

THE EFFECTS OF TEMPERATURE AND VARYING
SUBSTRATE CONCENTRATIONS
ON BIOLOGICAL TOWERS

By

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CHAPTER I

INTRODUCTION

Biological treatment was first initiated in 1877, when the function of bacteria in the purification or removal of organic matter was first understood (1). Before this work waste treatment was thought to be purely a physical process. The later developments in understanding of bacterial action brought about the trickling filter process. This consists of rock or stone in a basin with wastewater distributed on these stones allowing bacteria to grow utilizing the wastewater as a food. This process worked successfully until a greater understanding of the biological process was developed. This brought about the activated sludge treatment method. Activated sludge is nothing more than a fluidized basin of microorganisms into which wastewater is introduced as a food for the bacteria. The activated sludge wastewater treatment system works very well, but must have competent operators. The operation of an activated sludge system is much more difficult than a trickling filter.

The main problems with trickling filters are the clogging of the media, forming ponds, odors due to poor ventilation and poor distribution of wastewater over entire media; clogging of distribution nozzles, flies in vicinity of filter, and ice buildup on media during cold weather.

The above problems, plus the complexity of operation of an

activated sludge system led to the biological towers. The biological towers in industry today consist of plastic media and redwood media

The usefulness of biological towers lies in its ease of operation and in the fact that the common problems incurred in the trickling filter have been overcome. There is more surface area in the biological towers plastic media and more void space for greater air flow and no clogging.

Recent years of research at Oklahoma State University on biological towers as an effective biological waste treatment process has proven successful. The previous investigators' results of treatment efficiencies versus organic loading in $\text{lbs/dy}/1000 \text{ ft}^3$, has produced two distinctively different straight line plots. Most of the previous work has been done with temperature uncontrolled, whatever the water temperature was was the operating temperature. In reviewing the work of these previous investigators it is interesting to note that the plots look identical, except some work was one in the warmer months and some during the cooler months. The trend was that in the work performed during the colder months the efficiencies seem to be lower than work performed during the warmer months.

Therefore, the scope of the work to be presented here deals with the effects of temperature on the biological activity of the microorganisms ability to remove a common substrate when exposed to a change in temperature over an extended period of time.

Three different temperatures were used, and three different organic loadings were exposed to each temperature change.

CHAPTER II

LITERATURE REVIEW

A. Introduction

Research efforts previous to this paper on biological towers for temperature control are few in number. Most temperature control work has been done with pure cultures. In recent years, however, considerable research has been undertaken in temperature work with the activated sludge systems. For this reason most of the literature reviewed in this chapter deals with the activated sludge systems and pure culture work performed by microbiologists. The activated sludge work can be related directly to the biological towers, since both systems deal with a heterogeneous population.

Bacteria or microorganisms are categorized into three separate ranges of temperature.

Classification	Temp. Range °C
Psychrophils	4-25
Mesophils	25-40
Thermophils	40-75

The temperature ranges indicated above are not as clearly defined as the numbers indicate because many other factors must be taken into consideration to define the actual habits of growth and reproduction

of certain strands of microorganisms. For example, Brock (2) has found microorganisms in the Yellowstone National Park around the Hot Springs where temperature is in some places 99°C to 100°C.

B. Effects of Temperature on Growth Rate and Cell Composition

The importance of understanding the effects of temperature on a biological system is that temperature affects the chemical and biological reaction rates that take place. Farrell and Rose (3) have compiled an excellent review of prior published articles relating to temperature.

Changes in temperature can change the species of organisms utilizing the same substrate. Most bacteria can grow only over a restricted range of temperature. Their growth depends upon a complex sequence of enzymatic reactions whose rates are individually related to temperature. Pirt (4) claims that temperature, besides its general effect on reaction rates, can also exert highly selective effects on metabolic pathways by repressing a particular protein synthesis. This effect can lead to varied and complex responses when temperature is altered.

Ng's (5) research points out that it is difficult to interpret results due to changes in temperatures solely on this parameter because of the oxygen variations that occur when the temperature is changed. Increasing the temperature of a culture may change its physical properties appreciably and hence indirectly affect cell metabolism. Sinclair and Stokes (6) also have attributed greater yields at lower temperatures to higher solubilities of oxygen at lower temperatures. Yield in this case is mass of organisms rather than the true definition of yield

which is $dx/dt/ds/dt$ change in microorganisms with time divided by change in substrate with time.

Growth rates of microorganisms related to temperature have been studied by many investigators. Ng (5) studied the effect of growth temperature on the yield of E. Coli and found that it remained relatively constant over the range of temperature where the Arrhenius relationship between growth rate and temperature holds.

Hanus and Morita (7) using the Van't Hoff-Arrhenius plot in their investigations have obtained results that agree with previous investigators in indicating that there is no consistent difference in μ , temperature characteristic of growth rate, values of psychrophiles and mesophiles. Whereas μ values may be a property of the particular species studied or of the medium in which the organisms is grown, there appears to be little reason to try and correlate the μ of an organism with its temperature range of growth.

Ingraham and Bailey (8) point out that μ , temperature characteristic of growth rate of a given bacterial strain is relatively constant near the center of the temperature range over which growth of the strand occurs; it decreases at higher temperatures and at lower temperatures it increases dramatically. The temperature characteristic, μ , is defined as

$$\text{Log } k = \frac{\mu}{2.303 RT} + C$$

k = Specific growth rate constant

R = Gas constant

T = Absolute temperature

C = Constant

Roth and Wheaton (9) studied temperature effects on growth of the genus Arthobacter. Seven members of the genus Arthobacter were grown at 0, 7, 20, 30, and 37°C. In general, no sharp cut off point was observed between growth temperature requirements of psychrophilic and mesophilic bacteria. However, the time required for maximum growth at psychrophilic temperatures is longer than the time required for maximum growth at mesophilic temperatures.

Malcolm (10), using a refined medium, was able to maintain growth of a psychrophilic coccus over its entire temperature range -4 to 25°C. A rapid loss of viability occurred at temperatures above 25°C. Studies have shown cells were not killed by increase in temperature directly but instead by elevated levels of intercellular adenosine-5-triphosphate and amino acids. This finding was reflected in the reduced rate of amino acids synthesis during the recovery of heat shocked cells, and leakage of degraded ribonucleic acid products. Deoxyribonucleic acids synthesis was not affected by elevated temperature.

Ng, Ingraham, and Marr (11) used exponentially grown cultures of Escherichia Coli ML30, and subjected them to abrupt changes in temperature. If the change in temperature was made within the range of temperature in which the temperature characteristic, μ , is constant, exponential growth resumed immediately at a rate characteristic of the new temperature. If the shifts were made to or from a temperature below the range of constant μ , the initial growth rate was intermediate to the rates normal for the initial and final temperatures. The results indicate that growth at low temperature alters or damages the cell in a way that reduces the growth rate. A period of growth at higher temperature is required to correct the damage. Release from

glucose repression by the induction of β -Galactosidase occurs at a temperature coincident with low temperature damage. Derepression may be the damage that results from growth at low temperature.

Shaw's (12) experimental work with mesophilic and psychrophilic yeasts was similar to Ng, Ingraham, and Marr's work and produced results that agreed with these previous investigators. Shaw showed that shifts from high to low temperature or low to high temperature resulted in transient growth rates. Shaw also showed that transient growth rates follow the Arrhenius equation with modifications for cell damage at high temperature.

Topiwala and Sinclair (13) found in working with a pure culture that the observed yield, growth rate, and maximum dilution rate at which the culture could be operated were all functions of temperature. To obtain these results Topiwala and Sinclair fitted their data by a kinetic model involving Monod's relationship for growth dependence on substrate concentration together with an endogenous metabolism term.

$$\mu_N = \frac{\mu_M S}{K_S + S} - k_d$$

where:

μ_N = Net growth rate

μ_M = Maximum rate of growth with S, the limiting nutrient

S = Limiting nutrient

K_S = Saturation constant

k_d = Endogenous metabolism term

The kinetic parameters were determined by a variety of techniques and, with the exception of the potential yield constant, were found to

be strong functions of temperature.

George and Gaudy (14) found in their investigational work that a heterogeneous population responded more favorably to an increase in temperature than it did to a decrease in temperature, at the same dilution rate. However, at two different dilution rates the lower dilution rates handled temperature change most effectively. This means less organic substrate leaked out of the system at lower dilution rates.

Tempest and Hunter (15) suggest that the observed yield variation was due to temperature dependence of the maintenance energy required.

Temperature has been shown to have some effect on cell composition as shown by Brown and Rose (16) in their work with Candida utilis. A decrease in temperature resulted in an increase in ribonucleic acid and protein production while the deoxyribonucleic acid content remained unchanged. Evreinova et al., (17) working with Aspergillus fumigatus obtained results that agreed with Brown and Rose's work.

Pigment production was found to occur in Bacillus Cereus Var. Alesti by Uffen and Canale Parola (18). They determined that the enzymes directly or indirectly associated with pigment production are synthesized by growing cells at 15°C but not at 30°C. However, once these enzymes have been produced they are active at both temperatures.

Muck and Grady (19) studying the effects of temperature on the biologically determined characteristics, μ_m , Y_T , k_d , K_S of microbial growth, found that as temperature is increased between 10°C-30°C growth rate and the decay coefficient are increased. The true yield was found to increase with an increase in temperature from 10°C-20°C, but further increases in temperature decreased the true yield. The saturation constant (k_s) was found to decrease slightly with a temperature

increase from 10°C-20°C but rose substantially when temperature was elevated to 30°C.

C. Effects of Temperature on Substrate Removal

Novak (20) working with the Arrhenius form of the common temperature correction equation

$$K_T = K_{20}\theta^{(T-20)}$$

where:

K_T = Microorganism growth rate at some temperature, T

K_{20} = Microorganism growth rate at 20°C.

θ = Temperature coefficient constant

states that θ , temperature constant, is involved with other parameters. Eckenfelder and Englande (21) noted that food to microorganism ratio is the most important parameter to determine θ . Pohl (22) concluded that θ , was dependent upon the suspended solids concentration (MLSS). Novak also notes that low cell systems are more temperature dependent than systems with high suspended solids concentration.

Prasad and Jones (23), doing work with nitrogenous compound, found that temperature is an important parameter. Their results show that in spite of the apparent metabolic stability of protein at low temperatures, the psychrophilic bacteria may use other nitrogen sources such as urea, amino acids, and creatinine. Ammonia and nitrates may also be utilized. The comparative degradation rates of various organic nitrogen compounds by psychrophilic bacteria at 20°C and 2°C indicate psychrophiles play an important role in organic removal from wastewaters. However the ability or activity at 2°C is lower than at 20°C

which indicates that biological treatment can operate at lower temperatures provided sufficient time is given for the organisms to utilize the waste.

Previous investigators have studied kinetics of substrate removal in the biological towers and found first order kinetics to prevail. Deen (24) found that substrate removal was a function of total organic loading applied. Bentley (25) showed through experiments with the biological towers that parameters common to activated sludge were valid for the fixed bed reactors.

Kornegay and Andrews (26) found in their research that mass balance equations can be utilized for biological towers.

Cook (27) in his research confirmed the work of Kornegay and Andrews. Cook concluded that first order kinetics were followed and were a result of the microorganism concentration varying with depth. Cook also found evidence of non-carbohydrate intermediates.

Kepler's (28) work dealing with shock loads on biological towers provided substantial evidence that the fixed film reactor is a good method of dealing with shock loads.

Little's (29) research dealt with nitrification in the towers and found that there exists a definite COD concentration at which nitrification will begin.

CHAPTER III

MATERIALS AND METHODS

A. Experimental Approach

An existing 9-foot biological tower used for previous experimental work was given an additional foot of depth to 10-feet. Temperature control of the Stillwater tap water was used to measure the effect of temperature upon the substrate removal, of varying organic loading with three different temperatures, 10°C, 18°C and 23°C. Samples were taken of the influent and at every two feet of depth including the effluent for COD determinations.

B. Experimental Apparatus

The 10-foot biological tower used for this experiment consisted of two separate stacks of media supported by plexiglas towers. The towers are arranged such that four feet of the biological support media is located in the first tower and six feet of the biological support media is located in the second plexiglas tower. Figure 1 shows the experimental apparatus used. The towers are linked in series due to the limited height of the laboratory. The effluent from the collection through of the first tower is pumped to the top of the second tower through pyrex plastic tubing.

The cross-sectional area of the tower is 1 ft² and the depth of

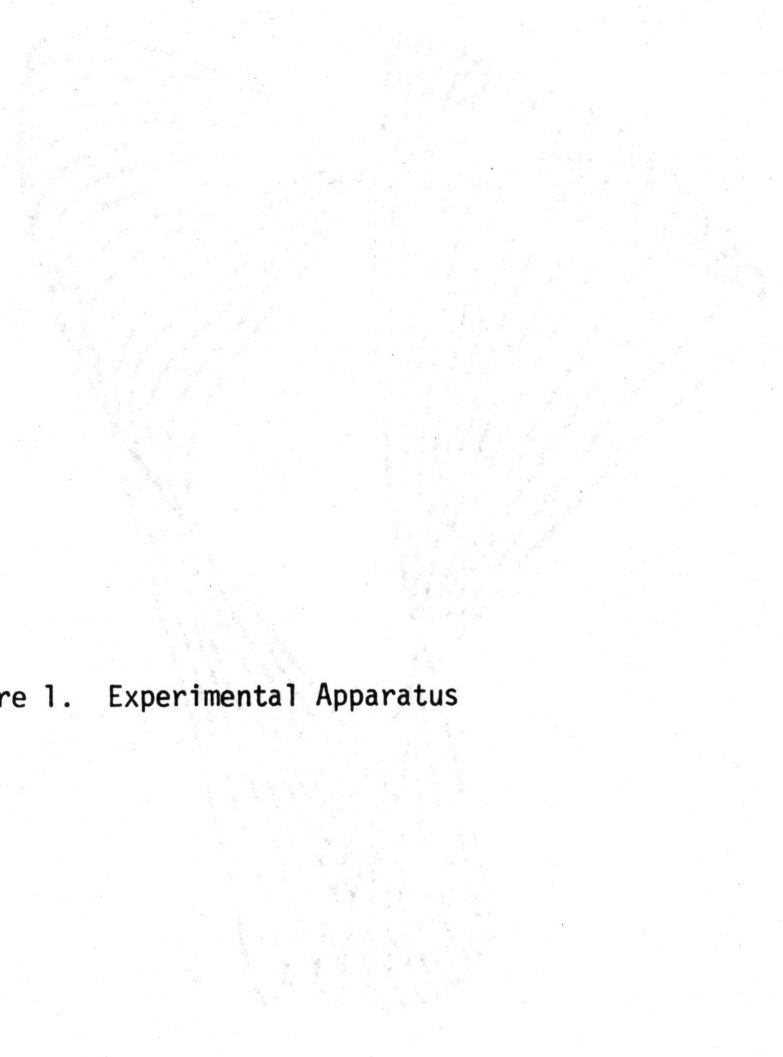
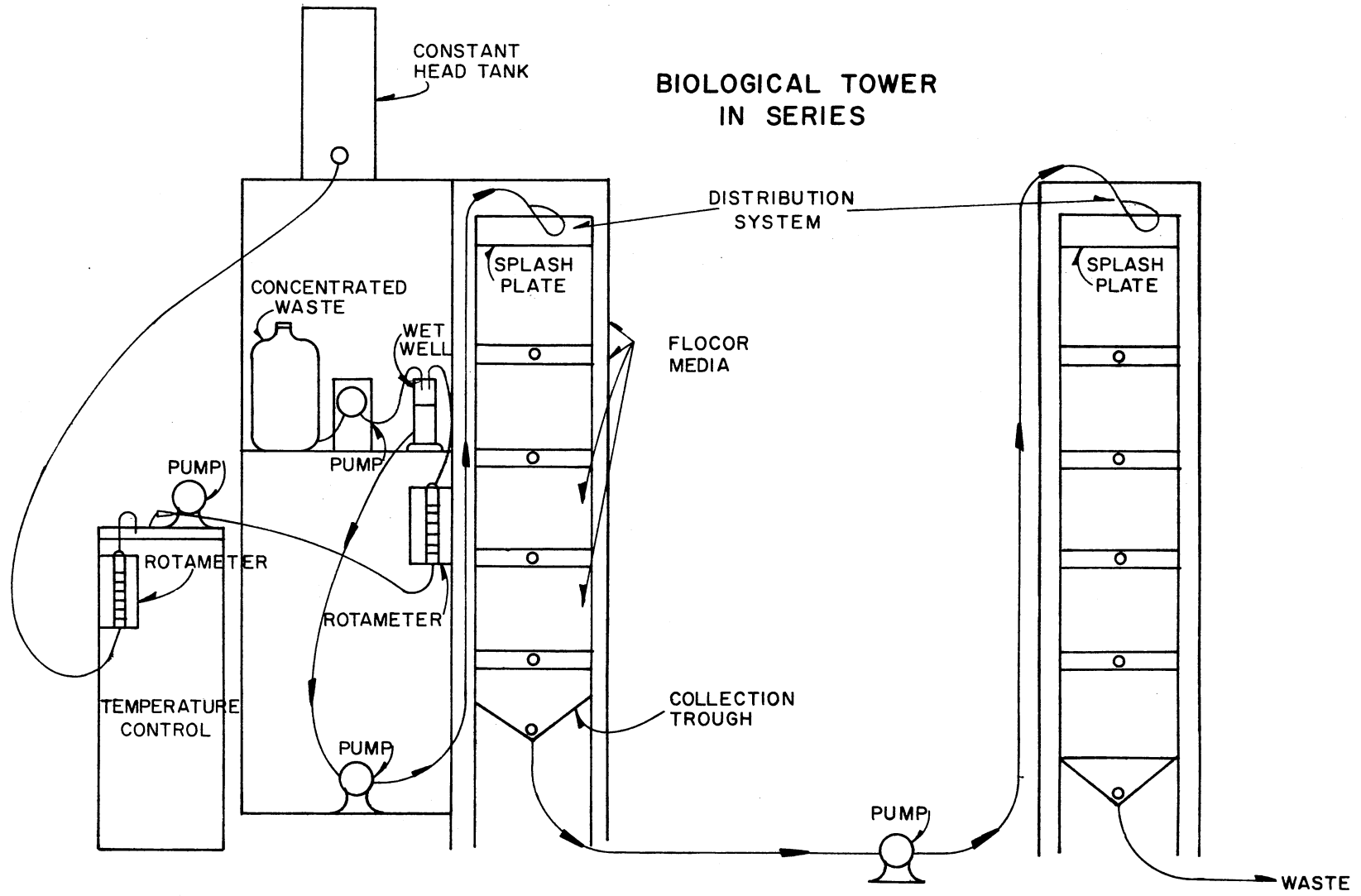


Figure 1. Experimental Apparatus

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one unit is 1 ft. The Cubic area is 1 ft³ (1.0 x 1.0 x 1.0). Between each foot of plastic media there is a 4 in. gap to provide for sampling and air circulation.

The plastic media used is the Flocor brand name which is a rigid polyvinyl chloride material. It was first developed by the Imperial Chemical Industries, Ltd., London, England, and has been previously licensed by the Ethyl Corporation in the United States. Flocor has 2-1/4 inch triangular openings, and each cubic foot has a maximum of 27 square feet surface on which biological activity may take place. The void ratio of 97% allows sufficient air flow for microorganism to utilize oxygen in their chemical oxidation reactions. Air moves upward like a forced draft system.

Hydraulic flow to the biological units was maintained by means of Stillwater tap water flowing into a constant head tank. The purpose of this research is temperature control, so from the constant head tank the water flowed by gravity through a rotameter set at 750 gal/dy to a temperature control device. In this experiment the precision scientific temptrol water bath wash was used. This piece of equipment has its own pump and the water can be elevated or lowered in temperature in this water bath. The water was pumped from the temperature control device through another rotameter set at 750 gal/dy into a mixing chamber, or wet well, where it was mixed with a concentrated synthetic waste.

The substrate or synthetic waste used in this experiment employed sucrose (C₁₂H₂₂O₁₁) as its main constiuent and source of carbon. Nitrogen was furnished by using an ammonium-nitrate fertilizer. Commercial grade fertilizer used for the lawn was employed with an analysis of 33% nitrogen that was readily obtainable. This 33%

nitrogen was composed of 16% nitrates, and 16% was in the form of the ammonium compound. The fertilizer is easily solubilized in solution, but due to the concentration involved, concentrated sulfuric acid was added to help maintain the solubility of the solution as well as prevent microbial activity from occurring in the feed bottle. The solution was stirred continuously by a mechanical mixer with a variable speed control.

The concentrated feed was pumped to the mixing chamber by a Milroyal pump, made by Milton Roy. The Milton Roy pump can be adjusted to obtain the desired flow from the feed bottle to reach a desired concentration after mixing with the hydraulic flow, from the following equation:

$$(Q_T + Q_W) S_i = Q_W C_W$$

$$Q_T = \text{Hydraulic flow}$$

$$Q_W = \text{Waste flow}$$

$$S_i = \text{Desired influent substrate level}$$

$$C_W = \text{Concentration of waste in feed}$$

Composition of the synthetic waste is given in Table I.

After reaching the desired concentration by diluting with tap water the mixture is pumped to the top of the first plexiglas tower by a Teel rotary-screw pump (Model Ip610). The pump was driven by a Dayton single speed motor (Model KSS5JXBJB-9B). Distribution at the top of the tower was accomplished by using a perforated circular section of tubing, through which the flow was transferred to a splash plate, which consisted of a plexiglas baffle with small hole to even out the flow to the plastic media.

Effluent from the first tower was pumped to the top of the second

tower by means of a similar Teel rotary-screw pump where a similar distribution system evened the flow to the media.

TABLE I
COMPOSITION OF SYNTHETIC WASTE RELATIVE TO
A SUCROSE CONCENTRATION OF 100 MG/L

Constiuent	Concentration
$C_{12}H_{22}O_{11}$	100 mg/l
Ammonium-Nitrate Fertilizer	64
$MgSO_4 \cdot 7H_2O$	10
K_2HPO_4	6
$MnSO_4 \cdot H_2O$	1
$CaCl_2$	0.75
$FeCl_3 \cdot 6H_2O$	0.05

C. Experimental and Analytical Procedures

Seed for the biological towers to obtain initial growth was taken from the primary clarifier effluent of the Stillwater municipal sewage treatment plant.

The initial temperature was $10^{\circ}C$ and the substrate level was 300 mg/l. The unit was operated several weeks before a sampling program was initiated. Consecutive days of sampling for COD were the

verification of steady state. After obtaining a steady state the feed rate was decreased to 200 mg/l and 100 mg/l to obtain steady states. The same means of determining steady state was used throughout this experiment.

Temperatures of 18°C and 23°C followed using the same organic loading that was applied at 10°C.

Sampling was accomplished by using a sampling wand. This consisted of a small piece of PVC tubing which had previously been cut along its longitudinal axis. To obtain a sample that should be representative of activity that has taken place above the sampling point the wand was moved back and forth between the media.

These samples were then filtered through Millipore membrane filters (0.45 μ). The filtrate was placed in its corresponding COD flask.

The chemical oxygen demand (COD) test was performed in accordance with standard methods (30).

CHAPTER IV

RESULTS

Temperature control experiments conducted during this research were restricted due to limitations of equipment, and season. The rate of flow of water into the temperature control device and back out into the unit left limited contact time to vary the temperature. The minimum temperature obtained was 10°C and the maximum was 23°C. Three different temperatures, 10°C, 18°C, and 23°C and three different substrate concentration levels, 100 mg/l, 200 mg/l and 300 mg/l were selected to identify the effect of temperature on substrate removal in the biological towers.

A. Effects of Temperature

During the month of February the temperature was controlled at 10°C to determine cold weather effects on substrate removal. Samples were taken on consecutive days for two or three days to obtain a steady state value.

Operation of the towers was very difficult at the colder temperature due to the fact that the microorganisms grow slower at the lower temperatures. Data from samples taken at substrate concentrations of 150, 200, and 300 mg/l for 10°C are presented in Table II. This same information is shown graphically in Figure 2. The extra straight line on Figure 2 is for a substrate concentration of 500 mg/l. This

TABLE II
COD REMAINING AT VARIOUS DEPTHS

TEMP	FLOW	DEPTH = 0	2	4	6	8	10
		<u>$S_i = 100$</u>					
10°C	750 gpd/ft ²	155	140	127	115	104	93
18°C	750 gpd/ft ²	103	79	62	48	37	29
23°C	750 gpd/ft ²	100	65	43	28	18	12
		<u>$S_i = 200$</u>					
10°C	750 gpd/ft ²	220	210	190	170	150	137
18°C	750 gpd/ft ²	210	165	130	99	76	58
23°C	750 gpd/ft ²	185	161	90	62	42	29
		<u>$S_i = 300$</u>					
10°C	750 gpd/ft ³	310	292	277	259	244	229
18°C	750 gpd/ft ³	310	238	180	135	103	78
23°C	750 gpd/ft ³	310	212	134	105	70	47

Convert to #COD/dy/1000 ft³

experimental run was performed to verify the performance of the biological tower at high organic loadings. At the higher organic loadings the efficiency of the towers was consistent at 25 per cent removal. The straight lines on Figure 2 indicates that first order kinetics does exist with respect to depth.

Table II also shows the data obtained at 18°C. The 18°C graphical representation is presented in Figure 3, where it can be seen that the straight lines indicate first order kinetics with respect to depth. It should be pointed out that the slopes are greater than in Figure 2.

Figure 4 expresses the data from the 23°C temperature experiments also contained in Table II. The straight line at 300 mg/l initial concentration shows first order kinetics. The straight line through the 200 mg/l data is representative even though the data was more scattered. The 100 mg/l initial concentration experiment does follow first order kinetics through the first six feet of removal. Then the COD concentration leaps upward at 8 feet and 10 feet possibly due to intermediate production or a remaining non-biodegradable fraction.

Figure 5 shows the overall efficiency of the entire tower depth versus total organic loading. The 10°C and 18°C lines definitely do not suggest first order kinetics with respect to total organic loading. The 23°C line drawn through the data is questionable.

Tables III, IV, and V show the data in Table II converted to lbs COD/dy/1000 ft³ (total organic loading). Shown in these tables is the per cent COD removed during each two incremental feet of depth.

In Figure 6, the 10°C temperature experiment data of per cent COD removed versus total organic loading, the 100 mg/l, 200 mg/l and 300 mg/l 1st order removal curves fell very close and could probably

Figure 2. COD Remaining Versus Depth at 10°C and 750 Gpd/ft²

○ - $S_i = 500$ mg/l
⬡ - $S_i = 300$ mg/l
△ - $S_i = 200$ mg/l
□ - $S_i = 100$ mg/l

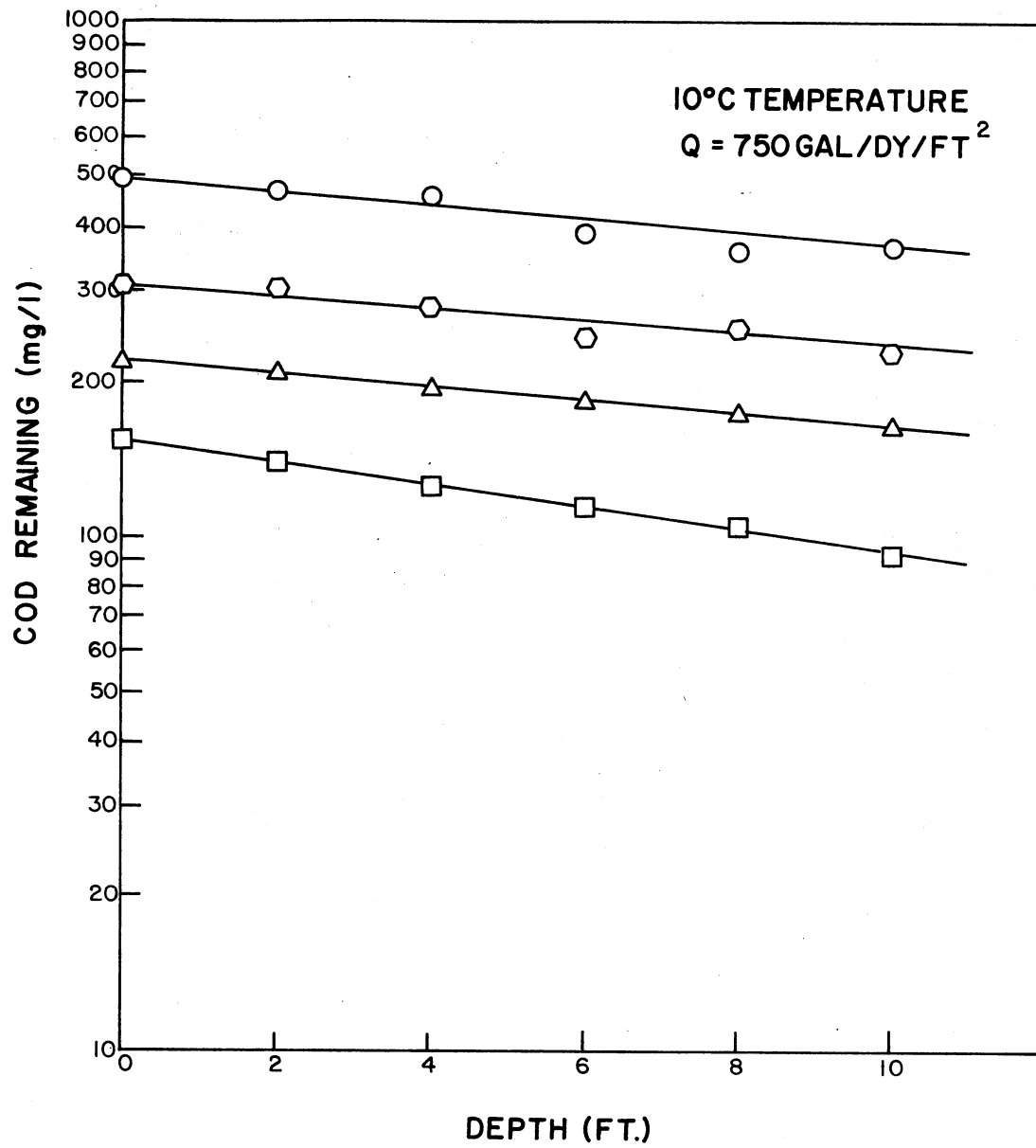


Figure 3. COD Remaining Versus Depth at 18°C and 750 Gpd/ft²

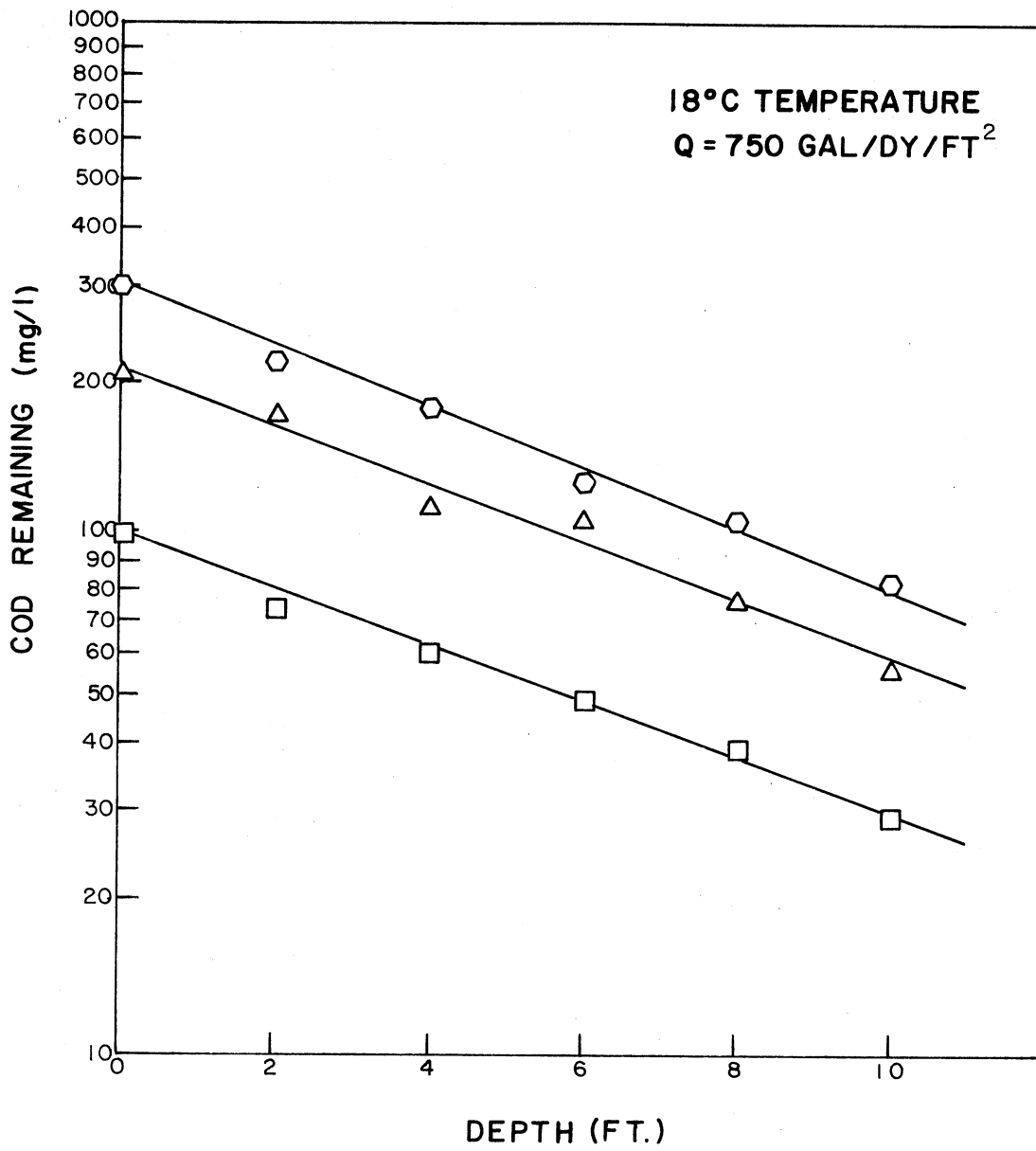


Figure 4. COD Remaining Versus Depth at 23°C and 750 Gpd/ft²

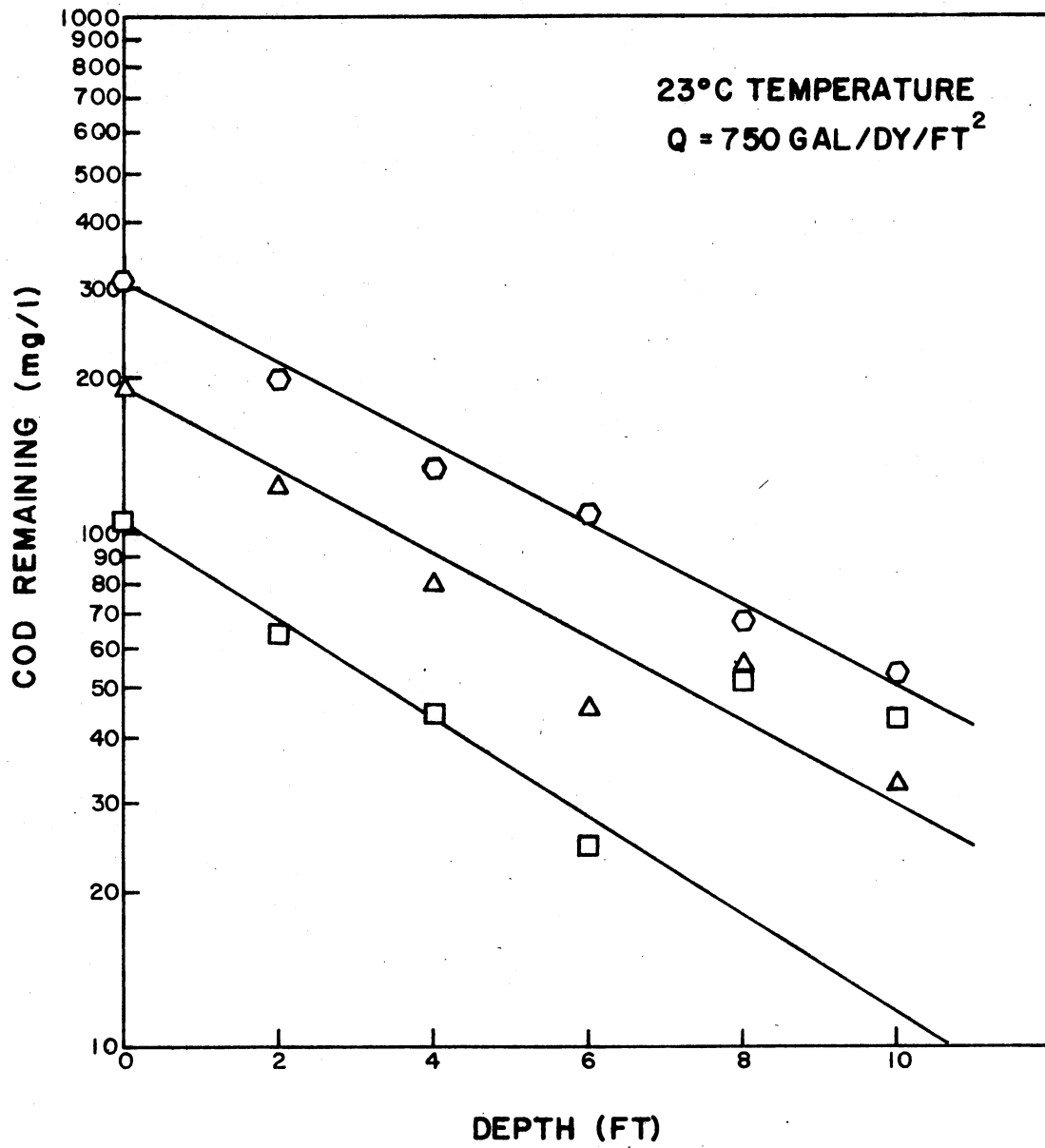


Figure 5. COD Removal Efficiency Versus Total Organic Loading

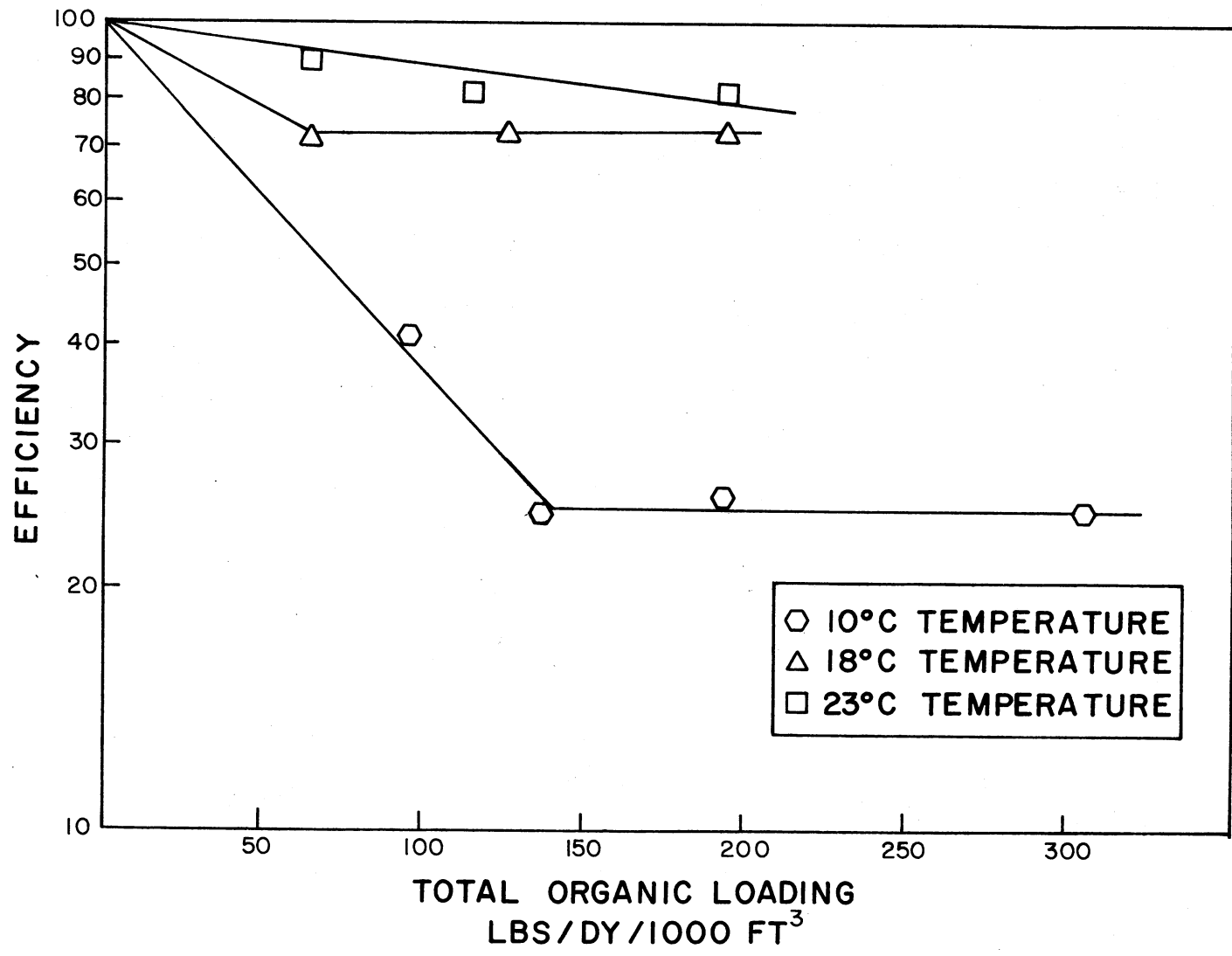


TABLE III
 LBS COD/DY/1000 FT³ VERSUS PER CENT COD
 REMOVED AT 100 mg/l CONCENTRATION

Temp	Depth (ft)	$S_i = 100$	
		lbs COD/Day/1000 ft ³	% COD Removed
10 ⁰ C	2	484.76	10
	4	242.38	18
	6	161.98	26
	8	121.2	33
	10	97.0	40
18 ⁰ C	2	322.13	23
	4	161.06	40
	6	107.37	93
	8	80.53	64
	10	64.4	72
23 ⁰ C	2	312.75	35
	4	156.38	57
	6	104.75	72
	8	78.18	82
	10	62.55	88

TABLE IV

LBS COD/DY/1000 FT³ VERSUS PER CENT COD
REMOVED AT 200 mg/l CONCENTRATION

Temp	Depth (ft)	$S_i = 200$ lbs COD/dy/1000 ft ³	% COD Removed
10 ⁰ C	2	688.05	4.5
	4	344.03	13.6
	6	229.35	22.7
	8	172.01	31.8
	10	137.61	37.7
18 ⁰ C	2	656.77	21.4
	4	328.38	38.0
	6	218.93	52.85
	8	164.19	63.8
	10	131.35	72.4
23 ⁰ C	2	578.58	13.0
	4	289.29	51.4
	6	192.86	66.5
	8	144.64	77.3
	10	115.71	84.3

TABLE V
 LBS COD/DY/1000 FT³ VERSUS PER CENT COD
 REMOVED AT 300 mg/l CONCENTRATION

Temp	Depth (ft)	$S_i = 300$ lbs COD/dy/1000 ft ³	% COD Removed
10°C	2	969.53	5.8
	4	484.76	10.6
	6	323.18	16.5
	8	242.38	21.3
	10	193.91	26.1
18°C	2	969.53	23.2
	4	484.76	41.9
	6	323.18	56.5
	8	242.38	66.7
	10	193.91	74.8
23°C	2	969.53	31.6
	4	484.76	56.7
	6	323.18	66.1
	8	242.38	77.4
	10	193.91	84.8

Figure 6. Per Cent COD Removed Versus Total Organic Loading
at 10°C and 750 Gpd/ft²

100	S_i	=	100 mg/l
200	S_i	=	200 mg/l
300	S_i	=	300 mg/l

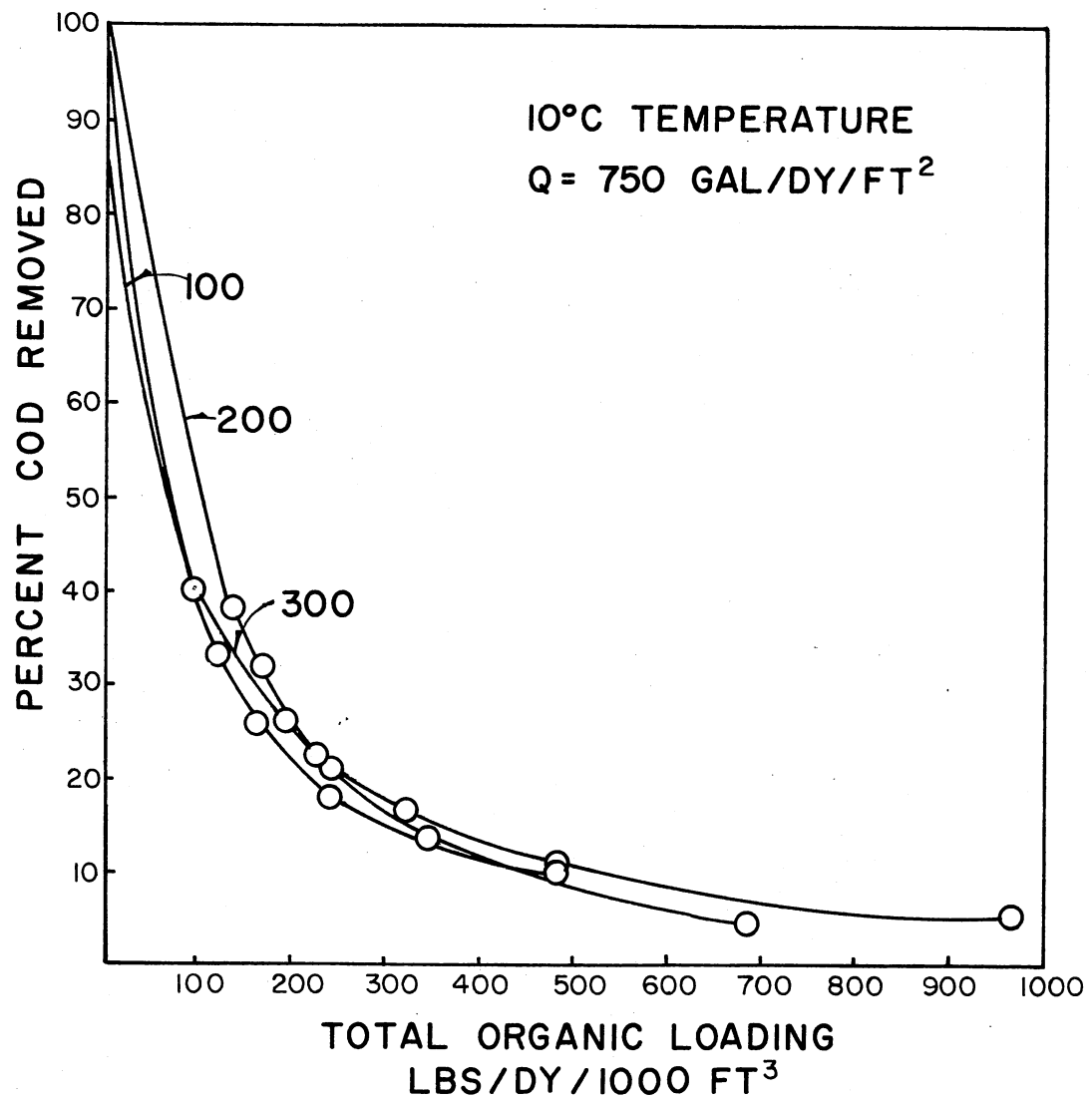


Figure 7. Per Cent COD Removed Versus Total Organic Loading
at 18°C and 750 Gpd/ft²

100	S _i	=	100 mg/l
200	S _i	=	200 mg/l
300	S _i	=	300 mg/l

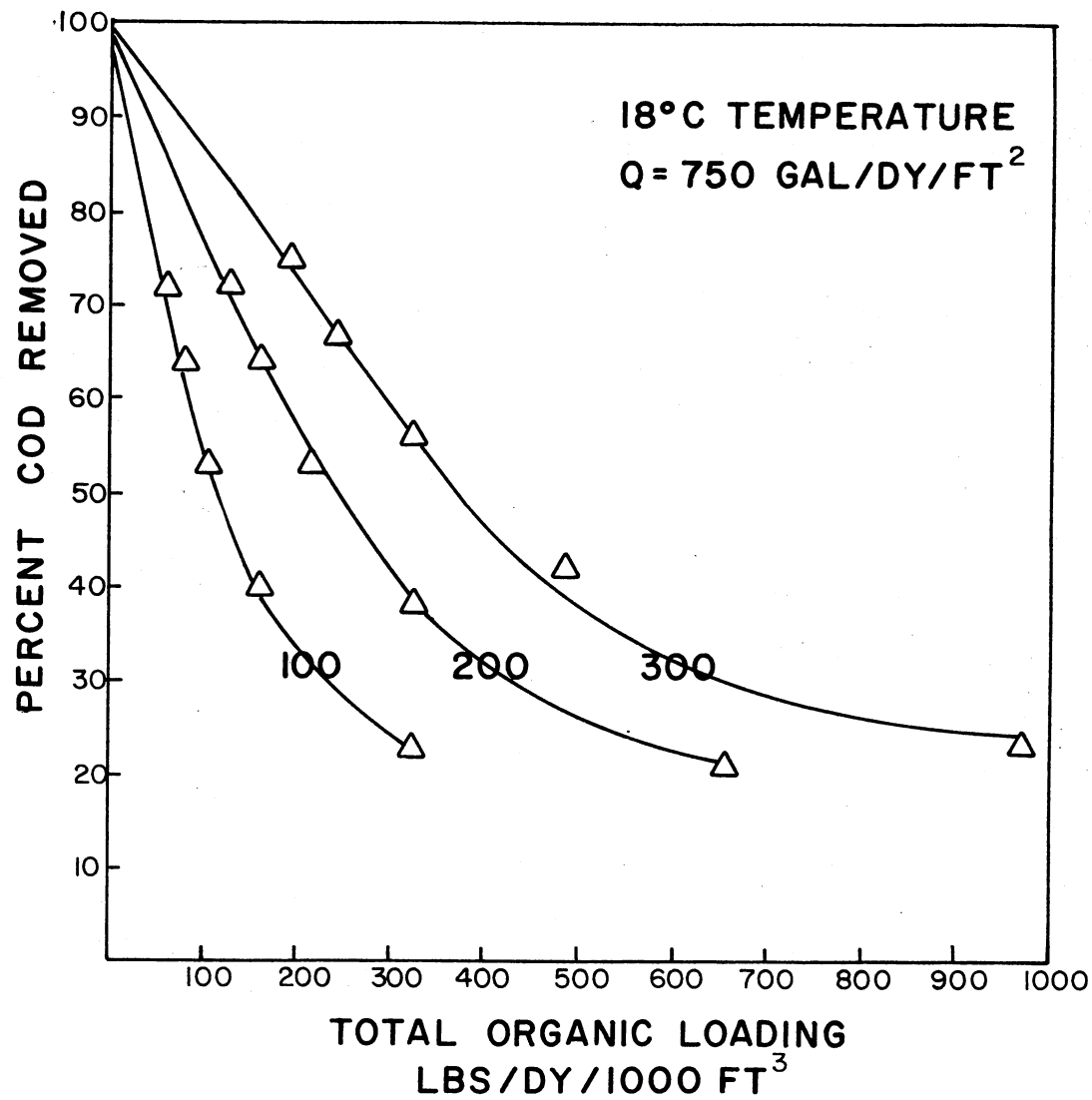
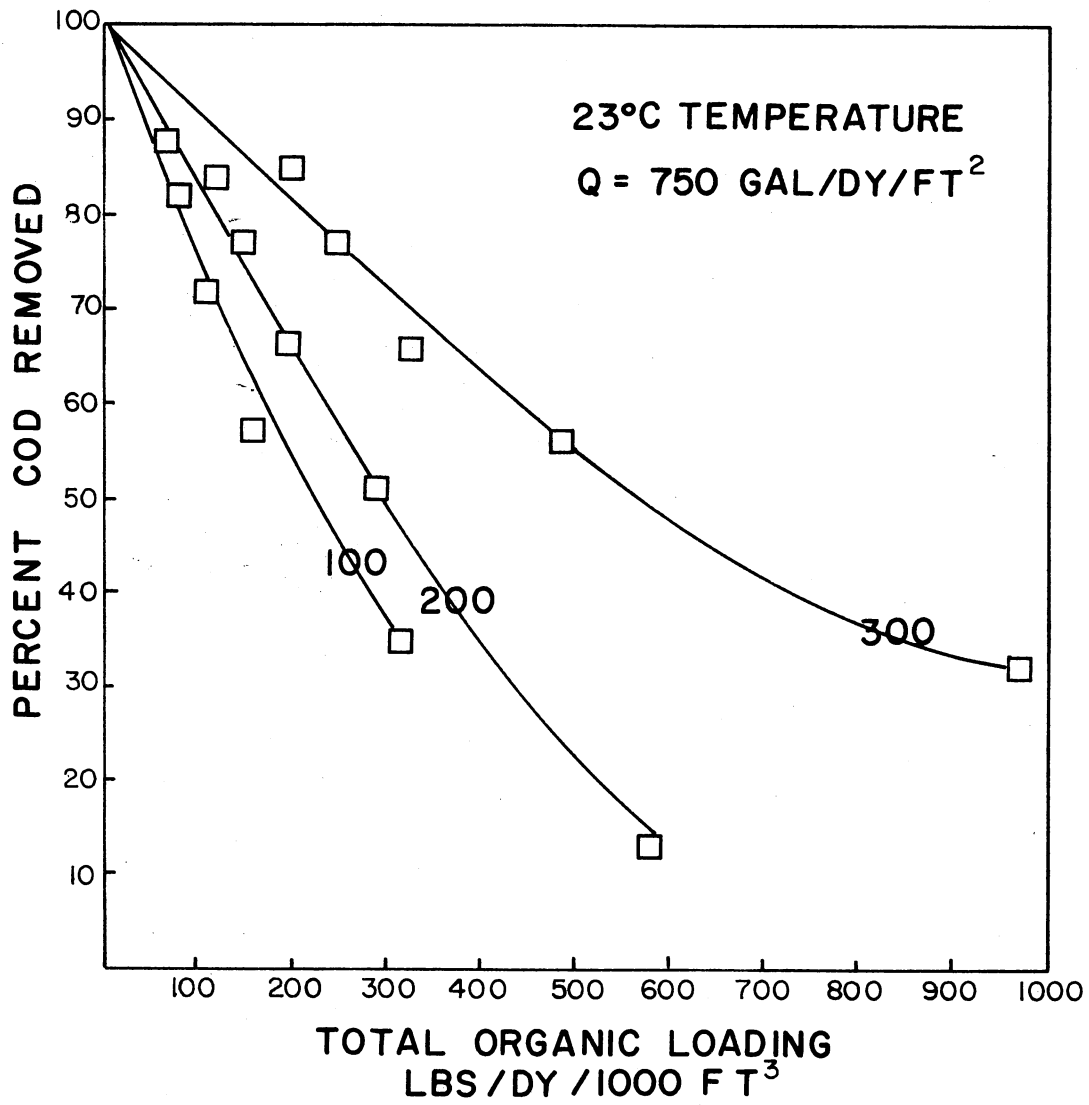


Figure 8. Per Cent COD Removed Versus Total Organic Loading
at 23°C and 750 Gpd/ft²

100	S _i	=	100 mg/l
200	S _i	=	200 mg/l
300	S _i	=	300 mg/l



be drawn as one single curve. However, in Figure 7 the 18^oC temperature data, and Figure 8 the 23^oC temperature data, the results show three distinctly different 1st order removal curves with respect to total organic loading. These figures suggest that the efficiency is also a function of initial substrate concentration.

CHAPTER V

DISCUSSION

Water temperature effects can be quite severe as the results depict. Cold temperature is the natural habitat of the psychrophilic organism which grows at a slower rate than the mesophiles and thermophile. Psychrophiles grow best at a high t , detention time, or low D , dilution rates.

$$\mu = \frac{\mu_m S}{K_S + S} = D$$

μ = growth rate

μ_m = maximum growth rate

K_S = saturation constant or value at which
 $\mu = 1/2 \mu_m$

S = limiting nutrient

D = dilution rate = $1/t$

The above equation is good for a once through system which was characteristic of the mode of operation of the biological towers for this experimental work.

The initial temperature at start-up was 10°C . This temperature took considerable time to obtain a good growth upon the media. This initial experiment was performed without the aid of the constant head flow reservoir to maintain a constant hydraulic flow. Without the constant head occasional shocks were applied to the towers due to

fluctuations in the water pressure. These shocks were not intentional and experimental data was not taken until a constant flow was applied and a steady state obtained. The data from the 10°C experiments express rather well the major effect of cold temperature in that efficiencies drop considerably at all substrate concentrations investigated. The effects seem worse at higher substrate concentrations, although from 200 mg/l up to 500 mg/l the removal efficiency was about 25% for the entire substrate range. From the literature review it is found that at the temperatures where psychrophilic bacteria grow best, a longer detention time is needed for them to utilize the substrate in the incoming waste, to obtain an acceptable effluent.

To obtain the higher efficiencies longer detention times would be required. This could be accomplished by the addition of more depth to the present biological towers. Several methods of design have been formulated to design biological towers. Eckenfelder suggests a design method that has a temperature related constant (31). The equation suggests first order kinetics.

$$\frac{S_e}{S_o} = e^{-\frac{KD}{Q^n}}$$

S_e = organic remaining (mg/l)

S_o = organics in raw wastewater (mg/l)

K = removal rate constant (temperature related)

D = tower depth

Q = hydraulic loading (gpm/ft²)

n = constant

The temperature related constant, removal rate, K is expressed by:

$$K_T = K_{20} 1.035^{(T-20)}$$

The total organic loading method combines both hydraulic and organic loadings. It has been researched extensively in the Oklahoma State Laboratories and prior research points total organic loading to exist as a useful method of design and is the method used to compose Table II, III, IV, V, Figure 6, 7, and 8 of the results.

Kincannon's design method says that treatment efficiency has a first order relationship to the total organic loading. When plotted on semi-log paper a straight line indicates first order. Kincannon's equation

$$S_e = S_o (1 - e^{-KL})$$

where

K = reaction rate constant

L = total organic loading

where

$$L = \frac{Q S_o 8.34}{DA}$$

Q = flow rate (MGD)

S_o = influent substrate concentration

D = tower depth

A = cross-sectional area of tower (ft²)

The temperature related constant in Kincannon's method is also incorporated in K, reaction rate constant. K is found by plotting per cent COD removal versus total organic loading. Figure V in the results chapter shows a logrimatic plot of the data collected in these experiments. From this plot it can be seen that the data collected in these experimental runs do not fit a good straight line plot in

some instances.

As temperature is increased the efficiency or COD removed increases. The 18⁰C temperature is still within the psychrophilic bacteria growing range. But some mesophilic bacteria will exist that grow at the lower end of the mesophilic range. The cutoff point of psychrophiles and mesophiles is not a clear cut definition of environments. The strict psychrophiles probably grow below 10⁰C. The increase in temperature increases the growth rate requiring less time to remove organics from the waste. This can be seen by viewing the graphs in Figures 3 and 4. The higher the temperature goes within a certain range the better the efficiency within a set depth of biological towers.

It can be seen in Figure 4 that as the efficiency increases at low initial substrate concentrations, the actual COD of the effluent is higher than the projected plot drawn through the first three points. This unexpected jump in COD remaining at eight feet is believed to be due to a by-product or intermediate formed as the substrate was decreasing. No analysis was made to verify that this was a by-product or to identify the content of the by-product but Cook (27) in his experimental work found the same phenomena. Additional work needs to be performed in this area to determine if the intermediates formed are biodegradeable or non-biodegradeable. It appeared from the data that a portion may be biodegradeable if the microorganisms were given enough time to acclimate themselves to use it as a substrate.

Figure 5 in the results chapter, efficiency versus total organic loading, the logarithmic graph, and Figures 6, 7, and 8 the arithmetic plots of the respective temperatures 10⁰C, 18⁰C and 23⁰C does not agree with the previous investigators work that efficiency of removal is

dependent on total organic loading.

Figure 6, the 10°C graph does show that efficiency is related to total organic loading in that at lower organic loading the efficiency does improve. This could be explained by the fact that the overall efficiency of the entire 10°C temperature range of substrate concentrations was ineffective in removing the organic matter, and consequently the original substrate concentration did not get low enough to detect substrates that could not be removed, that non-biodegradable, or intermediate production.

Figure 7, the 18°C temperature, arithmetic graph of per cent COD removed versus total organic loading, shows that the increase in temperature improves the removal efficiency, however, it does not show that an increase in efficiency is a function of the total organic loading. This can possibly be explained by the fact that intermediate production or the original non-biodegradable portion of the substrate kept the efficiency from increasing as the total organic loading decreased. This same effect can be seen in Figure 5, the logrimetic plot of efficiency versus total organic loading. The efficiencies remained approximately the same at all organic loadings.

Figure 8, the 23°C temperature arithmetic graph shows much the type data that was present in Figure 7. The results obtained with these varying substrate concentrations and temperature changes tend to disagree with the previous investigations into substrate removal kinetic on biological towers. A possible explanation for this occurrence is due to the fact that an original non-biodegradable portion of the substrate was carried throughout the experiment.

It is possible that the nitrogen source, fertilizer, for this

synthetic substrate, contained a residual, non-biodegradable portion of approximately 30 mg/l COD. If this had been determined during these experiments and subtracted from the soluble biodegradable portion, it is believed the curves in Figure 5 would have been straight lines as 1st order kinetics depict. Also it is believed that the three separate curves in Figures 6, 7, and 8 would have converged to a single curve. This is indicated in Figure 4 for the experiment using an initial COD of 100 mg/l. The COD was never removed below 30 mg/l, even though four feet of tower remained available for removing COD.

From this study it appears that the non-biodegradable portion a wastewater must be determined and considered when calculating the efficiency. If not, an erroneous value will be determined. This could explain some of the low efficiencies obtained at low loadings by previous investigators in the Oklahoma State University Bioenvironmental Engineering Laboratories.

CHAPTER VI

CONCLUSIONS

The results of experiments due to temperature change and varying substrate concentrations lead to the following observations:

1. Temperature does affect the treatment efficiency
2. Substrate removal follows first order kinetics with respect to depth.
3. The non-biodegradability portion of the feed affects the kinetics of substrate removal.

CHAPTER VII

SUGGESTIONS FOR FUTURE WORK

1. Experimental work should be performed to determine the intermediates produced and an analysis to determine the non-biodegradable content.
2. Additional temperature work at the low temperatures to verify or disprove the work in this paper.
3. A study to check the kinetics of temperature change and substrate removal simultaneously.

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