the values of K_La predicted from Eq. 12 is shown in Figure 10. These data plot as

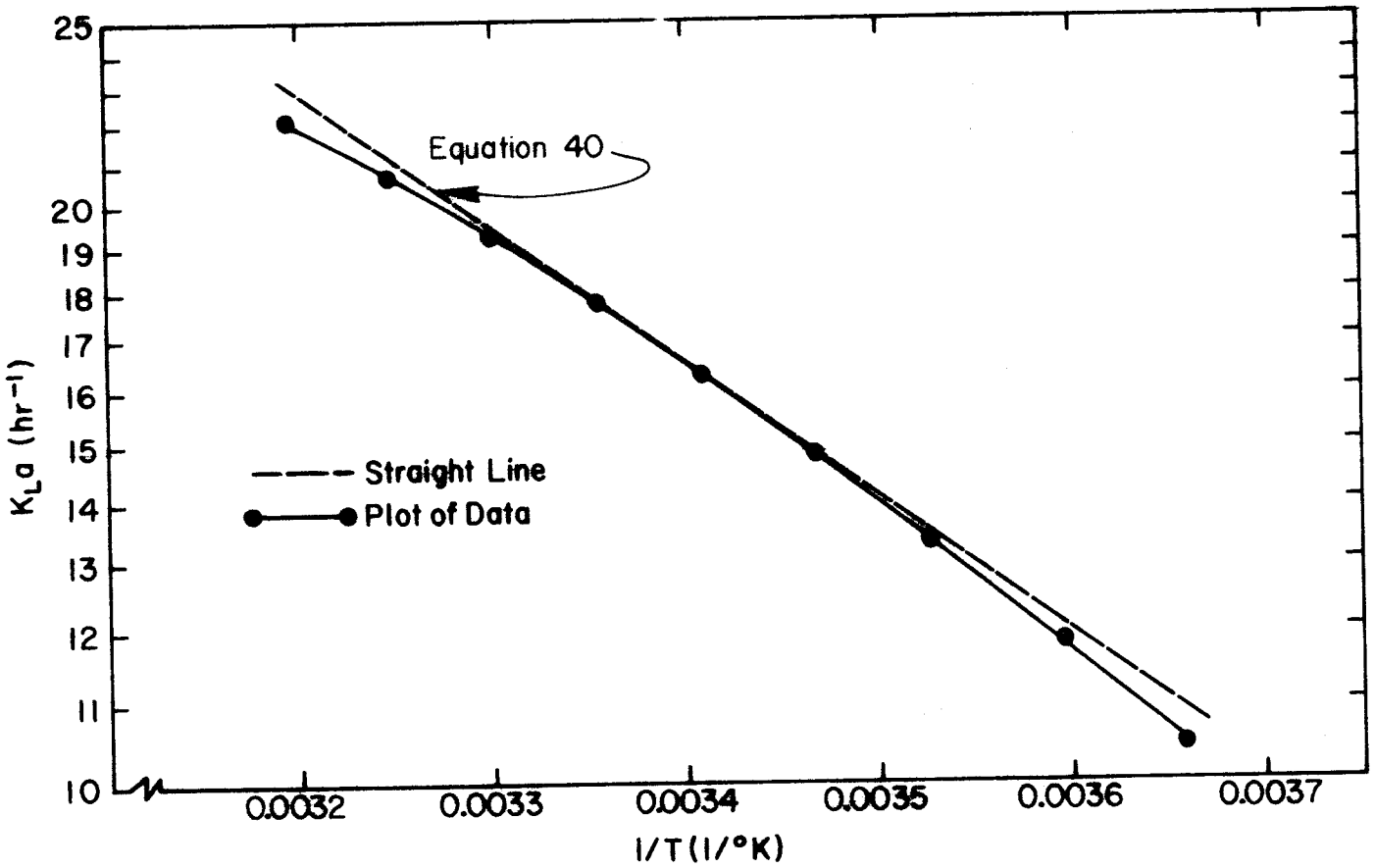

Fig. 10 Applicability Test for van't Hoff–Arrhenius Equation

a curve indicating that the activation energy, E_a, is not a constant as predicted by the ideal rate theory.

For chemical reactions and some biological processes, it has been found that the activation energy varies linearly with temperature[3] (Moore, 1962, page 568, and Ward and Jex, 1969). Activation energies were calculated over a wide range of temperatures using values of $K_L a$ predicted from Eq. 12 in order to find out if this relationship could be used to define the variation in activation energy, E_a, observed in these studies. This was done by starting with Eq. 37 and proceeding to the finite difference form assuming that E_a is constant over the temperature range being considered

$$\frac{d(\ln K_L a)}{dT} = \frac{E_a}{RT^2} = \frac{\Delta \ln(K_L a)}{\Delta T} . \tag{43}$$

For R equal to 1.99 cal/(mole)(^oK), Eq. 43 can be expressed in the form

$$E_a = (1.99)(T^2)(\Delta 2.3 \log K_L a)/(\Delta T) \tag{44}$$

where E_a is expressed in cal/mole and T in ^oK. Results obtained from Eq. 44 are shown in Table 12. No linear or exponential relationship between temperature and either E_a or changes in E_a could be identified from plots on either rectangular, semi-log, or log-log graph paper. This indicates that the van't Hoff-Arrhenius equation may be valid for any particular temperature, but it cannot be readily used for predicting the dependence of $K_L a$ values upon water temperature.

B. Linear Versus Exponential Variation in $K_L a$

The effect of water temperature upon $K_L a$ is commonly expressed in the following form:

$$K_L a_{(T)} = K_L a_{(20^oC)}\ \theta_e^{(T-20)} \tag{45}$$

where T is expressed in ^oC and θ_e is the dimensionless temperature coefficient. Equation 45 states that $K_L a$ changes in a constantly

---

3. For ordinary chemical reactions in liquid and solid systems near atmospheric pressure (Moore, 1962, page 568),

$$E_a = E_{ao} + RT = (E_{ao} + 544) + (1.99)\ (^oC) \tag{41}$$

where E_{ao} is the value of E_a at 0^oK. The rate of change in E_a is

$$d(E_a)/dT = R . \tag{42}$$

Table 12

Activation Energies

T (°C)	E_a (cal/mole)	ΔE_a (cal/mole)	ΔE_a/°C [cal/(mole)(°C)]
0.0	4221		
2.5		393	78.6
5.0	3828		
7.5		298	59.6
10.0	3530		
12.5		240	48.0
15.0	3290		
17.5		192	38.4
20.0	3098		
22.5		157	31.4
25.0	2941		
27.5		133	26.6
30.0	2808		
32.5		120	24.0
35	2688		
37.5		91	18.2
40.0	2597		
45.0		156	15.6
50.0	2441		
55.0		123	12.3
60.0	2318		
65.0		97	9.7
70.0	2221		
75.0		69	6.9
80.0	2152		
85.0		56	5.6
90.0	2096		
95.0		49	4.9
100.0	2047		

proportional (geometric) manner with respect to water temperature. Some investigators, however, have reported that $K_L a$ varies linearly (arithmetically) with temperature (ASCE Committee on Sanitary Engineering Research, 1961, pages 62-63). The linear expression takes the form of Eq. 17, i.e.,

$$K_L a_{(T)} = K_L a_{(20^oC)} [C + \theta_1 (T)] \qquad (46)$$

where C is a dimensionless constant which is the 0^oC intercept of the $K_L a_{(T)}/K_L a_{(20^oC)}$ versus T line, θ_1 is the temperature coefficient having units of $^oC^{-1}$, and T is water temperature in ^oC.

As pointed out by Fair, Geyer, and Okun (1968, page 28-45), the exponential form of the temperature correction relationship (Eq. 45) is limited in its applicability because θ_e is not a constant but varies with temperature. Use of either the exponential or linear relationship, however, gives results which are not significantly different over a moderate temperature range (Hull and Carbaugh, 1959). Calculations made from values of θ_e and θ_1 reported in the literature indicate that this temperature range is approximately 10 to 25°C (see Figure 11). It is significant that a review of the literature revealed that most studies which have been designed to date for studying the dependence of aeration upon water temperature have typically been carried out in systems where the water temperature was varied over the same range, $\pm$ 5°C in either direction. Therefore, there seems to be no real basis available for identifying the variation in K_La if large temperature corrections are to be made, i.e., if the expected temperature is more than about $\pm$ 10°C from 20°C, the standard reference temperature used in aeration calculations.

Field studies carried out under this project revealed that many wastewater treatment facilities located in the mountainous regions of Colorado aerate wastewaters having temperatures which are below 10°C during several months of the year. A few plants were found to be aerating wastewaters with temperatures below 1°C over the same period. Design of aerators for use in these extreme water temperatures has had to be based upon extrapolations of the moderate-temperature aeration data mentioned above. Standard practice has been to use the exponential relationship given in Eq. 45 for making these extrapolations (WPCF MOP 5, 1971, page 19, and Eckenfelder, 1970, page 136). However, Hull and Carbaugh (1959) state: "For large temperature changes, it appears that the available evidence favors the use of the linear relationship." The results of the study reported herein support Hull and Carbaugh's conclusion. It has been shown that a linear relationship exists between K_La and water temperature over the entire range of 0 to 40°C, a range almost twice as great as any tested to date. Besides testing the more general case by including a much greater temperature range, this study included a large number of data points, 68 to be exact, which produced a correlation coefficient for a straight line relationship of 0.995. From Table 3, it can be seen that the deviation of the data from the line of best fit was small [1 standard deviation equalled 2.3 percent of the best estimate of $K_La_{(20^oC)}$]. In short, there seems to be little reason to question the conclusion that the relationship between K_La and water temperature is linear.

VI. Summary

A laboratory-scale mechanical aeration system which simulated near perfect mixing conditions was used to study the variation in oxygen mass transfer rates into water with respect to changes in water temperature. A water temperature range of 0 to 40°C was tested. It was found that the variation of K_La for aeration systems varies linearly with temperature and that this variation can be predicted from the expression

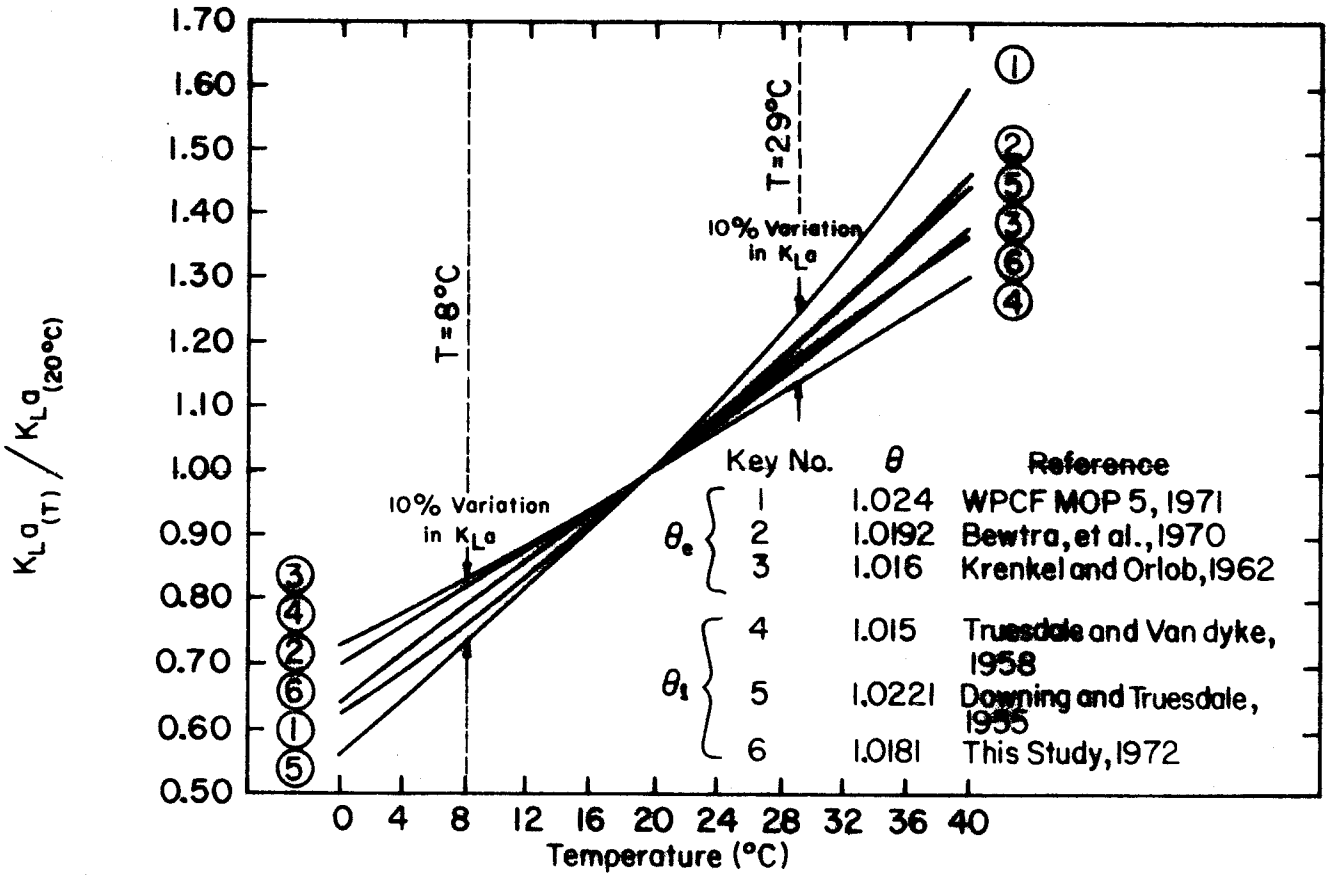

Fig. 11 Correction Factors for $K_L a$ from the Literature , 20°C Reference

$$K_L a_{(T)} = K_L a_{(20^oC)} \ (0.6375 + 0.0181\ T) \qquad (47)$$

where T is the water temperature in ^oC.

The above equation can be used in conjunction with Eqs. 35 and 28 to construct diagrams which show the combined effects that elevation and water temperature have upon aeration. Such diagrams show that those factors associated with increasing elevation which tend to reduce aerator output predominate over those factors which tend to enhance aerator output.

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Appendix A

CALIBRATION OF DISSOLVED OXYGEN METER AND STRIP CHART RECORDER

1. Set the dissolved oxygen meter control to "Red Line" and adjust the meter reading to the red mark.

2. Set the dissolved oxygen meter control to "Zero" and adjust the meter reading to 0.0 ppm.

3. Adjust the dissolved oxygen meter reading to the calculated saturated dissolved oxygen value for pure water at the sample temperature and measured barometric pressure (see Appendix B for calculating the saturated dissolved oxygen value).

4. Repeat Steps 1 through 3 until no further adjustment is necessary.

5. Set the dissolved oxygen meter control to "Zero" and adjust the zero setting on the strip chart recorder.

6. Set the dissolved oxygen meter control to an appropriate reading scale and adjust the strip chart recorder to record the calculated dissolved oxygen saturation value.

7. Repeat Steps 5 and 6 until no further adjustments need to be made on the strip chart recorder.

Appendix B

SATURATION DISSOLVED OXYGEN CONCENTRATIONS IN WATER

Standard Methods (Standard Methods for the Examination of Water and Wastewater. 1971. American Public Health Association, New York, page 480) recommends that the saturated dissolved oxygen concentration for water at a given barometric pressure be calculated according to the following formula:

$$C_s = C_1(P - p)/(1 - p) \qquad (48)$$

where C_s is the saturation DO concentration (mg/ℓ) in water at the stated barometric pressure, P, which is expressed in atmospheres. C_1 is the saturation DO concentration (mg/ℓ) in water at a barometric pressure of 1 atmosphere (mean sea level) and p is the vapor pressure of water in atmospheres at the temperature of the water being considered. Values of C_1 can be obtained from the following table (ASCE Committee on Sanitary Engineering Research. 1960. "Solubility of Atmospheric Oxygen in Water." Jour. Sanitary Engineering Division, Proceedings of ASCE SA4: 41-53, page 48) which is based on Eq. 21:

Table 13

Saturation Dissolved Oxygen Concentrations Used

Water Temperature, T, (^oC)	C_1 (mg/l)	Water Temperature, T, (^oC)	C_1 (mg/l)
0.0	14.65	8.0	11.84
0.5	14.45	9.0	11.55
1.0	14.25	10.0	11.27
2.0	13.86	15.0	10.03
3.0	13.49	20.0	9.02
4.0	13.13	25.0	8.18
5.0	12.79	30.0	7.44
6.0	12.46	35.0	6.75
7.0	12.14	40.0	6.05

Values of p can be obtained from Table 14 (Weast, Robert C. 1970. Handbook of Chemistry and Physics. The Chemical Rubber Co., Cleveland, Ohio, page D-143):

Table 14

Water Vapor Pressures Used

Water Temperature, T,(^{o}C)	(mm of Hg)	Water Temperature, T, (^{o}C)	(mm of Hg)
0.0	4.58	8.0	8.04
0.5	4.75	9.0	8.61
1.0	4.93	10.0	9.20
2.0	5.29	15.0	12.79
3.0	5.69	20.0	17.54
4.0	6.10	25.0	23.76
5.0	6.54	30.0	31.82
6.0	7.01	35.0	42.18
7.0	7.51	40.0	55.32

If one is willing to accept an error of 100(1-x)%, then

$$\frac{P-p}{1-p} = xP \quad \text{or} \quad p = \frac{P(1-x)}{1-xP} \tag{49}$$

For example, for an acceptable error of 1%,

$$p = \frac{0.01P}{1-0.99P} \quad . \tag{50}$$

Using Eq. 50, one can determine water temperatures (as a function of elevation) below which Eq. 48 may be ignored with an error of only 1%. These values are listed in Table 15.

Table 15

Maximum Water Temperatures for an Error of 1%

Elevation, feet	Maximum Water Temperature for which Eq. 48 can be Ignored with an Error of 1%, ^{o}F
0	212
1,000	150
2,000	125
3,000	111
4,000	99
5,000	90
6,000	84
7,000	78
8,000	74
9,000	70
10,000	66
11,000	63
12,000	60
13,00	57
14,000	55
15,000	53

Appendix C

ADJUSTMENT OF AIR FLOW

1. With the air flow set at approximately 1 SCFH, determine air pressure above existing atmospheric pressure at the discharge port of the flowmeter, P_A, by means of the water manometer connected to the air line at that point. Convert to pounds per square inch (psi) by means of the following formula:

$$P_A = \frac{0.036 \text{ psi}}{\text{in. of water}} \text{ X (inches of water)} . \quad (51)$$

2. Determine the existing barometric pressure, P_B, by means of a mercury barometer. Convert to psi by means of the following formula

$$P_B = \frac{0.0195 \text{ psi}}{\text{mm of Hg}} \text{ X (mm of Hg)} . \quad (52)$$

3. Determine the absolute pressure of the air at the discharge port of the flowmeter, P_D, by means of the following formula:

$$P_D = P_A + P_B . \quad (53)$$

4. Determine the difference between the absolute pressure of the air at the discharge port of the flowmeter, P_D, and standard atmospheric pressure by means of the following formula:

$$\Delta P = P_D - 14.70 \text{ psi} . \quad (54)$$

5. Correct the flowmeter reading for the air pressure at the flowmeter discharge port using ΔP from Step 4 and the following equation (valid only for ΔP between 0 and -4 psi):

$$\text{Actual Flow} = (1.00 - 0.0375\ \Delta P)(\text{Observed Flowmeter Reading}) \quad (55)$$

6. Determine the flowmeter reading under standard conditions for 1 SCFH from the flowmeter calibration curve.

7. Adjust the air flow until the flow calculated in Step 5 equals the flowmeter reading determined in Step 6.

Appendix D

DETERMINATION OF SODIUM SULFITE AND COBALT CHLORIDE DOSAGE

Experiments with actual test runs have shown that the use of 20 mg/l sodium sulfite (Na_2SO_3) per mg/l of DO contained in the agitated sample provides good dissolved oxygen annihilation and a short time lag before reaeration begins. Colbalt chloride ($CoCl_3$) was added as a catalyst at a ratio of 1 part $CoCl_3$ to 50 parts of Na_2SO_3 by weight.

Prepare a Na_2SO_3 solution with a concentration of 100g Na_2SO_3 per liter in distilled water. Also prepare a $CoCl_3$ solution of 20g $CoCl_3$ per liter in distilled water. The amounts of these solutions to be added to a sample under test can be calculated by means of the following formulas:

$$\text{ml } Na_2SO_3 \text{ solution} = 0.2 \text{ (sample DO)(sample volume)} \quad (56)$$

$$\text{ml } CoCl_3 \text{ solution} = 0.1 \text{ (ml } Na_2SO_3 \text{ solution used)} \quad (57)$$

where sample DO is in mg/l and sample volume is in liters.

Appendix E

DERIVATION OF AN EQUATION FOR PREDICTING ATMOSPHERIC PRESSURE AS A FUNCTION OF ELEVATION AND AIR TEMPERATURE

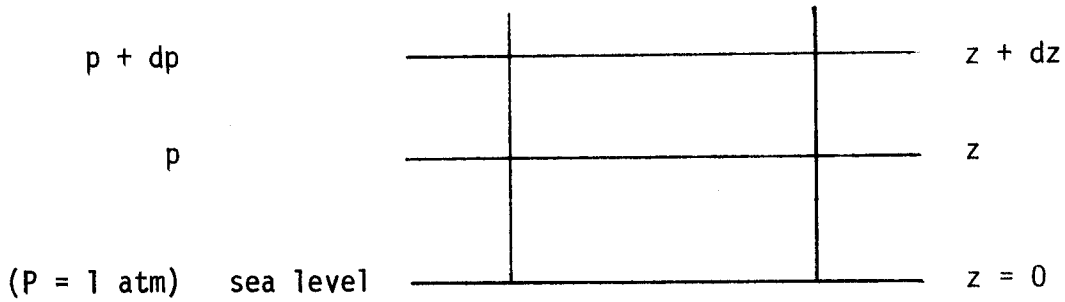

Figure 12 - Definition Sketch of Model Atmosphere

Let

p = atmospheric pressure, dynes/cm^2
z = elevation, cm
g = acceleration due to gravity, 981 cm/sec^2
ρ = density of air at elevation z, g/cm^3 .

Then, for a horizontal slice of atmosphere which is dz thick,

$$dp + \rho g\, dz = 0 \tag{58}$$

and

$$p(z + dz) = p(z) + dp \ . \tag{59}$$

From the ideal gas law,

$$pV = NRT \tag{60}$$

where V is volume in cm^3, N is moles of gas, R is the universal gas constant, 8.31×10^7 (dyne)(cm)/(mole)(^{o}K), and T is the absolute air temperature in ^{o}K(^{o}C + 273.1). By definition

$$\rho = \frac{MN}{V} \tag{61}$$

where M is the molecular weight of air (29 g/mole). Substituting Eq. 61 into Eq. 60 gives

$$p = \frac{\rho}{M} RT \ . \tag{62}$$

From Eq. 58,

$$dp = -\rho g\, dz \ . \tag{63}$$

Dividing Eq. 58 by Eq. 62 gives

$$\frac{dp}{p} = - \frac{gM\ dz}{RT} \quad . \tag{64}$$

Ordinarily,

$$T_z = T_o - (\frac{\Delta T}{\Delta z})\ z \tag{65}$$

where $\Delta T/\Delta z$ is 6.48×10^{-5} $^oK/cm$, T_z is the air temperature at elevation z in ^oK, and T_o is the corresponding air temperature at mean sea level in ^oK (for the U.S. standard atmosphere $T_o = 15 + 273 = 288\ ^oK$). Substituting Eq. 65 into Eq. 64 and integrating between the appropriate limits gives

$$\int_{p_o}^{p} \frac{dp}{p} = - \frac{gM}{R} \int_{o}^{z} \frac{dz}{T_o - (\Delta T/\Delta z)z} \tag{66}$$

which yields

$$p = p_o[1 - (\Delta T/\Delta z)(z/T_o)]^{\ gM/R(\Delta T/\Delta z)} \tag{67}$$

or,

$$p = p_o[1 + (\Delta T/\Delta z)\ \frac{z}{T_z}\ ]^{-gM/R(\Delta T/\Delta z)} . \tag{68}$$

Substituting the numerical values of the constants into the exponent of Eqs. 67 and 68 gives

$$\frac{gM}{R(\Delta T/\Delta z)} = \frac{(981)(29)}{(8.31 \times 10^7)(6.48 \times 10^{-5})} = 5.29 \ .$$

For the altitude A in feet,

$$A = z/30.5 \quad \text{or} \quad z = 30.5\ A \quad . \tag{69}$$

Substituting Eq. 69 into Eq. 67 along with the values previously given for the remaining constants and changing the units of p and p_o to atm, one obtains Eq. 35 which gives the correct value of P (in atm) for the U.S. standard atmosphere.

A more precise estimate of barometric pressure can be made with Eq. 68 because it takes into account the actual absolute air temperature T_z. Again, substituting Eq. 69 into Eq. 68 along with the values of the constants already given, and converting p and p_o to atm, one obtains

$$P = [1 + \frac{1.98 \times 10^{-3}\ A}{T_A}]^{-5.29} \tag{70}$$

where T_A is the air temperature in ^{o}K at altitude A, where A is in feet.

PART 2 - BOD AND COD REMOVAL FROM WASTEWATER BY AERATION WITH AIR AND OXYGEN

by

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The primary purpose of this study was to determine if the higher DO (dissolved oxygen) concentrations obtainable by using pure oxygen (O_2) for aeration would increase the removal rate of BOD (biochemical oxygen demand) and COD (chemical oxygen demand) in settled, municipal wastewater. All experimentation was carried out at an elevation of 5,226 feet above sea level where the annual average atmospheric pressure is 0.833 atm (atmospheres). All experimental work was done at room temperature, and the wastewater temperature was approximately 20°C (degrees centigrade). At this temperature and elevation, the saturation DO concentration is about (9 X 0.833 =) 7.5 mg/ℓ (milligrams per liter) when air is used for aeration, and about (7.5 X 100/20.9 =) 35.8 mg/ℓ when oxygen is used for aeration.

REASON FOR THE AERATION STUDY

From Figure 1, it is clear that the annual average air temperature decreases at a rate of about 3.6°F (degrees Fahrenheit) per 1,000 feet increase in elevation or per 300 miles toward true north. For example, the following are the approximate latitude equivalents of the corresponding elevations:

Elevation, feet	Latitude equivalent
5,000	Fort Collins, Colorado
6,000	Northern Wyoming Border
7,000	Canadian Border
8,000	Edmonton, Alberta, Canada
9,000	Northern Saskatchewan, Canada
10,000	Anchorage, Alaska
11,000	Artic Circle
12,000	Point Barrow, Alaska
13,000	75° North Latitude

When BOD is removed from wastewater by aeration, the rate of removal is considerably less at lower wastewater temperatures. In addition, while the saturation DO concentration is higher at low temperatures, it decreases with elevation in a manner directly proportional to atmospheric pressure. For example, for a wastewater at a temperature that is the same as the air temperature for a given elevation, the saturation DO concentrations would tend to be as follows:

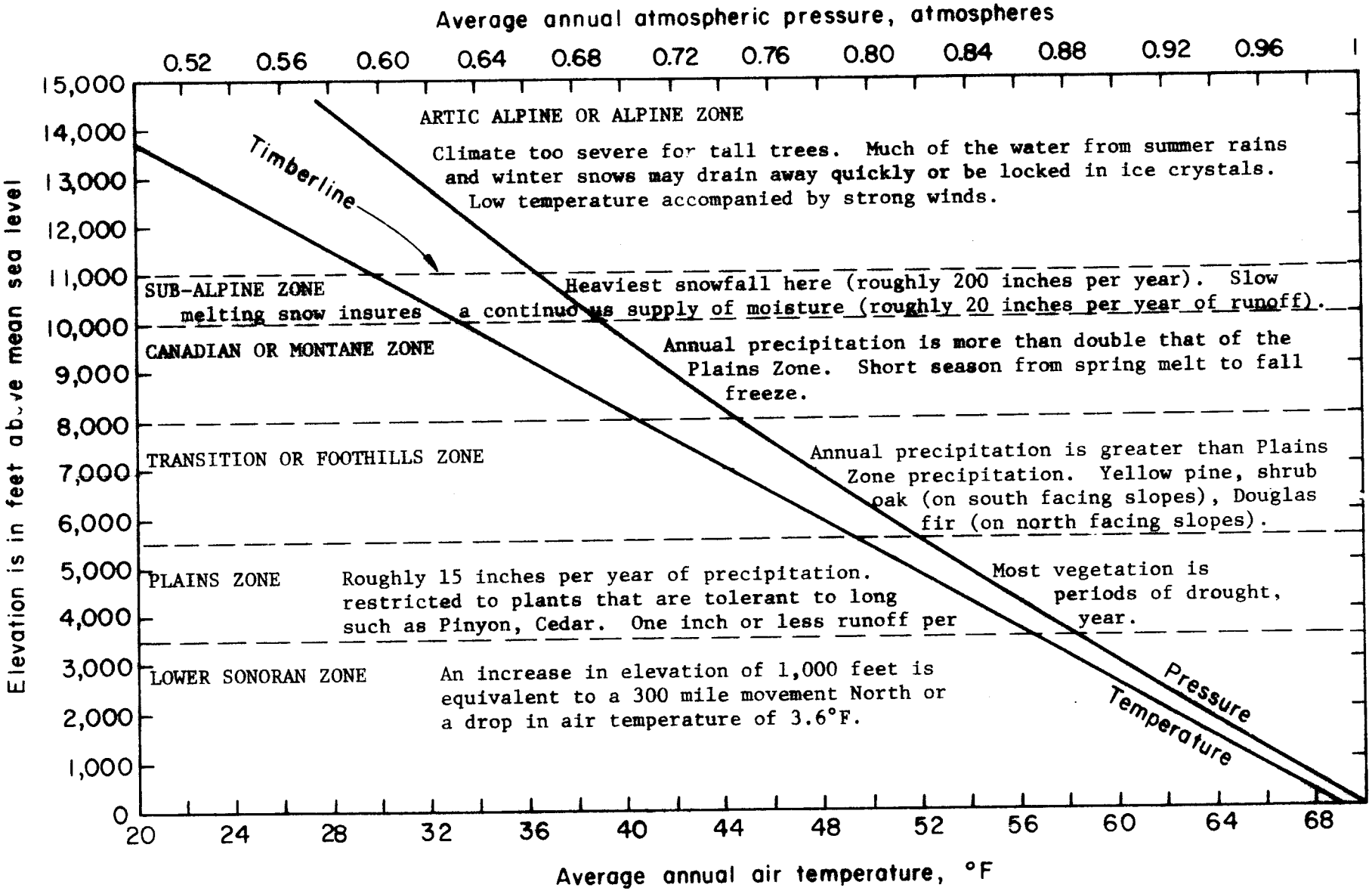

Figure 1. ELEVATION CLASSIFICATION FOR COLORADO

Elevation, feet	Saturation DO concentration corresponding to wastewater temperature equal to mean annual air temperature, mg/ℓ*
0	10.0
1,000	10.1
2,000	10.2
3,000	10.4
4,000	10.4
5,000	10.7
6,000	10.8
7,000	11.0

*Air temperatures used were those of the U.S. standard atmosphere which are 10°F less than those shown in Figure 1.

EXPERIMENTAL PROCEDURE

The wastewater used was the effluent from the primary settling basin of the Fort Collins, Colorado, wastewater treatment plant. Each sample was divided in half volumetrically and each half was placed into a battery jar. One jar was aerated with air and the other was aerated with industrial grade pure oxygen gas.

EXPERIMENTAL CONDITIONS

The experimental conditions for each of 6 separate tests are given in Table 1. Detailed conditions for each test follow.

Table 1

Experimental Conditions

Test Number	Initial volume in each battery jar, liters	Evaporation rate, liters per day		Gas flow rate, liters per hour		DO concentration mg/ℓ		Total volume loss due to sampling, liters	
		Air	Oxygen	Air	Oxygen	Air	Oxygen	COD	BOD
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	6.325	0.290	0.290	80	80	6.6	29	0.05	
2	8.045	0.445	0.310	80	80	7.0	30	0.03	
3	7.630	0.170	0.170	80	50	6.9	32	0.05	
4	9.050	0.360	0.430	15	15	7.0	29		0.785
5	8.800	0.240	0.240	4	4	5.2	22	0.11	
6	9.230	0.300	0.290	4	4	3.4	19	0.03	

In tests 1 and 2, aeration was by means of one inch diameter spherical stone diffusers placed at the bottom center of each battery jar. The samples from run 2 were all filtered before the COD determinations. This filtering removed up to 80% of the sample COD, so the data for this test is considered unrepresentative.

In test 3, a circumferential diffusor of plastic tubing perforated with small holes was used. This prevented a floc buildup on the jar walls observed in tests 1 and 2. In tests 1, 2, and 3, the COD was determined colorimetrically using a Hach colorimeter.

The same circumferential diffusor was used again for test 4. The BOD values are the ultimate BOD at 20^{0}C. Sample volumes for this test were 0.157 liters each, and the BOD concentration was determined manometrically.

For tests 5 and 6, the stone diffusors used in tests 1 and 2 were placed at the bottom edge of the circular jars and stirring was accomplished by mechanical means. The COD determinations for tests 5 and 6 were done potentiometrically using an oxidation-reduction probe and a recording automatic titrator. In test 6, the wastewater in the jars was permitted to settle about 30 minutes before the samples were taken from the supernatant.

EXPERIMENTAL RESULTS

The experimental results are given in Table 2. Sample volumes for the COD determination were 0.01 liters each. The evaporation rates given in Table 1 include the volume loss due to sampling except for test 4. The total volume loss due to sampling was less than 1% of the original volume for every test with the exception of tests 4 and 5 where it was 9 and 1% respectively.

In test 5 (observation numbers 36 and 37), distilled water was added to bring the volume back to the original volume before sampling. In test 6, distilled water was added to bring the volume back to the original volume after taking the sample.

ANALYSIS OF EXPERIMENTAL RESULTS

Table 3 shows the mass (in mg) of oxygen demand remaining as a function of time. These values were obtained by multiplying the mg/ℓ of oxygen demand by the corresponding volume in liters. The oxygen demand remaining is expressed as a fraction of the original mass of oxygen demand in columns 8 and 9 of Table 3.

Using the data in columns 2, 8, and 9 of Table 3, the time required for removal of 1/2 of the initial oxygen demand ($t_{1/2}$) in days was determined for each of the 6 tests, and these values are listed in Table 4.

Table 2 - EXPERIMENTAL RESULTS

Test	Time, Days	Oxygen Demand, mg/ℓ		Volume Before Taking Sample, liters		Observation Number
		Air	Oxygen	Air	Oxygen	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	0	250	250	6.325	6.325	1
1	0.94	220	100	6.052	6.052	2
1	1.87	100	75	5.783	5.783	3
1	2.94	175	120	5.473	5.473	4
1	3.87	60	90	5.203	5.203	5
1	4.00	75	75	5.165	5.165	6
1	5.00	50	25	4.875	4.875	7
2	0	100	80	8.045	8.045	8
2	0.17	35	25	7.969	7.992	9
2	1.17	30	30	7.524	7.682	10
2	2.17	30	25	7.080	7.371	11
2	4.0	30	30	6.265	6.805	12
3	0	180	170	7.630	7.630	13
3	0.82	120	100	7.491	7.491	14
3	2.0	55	25	7.290	7.290	15
3	3.86	25	35	6.974	6.974	16
3	4.93	30	25	6.794	6.794	17
3	5.94	20	20	6.621	6.621	18
3	12.2	20	20	5.550	5.550	19
4	0	215	170	9.050	9.050	20
4	0.98	84	94	8.540	8.472	21
4	1.95	42	52	8.033	7.897	22
4	2.99	0	10	7.502	7.292	23
4	4.03	0	6	6.972	6.690	24
5	0	283	259	8.800	8.800	25
5	0.35	358	362	8.716	8.716	26
5	1.04	269	260	8.550	8.550	27
5	2.06	232	220	8.305	8.305	28
5	3.33	246	237	8.000	8.000	29
5	3.92	230	222	7.858	7.858	30
5	4.02	201	176	7.835	7.835	31
5	4.83	282	249	7.640	7.640	32
5	5.81	193	202	7.406	7.406	33
5	6.85	169	200	7.158	7.158	34
5	17.85	307	295	4.510	4.510	35
5	38	127	133	8.800	8.800	36
5	44	26	29	8.800	8.800	37
6	0	322	329	9.190	9.230	38
6	0.95	275	282	8.905	8.955	39
6	1.88	183	182	8.911	8.960	40
6	7.08	222	233	7.630	7.720	41
6	9.08	187	191	8.590	8.650	42

Table 3 - ANALYSIS OF EXPERIMENTAL RESULTS

Test	Time, Days	Oxygen Demand Remaining, mg		Oxygen Demand Remaining, Fraction of Original		$\frac{t}{t_{1/2}}$
		Air	Oxygen	Air	Oxygen	
(1)	(2)	(3x5)	(4x6)	(8)	(9)	(10)
1	0	1582	1582	1.000	1.000	0
1	0.94	1330	605	0.840	0.382	0.467
1	1.87	578	434	0.365	0.274	0.931
1	2.94	957	657	0.605	0.415	1.462
1	3.87	312	468	0.197	0.295	1.923
1	4.00	388	388	0.245	0.245	1.990
1	5.00	244	122	0.154	0.077	2.49
2	0	805	644	1.000	1.000	0
2	0.17	279	200	0.347	0.311	0.159
2	1.17	225	231	0.280	0.359	1.093
2	2.17	213	184	0.265	0.286	2.03
2	4.0	188	204	0.233	0.317	3.74
3	0	1373	1296	1.000	1.000	0
3	0.82	900	749	0.656	0.577	0.377
3	2.0	401	182	0.292	0.140	0.921
3	3.86	174	244	0.127	0.180	1.778
3	4.93	204	170	0.148	0.131	2.27
3	5.94	132	132	0.096	0.102	2.74
3	12.2	111	111	0.088	0.086	5.62
4	0	1950	1540	1.000	1.000	0
4	0.98	717	795	0.368	0.516	0.98
4	1.95	338	411	0.173	0.267	1.95
4	2.99	0	73	0.000	0.047	2.99
4	4.03	0	40	0.000	0.026	4.03
5	0	2490	2280	1.000	1.000	0
5	0.35	3120	3160	1.252	1.386	0.016
5	1.04	2300	2220	0.924	0.974	0.049
5	2.06	1928	1828	0.774	0.802	0.097
5	3.33	1970	1898	0.792	0.832	0.156
5	3.92	1810	1745	0.727	0.765	0.184
5	4.02	1573	1378	0.632	0.604	0.189
5	4.83	2150	1903	0.864	0.835	0.226
5	5.81	1430	1497	0.575	0.606	0.272
5	6.85	1210	1430	0.486	0.627	0.321
5	17.85	1383	1330	0.556	0.583	0.839
5	38	1118	1170	0.449	0.513	1.783
5	44	229	255	0.092	0.112	2.06
6	0	2960	3040	1.000	1.000	0
6	0.95	2450	2520	0.828	0.829	0.134
6	1.88	1630	1630	0.551	0.537	0.265
6	7.08	1695	1800	0.573	0.592	0.995
6	9.08	1608	1650	0.544	0.543	1.280

Table 4

Time Required for Removal of 50% of the Oxygen Demand

Test	$t_{1/2}$, days		$\frac{t_{1/2}\ (oxygen)}{t_{1/2}\ (air)}$	Average $t_{1/2}$, days
(1)	Air (2)	Oxygen (3)	(4)	(5)
1	2.36	1.67	0.708	2.01
2	1.01	1.14	1.128	1.07
3	2.28	2.06	0.904	2.17
4	0.86	1.13	1.314	1.00
5	19.5	23.2	1.190	21.3
6	7.1	7.1	1.000	7.1
Average			1.040	

Clearly, the samples varied considerably in their biodegradability. Regardless, as column 4 of Table 4 shows, no improvement is obtained by the use of oxygen in place of air as far as rate of removal is concerned.

Using t to represent the time of aeration in days, the average values of $t_{1/2}$ from column 5 of Table 4 along with column 2 of Table 3 were used to calculate the ratio $t/t_{1/2}$ given in column 10 of Table 3. The average of columns 8 and 9 of Table 3 is plotted in Figure 2 versus column 10 of Table 3.

Because, from the foregoing discussion, the use of oxygen does not increase the rate or oxygen demand removal, it would appear that the most beneficial use of oxygen in wastewater treatment is where conventional methods of aeration can maintain minimum DO concentration only with great difficulty. In the constact-stablization activated-sludge wastewater treatment plant, there are two possible uses of oxygen that may compare favorably with conventional methods of aeration. One application of oxygen would be in the activated-sludge stabilization basin where part of the underflow from the secondary sedimentation basin is aerated separately before being returned to the activated sludge contact basin. The other application of oxygen would be in the activated-sludge contact basin itself in order to permit much higher concentrations of mixed-liquor suspended solids and consequently greater oxygen demand removal. Use of oxygen in the activated-sludge stabilization basin permits addition of sludge digester supernatant as a nutrient.

While air is 20.9% oxygen by volume, it is 23% oxygen by weight. As a result, one might expect that in Table 1, division of column 7 by the corresponding value in column 8 would yield a constant value of 0.23. Column 2 of Table 5 shows that this is the case with the exception of test number 6.

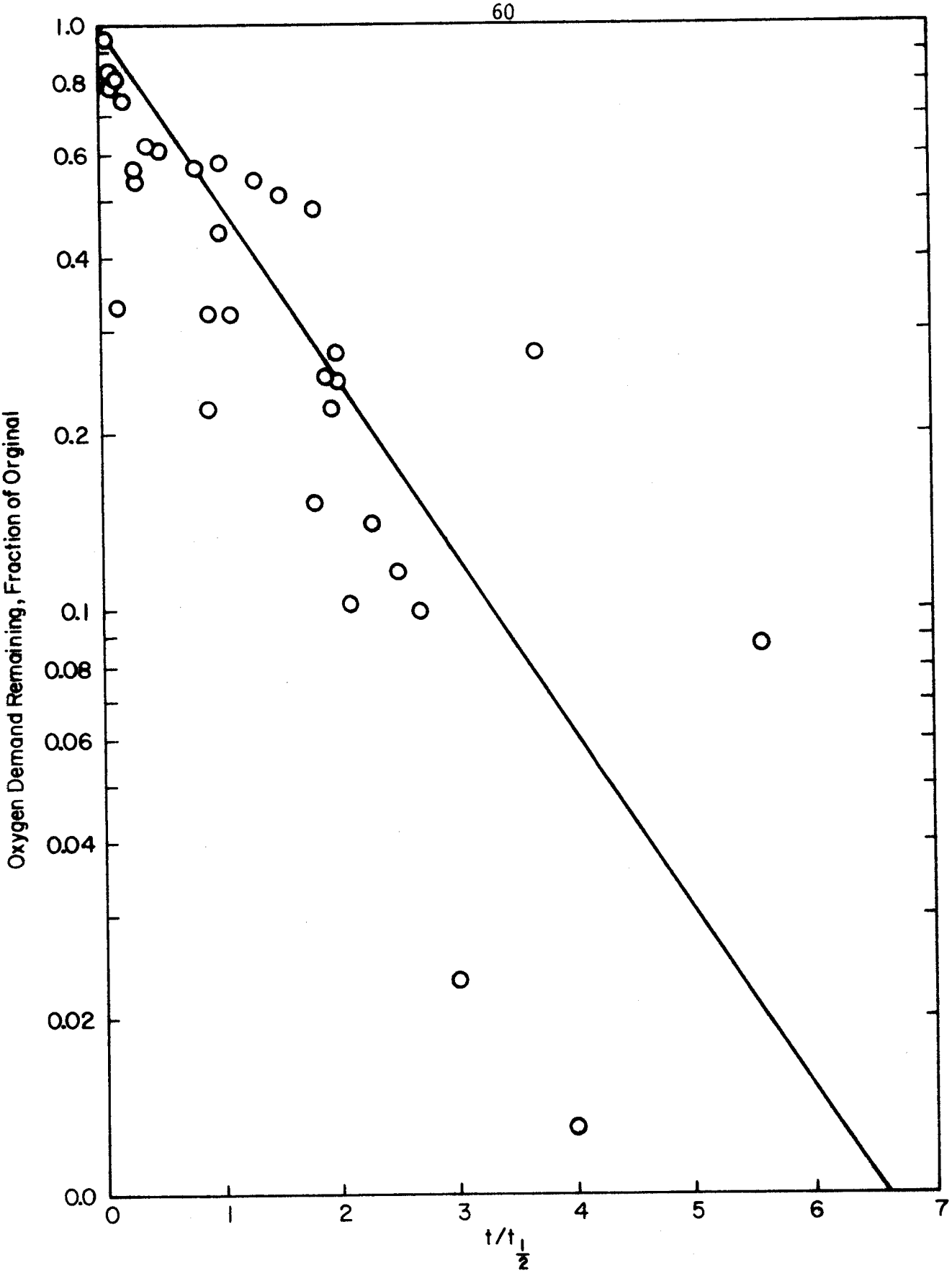

Fig. 2 - Fraction of Oxygen Demand Remaining Versus $t/t_{\frac{1}{2}}$

Table 5 - ANALYSIS OF EXPERIMENTAL CONDITIONS

Test Number	DO Air / DO Oxygen	Test Duration, $t/t_{1/2}$	Average OD Remaining, grams		Gas Flow Rate, Liters per Hour per Gram OD Remaining (Average)		Fraction of Saturation DO Concentration	
			Air	Oxygen	Air	Oxygen	Air	Oxygen
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	0.23	2.49	0.750	0.750	106	106	0.880	0.810
2	0.23	3.74	0.286	0.228	280	350	0.933	0.839
3	0.22	5.62	0.345	0.325	232	145	0.920	0.894
4	0.24	4.03	0.654	0.516	23.0	29.0	0.933	0.810
5	0.24	2.06	1.320	1.210	3.03	3.30	0.693	0.615
6	0.18	1.28	1.953	2.01	2.04	1.99	0.454	0.531

Table 6 - EFFICIENCY OF OXYGEN TRANSFER

Test Number	Initial Oxygen Consumption Rate, Grams per Hour		Oxygen Supply Rate, Grams per Hour		Oxygen Supply Rate ÷ Initial Oxygen Consumption Rate		Efficiency of Oxygen Transfer, Percent	
	Air	Oxygen	Air	Oxygen	Air	Oxygen	Air	Oxygen
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	0.0228	0.0228	16.8	38	738	3860	0.136	0.0259
2	0.0217	0.0174	16.8	88	775	5060	0.129	0.0198
3	0.0183	0.0146	16.8	55	919	3410	0.109	0.0293
4	0.0565	0.0445	3.15	16.5	56	371	1.785	0.269
5	0.0034	0.0031	0.84	4.4	248	1422	0.404	0.0704
6	0.0121	0.0124	0.84	4.4	69	355	1.357	0.282

For a test of duration $t/t_{1/2}$, the average oxygen demand (OD) remaining expressed as a fraction of the initial oxygen demand is

$$\frac{\text{average oxygen demand}}{\text{initial oxygen demand}} = \frac{1 - 10^{-0.3\ t/t_{1/2}}}{0.695\ t/t_{1/2}} \qquad (1)$$

Equation 1 gives the average fraction of the initial oxygen demand remaining and is plotted in Figure 3. The average oxygen demand remaining (given in columns 4 and 5 of Table 5) is the product of the initial oxygen demand (given in Table 3) times the average fraction of initial oxygen demand remaining (given in Figure 3) corresponding to the $t/t_{1/2}$ values given in column 3 of Table 5. The gas flow rate given in columns 6 and 7 of Table 5 are the values given in columns 5 and 6 of Table 1 divided by the average oxygen demand remaining given in columns 4 and 5 of Table 5. The fraction of saturation dissolved oxygen concentration given in columns 8 and 9 of Table 5 are the values given in columns 7 and 8 of Table 1 divided by 7.5 mg/ℓ and 35.8 mg/ℓ, respectively.

In Figure 4, the fraction of saturation dissolved oxygen concentration achieved (columns 8 and 9 of Table 5) is plotted versus the gas flow rate (columns 6 and 7 of Table 5). Because the rate of oxygen demand removal has been shown to be independent of the dissolved oxygen concentration, one might as well use relatively low dissolved oxygen concentrations and achieve the same results. In any event, Figure 4 suggests that attempting to maintain dissolved oxygen concentrations greater than about 70% of saturation requires excessive gas flow rates.

Under the test conditions, each liter of air contained 0.21 grams of oxygen, and each liter of oxygen contained about 5 times this amount or 1.1 grams of oxygen. The rate at which oxygen was being consumed was

$$\text{oxygen consumption rate} = \frac{(0.693)(\text{oxygen demand remaining})}{t_{1/2}} \qquad (2)$$

A special case of Eq. 2 is the following

$$\text{initial oxygen consumption rate} = \frac{(0.693)(\text{initial oxygen demand})}{t_{1/2}} \qquad (3)$$

Equation 3 was used along with the data in Tables 3 and 4 (column 5) to calculate the initial oxygen consumption rates given in columns 2 and 3 of Table 6. The data in columns 5 and 6 of Table 1 was used to calculate the data in columns 4 and 5 of Table 6. Columns 6 and 7 in Table 6 were calculated using the data in columns 2, 3, 4, and 5 of Table 6. Columns 8 and 9 of Table 6 are the recipricals of columns 6 and 7 multiplied by 100%. The data in columns 6 and 7 of Table 6 and columns 7 and 8 of Table 1 are plotted in Figure 5.

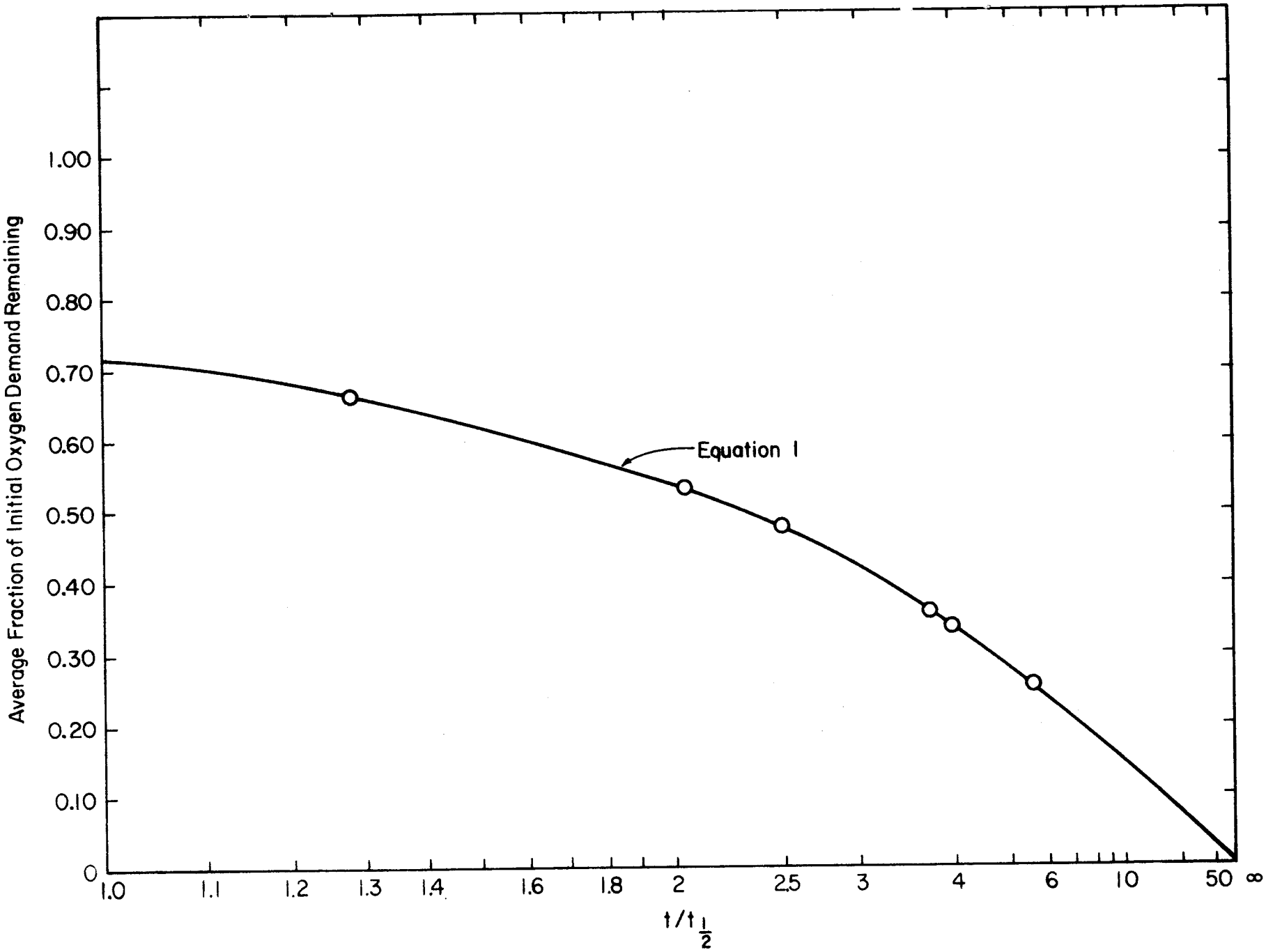

Fig.3- Average Fraction of Initial Oxygen Demand Remaining.

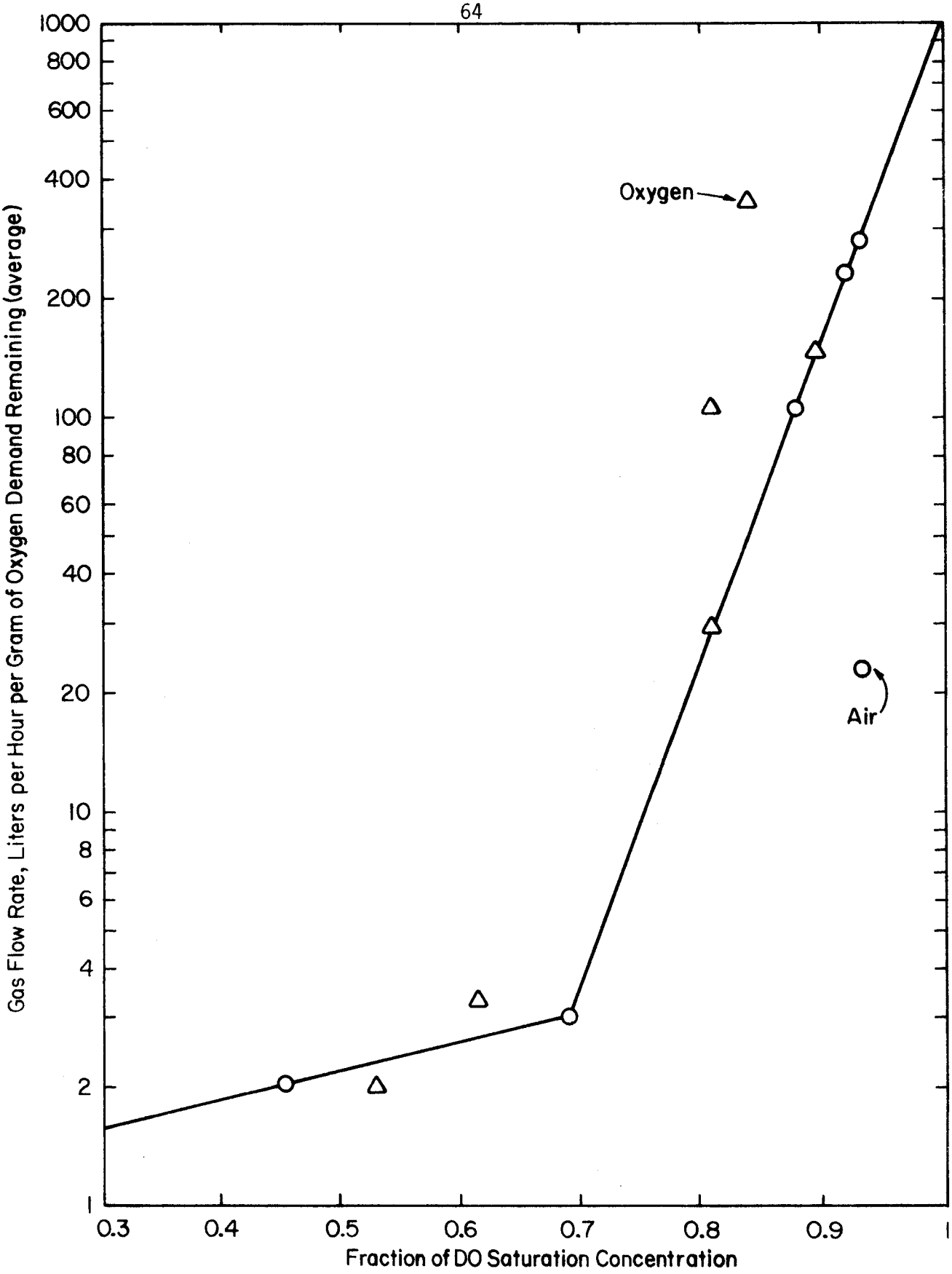

Fig. 4- Effect of Gas Flow Rate on Fraction of Dissolved Oxygen Saturation Concentration.

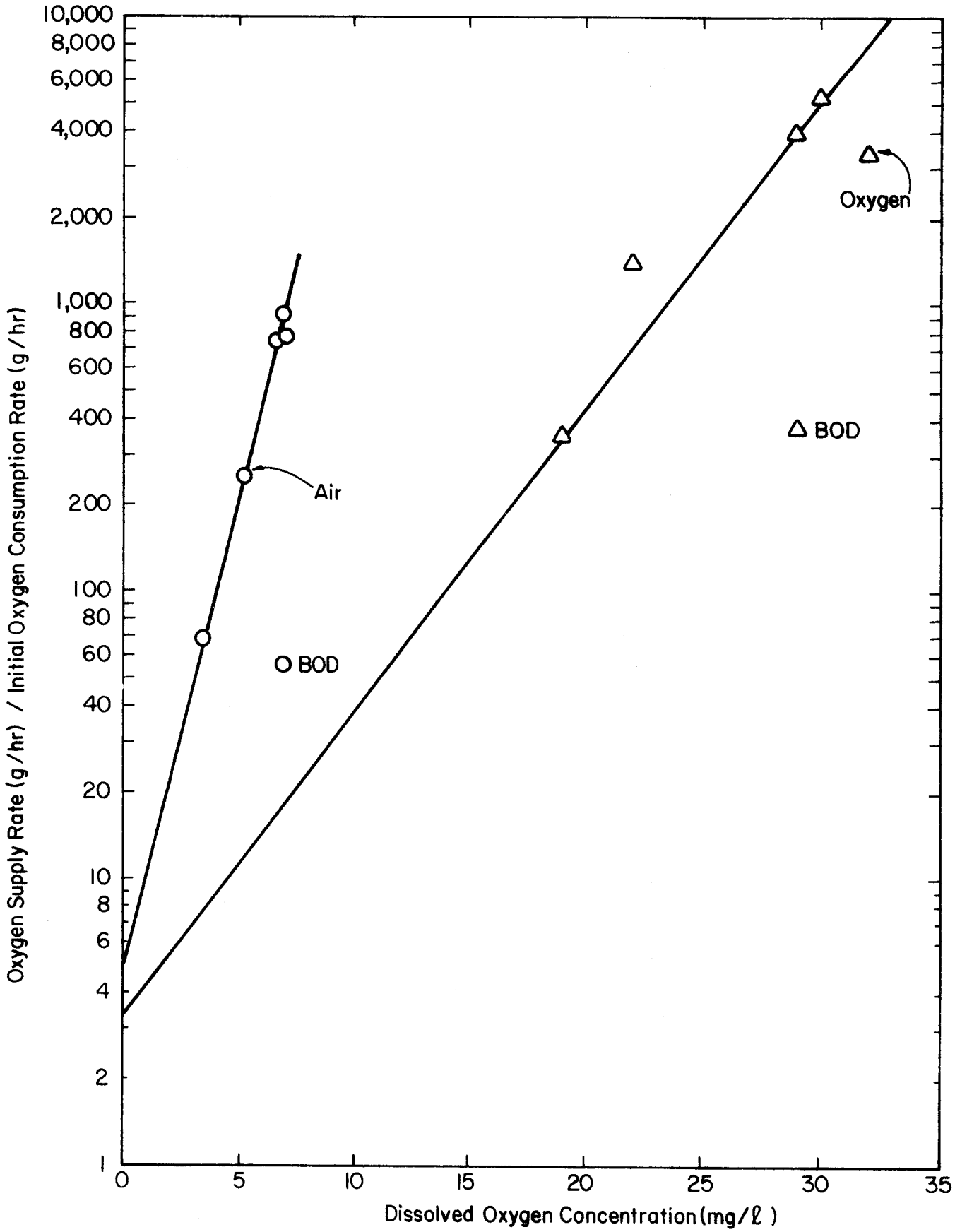

Fig. 5- Dissolved Oxygen Concentration Resulting from a Given Ratio of Oxygen Supply Rate to Oxygen Consumption Rate (by weight).

Figure 5 indicates that the maximum possible efficiency using air is about 20%, while the maximum possible efficiency using oxygen appears to be about 28%. The minimum concentration of dissolved oxygen ordinarily needed in aeration units is about 1 mg/ℓ. At this concentration, the efficiency using air is about 9% (the oxygen-transfer efficiency of bubble aeration is usually between 5 and 15%), while the efficiency using oxygen is about 22%.

Most of the plotted points in Figure 5 lie close to the empirical straight lines with the exception of the 2 points (labeled BOD) from test 4, which was the only test where the oxygen demand was measured in terms of BOD.

If oxygen is used, the effluent to be discharged could be saturated with dissolved oxygen (the saturation concentration of dissolved oxygen under the conditions of these 6 tests is about 7.5 mg/ℓ) and still have an efficiency of about 5%.

CONCLUSIONS

The first-stage BOD has generally been formulated as a first-order reaction. The concentration of oxidizable organic material present is the rate-determining factor, provided that the dissolved oxygen concentration is greater than a critical value of about 4 mg/ℓ at 20°C, for example. This work has shown that this statement is true (at 20°C) for dissolved oxygen concentrations all the way from 3.4 mg/ℓ up to at least 32 mg/ℓ . The use of oxygen for aeration does not have an effect on the rate of oxygen demand removal by aeration.

The rate of oxygen demand removal by aeration can vary enormously (by a factor of 27 in this study) for different samples of the same municipal effluent. In this study, it was shown that the oxygen demand rate constant could vary from 0.807 per day at 20°C (for BOD removal) down to as low as 0.0298 per day at 20°C (for COD removal) for a given municipal wastewater. For this reason, oxygen demand as a function of time for different samples is best compared as illustrated in Figure 2. The use of the 5-day, 20°C BOD is clearly of little value. Oxygen demands should either be expressed in terms of COD and/or in terms of ultimate BOD at a given temperature. Manometric and/or electrolytic respirometric determination of BOD is clearly preferable to other methods, because both the ultimate BOD and the BOD rate constant at a given temperature are necessary for stream pollution work.

Even when 90% of the oxygen demand is removed by aeration (see Figure 2), the average oxygen demand during the aeration period is about 40% of the initial oxygen demand (see Figure 3). Attempting to maintain a dissolved oxygen concentration greater than about 70% of saturation appears to require excessive gas flow rates (see Figure 4). Maintaining 4 mg/ℓ (at 20°C) of dissolved oxygen in a wastewater can be accomplished with 1% efficiency using air and 11% efficiency using oxygen (see Figure 5).

Even when air is used for aeration, contact-stabilization appears to be the best type of activated-sludge treatment from the standpoint of cost effectiveness. Further, if oxygen can be used advantageously in wastewater treatment, the contact-stabilization activated-sludge wastewater treatment plant offers the best possibilities for the advantageous use of oxygen as shown in Figure 6. In regions where extended periods of extremely low air temperatures are encountered, small activated-sludge wastewater treatment plants are often entirely enclosed. The building size necessary to enclose an activated-sludge wastewater treatment plant is smallest when contact-stabilization is the type of activated-sludge treatment employed. Enclosure of the activated-sludge contact basin and the activated-sludge stabilization basin (see Figure 6) would permit the most economical use of oxygen, because the overlying enclosed atmosphere could be passed through a packed tower countercurrent to potassium hydroxide in order to remove the carbon dioxide produced by microorganism respiration. This gas stream could then be reused for aeration.

In Table 7, the data in columns 2, 3, and 4 were obtained from Table 3. Column 5 of Table 7 shows that the maximum oxygen demand removal percent is the same for both air and oxygen. Column 6 of Table 7 is the average of columns 3 and 4 and indicates that the maximum COD removal by aeration is likely to be roughly 90%.

Table 7

Oxygen Demand Removal

Test Number	Test Duration, Days	Oxygen Demand Removal, Percent		Removal Ratio, $\frac{\% O_2}{\% \text{ air}}$	Average OD Removal, %
		Air	Oxygen		
(1)	(2)	(3)	(4)	(5)	(6)
1	5	84.6	92.3	1.091	88.4
2	4	76.7	68.3	0.891	72.5
3	12.2	91.2	91.4	1.002	91.3
4	4.03	100	97.4	0.974	98.6
5	44	90.8	88.8	0.978	89.7
6	9.08	45.6	45.7	1.002	45.6
Average				0.990	

RECOMMENDATIONS

Research should be carried out at the earliest possible time to determine the economic feasibility of using oxygen in connection with an enclosed contact-stabilization activated-sludge wastewater treatment plant of the type discussed in the conclusions section above and

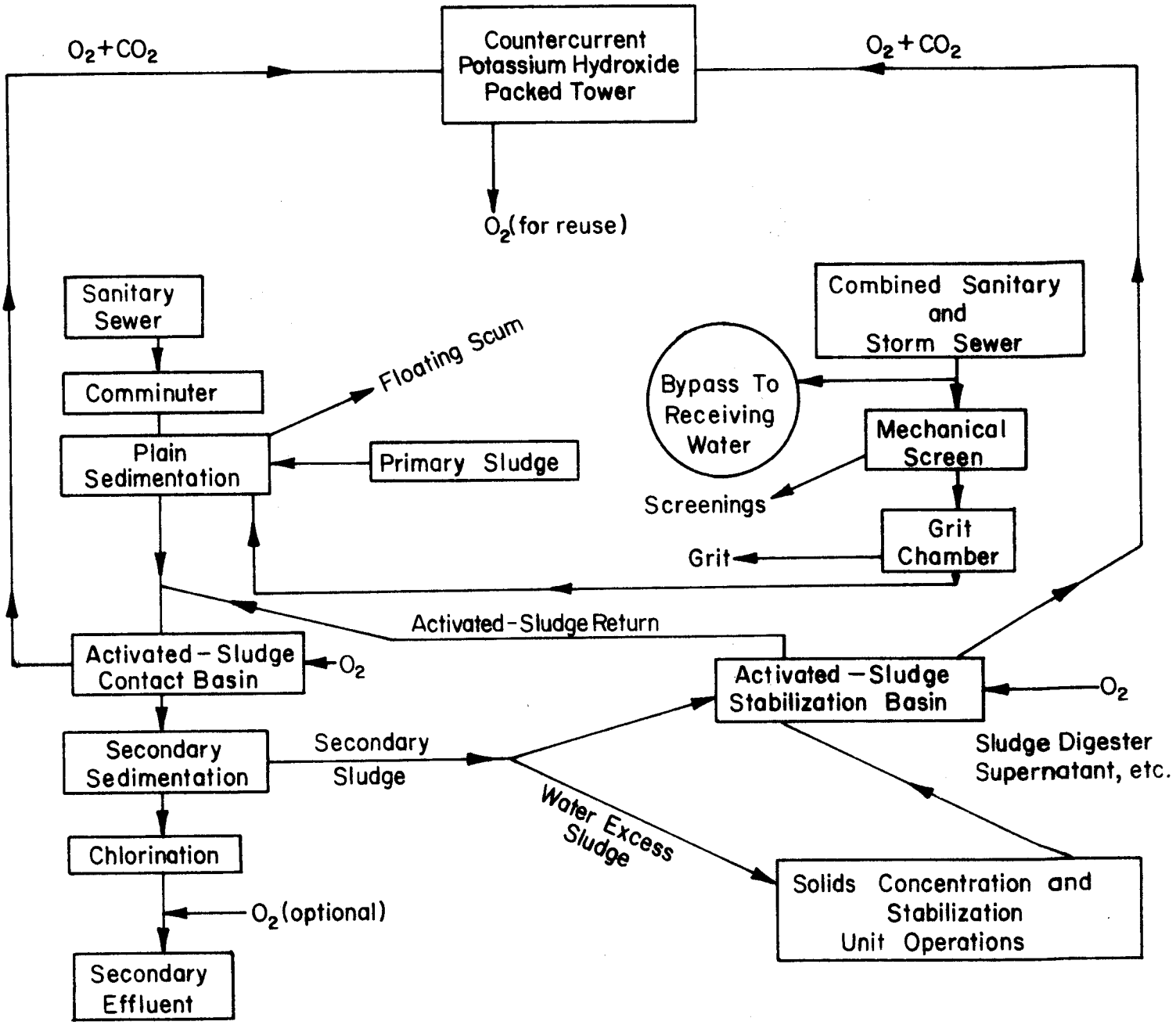

Fig.6- Enclosed Contact-Stabilization Activated-Sludge Wastewater Treatment Plant Using Oxygen Instead of Air for Aeration.

illustrated in Figure 6. This research sould be carried out in three phases as follows:

(1) Laboratory scale to determine the technical feasibility of the foregoing and to obtain design data for the second phase of the research which is;

(2) Pilot plant scale to estimate the economic feasibility of the foregoing and to obtain design data for the third phase of the research which is;

(3) Full scale plant to demonstrate the economic feasibility of the foregoing and to obtain accurate cost-effectiveness information.

SELECTED WATER RESOURCES ABSTRACTS
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15. Supplementary Notes: Part A by S. M. Morrison, G. C. Newton, G. D. Boone, and K. L. Martin, Microbiology

16. Abstract

Part 1 (The Effects of Water Temperature and Elevation upon Aeration): A laboratory-scale mechanical aeration system was used to examine the variation in oxygen transfer rates into water as a function of water temperature (0 to 40°C). The over-all volumetric mass transfer coefficient, liquid phase base, increased linearly with water temperature at a rate of 2.84% per °C. The relative aeration efficiency decreases with increasing elevation, water temperature, and dissolved oxygen concentration. For zero dissolved oxygen and elevation, the rate of oxygen transfer per unit volume and time is independent of water temperature for 0 to 60°C. Equations were developed relating saturation dissolved oxygen concentration to temperature and atmospheric pressure to elevation and air temperature.

Part 2 (BOD and COD Removal from Wastewater by Aeration with Air and Oxygen): Both air and oxygen were used under identical conditions to determine the effect (if any) of dissolved oxygen concentration on the rate of oxygen demand removal. At 20°C, the rate of oxygen demand removal was independent of the dissolved oxygen concentration over the range 3.4 to 32 mg/ℓ . The use of oxygen in an enclosed contact-stabilization activated sludge wastewater treatment plant suitable for high altitudes and latitudes is snown.

17a. Descriptors: *Aeration, *Water Temperature, *Altitude, *Oxygen, Dissolved Oxygen, Chemical Oxygen Demand, Biochemical Oxygen Demand

17b. Identifiers: *Oxygen Transfer, *Low Water Temperature, *High Elevation, *Oxygen Aeration, *Mechanical Aeration, Atomospheric Pressure, Air Temperature

17c. COWRR Field & Group: V D

18. Availability

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