This dissertation has been microfilmed exactly as received

69-12,179

McCLELLAND, Nina Irene, 1929-

THE EFFECT OF SURFACE ACTIVE AGENTS ON SUBSTRATE UTILIZATION IN AN EXPERIMENTAL ACTIVATED SLUDGE SYSTEM.

The University of Michigan, Ph.D., 1968
Engineering, sanitary and municipal

University Microfilms, Inc., Ann Arbor, Michigan

THE EFFECT OF SURFACE ACTIVE AGENTS ON SUBSTRATE UTILIZATION IN AN EXPERIMENTAL ACTIVATED SLUDGE SYSTEM

by

Nina Irene McClelland

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in
The University of Michigan
1968

Doctoral Committee:

Associate Professor K. H. Mancy, Chairman
Mister F. J. Coughlin, Procter and Gamble Company
Mister George H. Eagle, Ohio Department of Health
Professor John J. Gannon
Professor Lloyd L. Kempe
Assistant Professor Theodore F. Wetzler

A B S T R A C T

THE EFFECT OF SURFACE ACTIVE AGENTS ON SUBSTRATE UTILIZATION IN AN EXPERIMENTAL ACTIVATED SLUDGE SYSTEM

by

Nina Irene McClelland

Chairman: K. H. Mancy

Since June, 1965, linear surface active agents have been used as the active ingredient in most household detergent formulations. Prior to marketing, testing of these compounds was limited almost exclusively to measuring the rates at which they were oxidized in conventional biological waste treatment facilities. The purpose of this investigation was to determine the effect of surface active agents, both hard and soft, on substrate oxidation, selected biomass characteristics, and oxygen utilization in an experimental activated sludge system.

Six continuous flow units similar to those developed in Germany for testing surfactant biodegradability were used for measuring substrate oxidation. Activated sludge obtained from the field was used as the initial inoculum. A synthetic substrate of powdered dry milk solution was fed continuously to each reactor. Two units served as controls, and LAS or ABS in concentrations from 5 to 20 milligrams per liter was added to the other four units. Substrate levels in the influent and filtered effluent were measured daily on a grab sample basis, and reported in terms of BOD, TOC, and COD. Changes in the structure of the biomass were observed microscopically. Oxygen utilization rates were determined in a batch system with a membrane electrode, using lactose as the substrate

and 20 milligrams per liter of each of the test surfactants.

Results indicate that substrate utilization in the experimental activated sludge system was significantly retarded in the presence of surface active agents. The extent of retardation was related to the structure and concentration of the surfactant. The effect of LAS was less than that of an equivalent concentration of ABS because of its more rapid rate of biodegradation. Surfactant adsorbed films at the floc-solution interface were effective in reducing mass transport across the phase boundary as well as in increasing the surface viscosity of the biological floc. As a result, the elasticity of the floc was reduced, and its hydrodynamic characteristics were affected. In becoming more rigid, the floc was easily fragmented, less settleable, and readily washed out of the system. This effect was more apparent in the presence of ABS than in the presence of LAS, at the specific levels tested.

ACKNOWLEDGEMENTS

I wish to express my appreciation to the members of my committee for their guidance and support throughout the course of this investigation. The assistance of Professors-Emeritus Clarence J. Velz and Gerald M. Ridenour, and of Professor Morton S. Hilbert is also gratefully acknowledged.

In addition, I wish to thank my family and close friends for their patience and tolerance throughout my entire graduate program. Without their encouragement, its completion would not have been possible.

This dissertation is dedicated to the memory of the late Walter F. Snyder, philanthropist, leader, teacher, and friend.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
INTRODUCTION	1
Purpose	1
Motivation	1
AEROBIC BIOLOGICAL OXIDATION	3
ACTIVATED SLUDGE	6
KINETIC RELATIONSHIPS	8
OXYGEN TRANSFER	18
SURFACE ACTIVE AGENTS	23
Synthetic Surface Active Agents	25
A SIMPLIFIED MODEL	29
MATERIALS AND METHODS	30
Substrate Oxidation	30
Experimental Apparatus	30
Initial Inoculum	30
Substrate	33
Nutritional Requirements	37
Surfactants	39
Measurement Parameters	39
Experimental Procedure	41
Biomass Characteristics	42
Oxygen Utilization	43
Experimental Apparatus	43
Initial Inoculum	43
Substrate and Surfactants	45
Measurement Parameters	45
Experimental Procedure	45
Statistical Interpretation of Results	46

TABLE OF CONTENTS (Continued) Page

RESULTS 49

Substrate Utilization 49
Biochemical Oxygen Demand 49
Total Organic Carbon 52
Chemical Oxygen Demand 52
Surface Active Agents 60

Biomass Characteristics 65

Oxygen Utilization 72

DISCUSSION 79

CONCLUSIONS 83

REFERENCES 84

LIST OF TABLES

TABLE		Page
I	Summary of Theories for Gas Transfer Mechanism	21
II	Approximate Analysis of White House Instant Nonfat Dry Milk	34
III	Theoretical Composition of Experimental Substrate Containing 0.025 Percent White House Brand Nonfat Dry Milk	35
IV	The Effect of 10 and 20 Milligrams per Liter ABS and 10 and 20 Milligrams per Liter LAS on Substrate Utilization, Expressed in Terms of BOD	50
V	Theoretical Total Organic Carbon in the Substrate and Observed Total Organic Carbon Content of the Substrates and Effluents for Twenty-two Days of Operation in the Control System and in the Presence of ABS and LAS	53
VI	The Effect of ABS and LAS on Substrate Utilization, Expressed in Terms of COD	57
VII	Summary of Statistical Significance of the Effect of ABS and LAS on Substrate Utilization	63
VIII	Mean Percent Reduction of 10 and 20 Milligrams per Liter of ABS and LAS in the Experimental System	64

LIST OF FIGURES

Figure		Page
1	Schematic Illustration of the Aerobic Biological Oxidation of Organic Matter	4
2	Growth and Substrate Utilization in a Biological System	9
3	Schematic Illustration of the Physical System Used by Gates for Developing a Kinetic Model	14
4	Structure of the Air-Water Interface of a Solution Which Contains Surface Active Agents	14
5	The Structures of Typical Molecules of ABS and LAS	28
6	Reactor Assembly	31
7	Experimental Continuous Flow Activated Sludge Units	32
8	The Role of Nitrogen in Aerobic Biological Waste Treatment Processes	38
9	Experimental Apparatus Used for Measuring Oxygen Utilization	44
10	Example of Statistical Plot of Control Data	48
11	The Effect of Hard vs. Soft Surfactants on Substrate Utilization, Expressed as Percentage Reduction of BOD	51
12	The Effect of ABS on Substrate Utilization, Expressed as Percentage Reduction of COD	54
13	The Effect of LAS on Substrate Utilization, Expressed as Percentage Reduction of COD	56
14	The Effect of 10 Milligrams per Liter of Hard vs. Soft Surfactants on Substrate Utilization, Expressed as Percentage Reduction of COD	58
15	The Effect of 20 Milligrams per Liter of Hard vs. Soft Surfactants on Substrate Utilization, Expressed as Percentage Reduction of COD	59
16	Relative Effect of Retardation of 10 Milligrams per Liter of ABS and LAS on Substrate Utilization	61
17	Relative Effect of Retardation of 20 Milligrams per Liter of ABS and LAS on Substrate Utilization	62

LIST OF FIGURES (Continued)

Figure		Page
18	Photomicrograph of Fresh Initial Inoculum, 970X	66
19	Photomicrograph of Biomass with Paramecia Predominating, 470X	67
20	Aeolosoma quaternarium in the Biomass, 470X	68
21	Aeolosoma quaternarium, 970X	68
22	Filamentous Biomass, 970X	70
23	Bulking in the Experimental System	71
24	A Typical Control Reactor with Clear Supernatant in the Settling Unit	73
25	A Typical Reactor with 20 Milligrams per Liter ABS in the Substrate	74
26	The Effect of 20 Milligrams per Liter ABS on Oxygen Utilization in a Mixed Culture of Microorganisms, Measured with a GCOA	75
27	The Effect of 20 Milligrams per Liter LAS on Oxygen Utilization in a Mixed Culture of Microorganisms, Measured with a GCOA	77
28	The Effect of 20 Milligrams per Liter ABS on Lactose Utilization in a Mixed Culture of Microorganisms	78

INTRODUCTION

Purpose

The purpose of this investigation is to determine the effect of surface active agents on substrate oxidation, selected biomass characteristics, and the rate of oxygen utilization in an experimental activated sludge system.

Motivation

Public interest in the detergent "problem" was stimulated by a lack of understanding of the effect of surface active agents on biological waste treatment processes. Until June, 1965, alkylbenzene sulfonate (ABS) was the surfactant most commonly used in packaged household detergents. This compound is a paraffin derivative with a highly branched molecular configuration. In conventional aerobic biological treatment systems, it degrades relatively slowly. Linear alkylate sulfonate (LAS) was developed in response to public demands for "biodegradable" detergents. A compound such as ABS, which is more difficult to degrade than normal domestic waste water, is sometimes referred to as "hard". Compounds which degrade more readily are called biologically "soft".

Pre-market testing of detergent formulations with linear surface active agents was limited almost exclusively to biodegradation rates and foaming characteristics. A search of the literature failed to provide significant information on how the performance characteristics of conventional activated sludge waste treatment systems would be affected by their general use.

In 1967, a paper which described an investigation carried out in Russia in 1965 was abstracted in the literature (1). Bolotina et al. studied the effect of various concentrations of a surfactant, Sulfonol NP-1,

on the aerobic and anaerobic biological purification of waste water. A detergent formulation containing 5 milligrams per liter of this surfactant as its active ingredient, when added to a municipal waste water, was reported to have no adverse effect on the aerobic treatment process. At levels greater than 10 milligrams per liter, biological purification was said to be affected in the following ways: (1) the concentration of suspended solids and the oxygen demand in the effluent were increased, (2) nitrification was retarded, and (3) a froth was produced. These effects were attributed to an accumulation of the surfactant in the activated sludge. The level of NP-1 in the waste was reduced by 40 percent. The efficiency of the treatment process was inhibited when the level of NP-1 was greater than 7 milligrams per gram of mixed liquor suspended solids. From these effects, it can be assumed that NP-1 was a "hard" surfactant with a branched molecular configuration.

Two field studies with linear surfactants were reported (2, 3); however, it is difficult to interpret observations made in the field where no control can be imposed on the environment, and operational changes are limited to the design capabilities of the test units.

This investigation was designed to determine: (1) to what extent "soft" detergents affect the performance characteristics of an experimental activated sludge system, (2) how this effect compares with that of a "hard" detergent, and (3) to provide a conceptual understanding of the mechanism of interference of compounds with surface active characteristics on this type of treatment process.

AEROBIC BIOLOGICAL OXIDATION

Dissolved organic substrates are decomposed by aerobic biological oxidation, as shown schematically in Figure 1. Two interrelated processes are involved: metabolism, in which the substrate molecules are oxidized and energy is released to the system; and synthesis, in which characteristic cellular components are formed from simple precursors and assembled into specialized cell structures. Using lactose as an example, the overall reactions for substrate oxidation and biosynthesis can be expressed as follows:

Substrate oxidation (Metabolism):

$$C_{12}H_{22}O_{11}.H_2O + 12\ O_2 \rightarrow 12\ CO_2 + 12\ H_2O \qquad (1)$$

or, simplifying:

$$(CH_2O) + O_2 \rightarrow CO_2 + H_2O \qquad (2)$$

Biosynthesis:

$$8\ (CH_2O) + 3\ O_2 + NH_3 \rightarrow \underset{\text{Bacterial cells}}{C_5H_7NO_2} + 3\ CO_2 + 6\ H_2O\ . \qquad (3)$$

In addition, cellular material is oxidized by endogenous respiration (auto-oxidation):

$$C_5H_7NO_2 + 5\ O_2 \rightarrow 5\ CO_2 + NH_3 + 2\ H_2O \qquad (4)$$

The mechanism of biological oxidation has been the subject of numerous investigations (4, 5, 6, 7). Following the extracellular hydrolysis of macromolecules, it has now been shown that substrate molecules are linked to their respective enzyme systems at specific sites on the

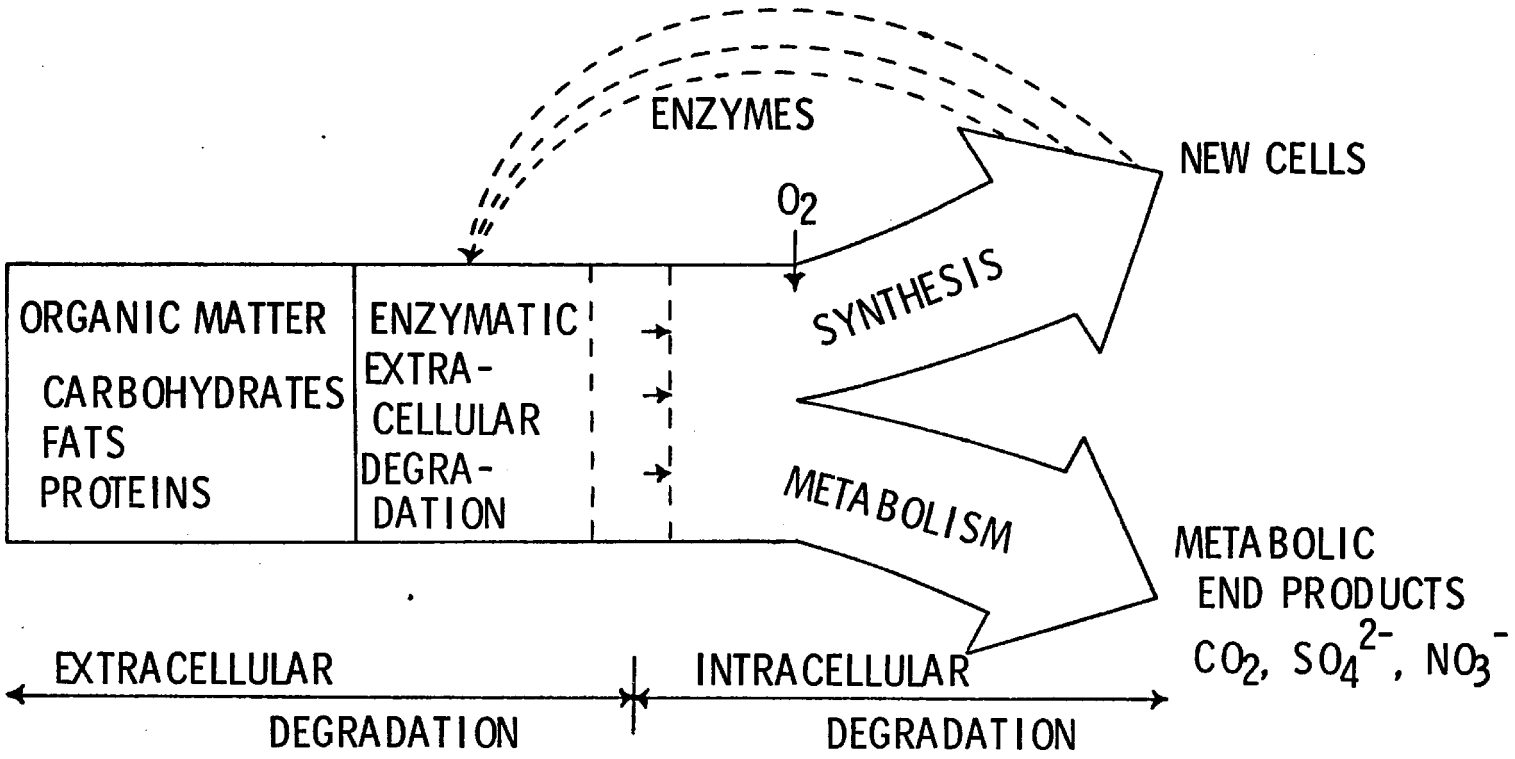


Figure 1. Schematic illustration of the aerobic biological oxidation of organic matter.

surface of the cell (8). Transport across the cell membrane occurs by diffusion or by active transport, an endergonic mechanism which transfers molecules against a concentration gradient when metabolic energy is available. The reaction sequence which occurs within the cell is determined by the nature of the substrate being oxidized.

ACTIVATED SLUDGE

Aerobic bio-oxidation systems are used extensively for treating domestic and industrial wastes. In the activated sludge process, a flocculant suspension of microorganisms is developed in a fluidized, continuous flow reactor system. Organic pollutants in the waste water serve as substrates, and are transferred to the biomass matrices. Air is supplied to keep the system aerobic, and to provide a dynamic interface (contact opportunity) across which substrate transfer can occur.

The active biomass is a physiologically mature, heterogeneous population of bacteria, fungi, protozoa, rotifers, and nematodes. After a defined aeration period, the flocculent biomass is removed from its suspending medium by sedimentation. Recirculation of part of the settled floc to the aerator as "seed" insures the continuing proliferation of a physiologically mature culture with floc-forming potential.

The ultimate goal of an activated sludge system is to produce a relatively clear, stable effluent. Although Kountz and Forney (9) showed that total oxidation of the waste does not occur, an ecological system is developed, which will utilize a complex, dilute, and often highly variable substrate as a food source while being held in intimate contact with the substrate for a defined period of time.

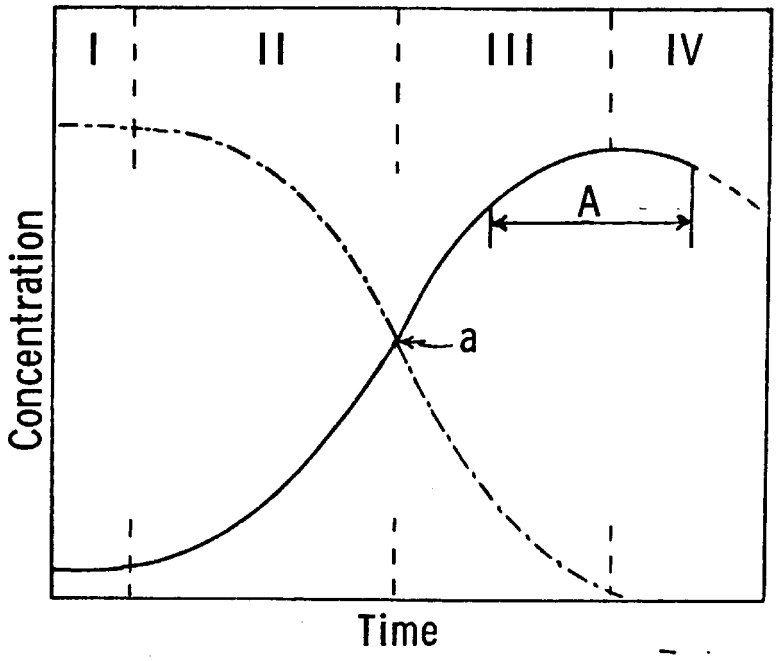
In an effectively designed and operated activated sludge system, flocculation and sedimentation of the biomass, oxygen transfer, and substrate transport are important phenomena. Some degree of operational flexibility can be attained by varying the contact period (hydraulic loadings), substrate level (organic loadings), turbulence, concentration of biomass, and the physiological age of the culture. An adequate supply

of essential nutrients must be available, and hydrogen ion concentration (pH) should be controlled within certain limits. Temperature is equally important to the system, but under field conditions, it is difficult, if not impossible, to adjust.

KINETIC RELATIONSHIPS

In a biological system, growth and substrate utilization are related directly. According to the classical concept, graphically shown in Figure 2, there is little or no increase in cell number and no substrate is consumed in the initial lag phase. During the logarithmic phase, an excess of substrate is present and the organisms multiply geometrically. Growth and substrate oxidation rates are constant and are a function only of the number of cells present in the system. At the point of inflection "a" in Figure 2, the food level is just adequate to maintain the maximum rate of growth; beyond this point it is limiting, and the growth rate declines. When the substrate has been exhausted, the number of organisms is reduced by endogenous respiration (cellular auto-oxidation) and the death phase occurs.

Line "A" in Figure 2 illustrates the ideal growth-substrate utilization relationships which exist in a conventional, continuous flow activated sludge system. The lag phase should be expected to occur only if the organisms are acclimating to a characteristically different substrate, or if the system is being operated with the biomass in the advanced endogenous phase. When a high initial rate of substrate utilization occurs for a short period of time, it can be attributed to the flocculation and adsorption of suspended and colloidal solids and the biosorption of soluble substrate. Log growth is eliminated when the organic strength of the raw waste is low or when return sludge is recirculated at a high rate.



——	Biomass
—·—	Substrate
I	Lag Growth Phase
II	Logarithmic Growth Phase
III	Declining Growth Phase
IV	Endogenous Respiration Phase
a	Inflection Point
A	Range of Operation of a Conventional Activated Sludge Plant

Figure 2. Growth and substrate utilization in a biological system.

Numerous attempts have been made to express the kinetics of biological oxidation. Basically, when the organic loading is high (excess substrate is available) and log growth occurs, the kinetics of growth and substrate utilization can be described by the following expressions:

Growth:

$$\frac{dX_1^O}{d\theta} = k^O X_1^O \tag{5}$$

which integrates to:

$$\ln \frac{X_0^O + X_1^O}{X_0^O} = k^O \theta \tag{6}$$

Substrate:

$$- \frac{dX_1^N}{d\theta} = k^N X_1^N \tag{7}$$

which integrates to:

$$\ln \frac{X_0^O + aX_2^N}{X_0^O} = - k^N \theta = \ln \left[1 + \frac{aX_2^N}{X_0^O} \right] \tag{8}$$

where:

X_0^O = initial concentration of biomass, (mg/l)

X_0^N = total oxidizable substrate, (mg/l)

and , at any time θ:

X_1^O = concentration of biomass, (mg/l)

$\Delta X_1^O = X_1^O - X_0^O$ = increase in biomass, (mg/l)

X_2^N = substrate oxidized, (mg/l)

$X_1^N = X_0^N - X_2^N$ = oxidizable substrate remaining, (mg/l)

k^O = growth rate constant, (time $^{-1}$)

k^N = substrate utilization rate constant, (time $^{-1}$)

Both Monod (10) and Hinshelwood (11) reported that growth is limited primarily by the depletion of substrate or by the accumulation of toxic metabollic products. When the declining growth phase is principally the result of substrate depletion, the rate of growth is a function of, and directly proportional to, the concentration of substrate remaining.

In the endogenous phase, the available substrate has been depleted. Organisms which were synthesized during the growth phases undergo continuous auto-oxidation, the rate of which approximates first order initially ($-dX_I^O / d\theta = k^O X_I^O$), then decreases as the cellular constituents remaining become more difficult to oxidize.

Non-specific terms are used to describe growth and substrate utilization in an activated sludge system. Because of the numbers and varieties of organisms involved, growth is determined most frequently on a weight basis. Substrate utilization is measured in terms of reduction of biochemical oxygen demand (BOD) (12, 13, 14, 15) or chemical oxygen demand (COD) (16, 17, 18), oxygen consumption (15), adenosine triphosphate (ATP) production (19), or release of free energy to the system (16, 17).

Gates (20) reviewed the kinetic models of McKinney (21), Eckenfelder and O'Connor (22), and Monod (10), and concluded that the Monod model was applicable for all situations. McKinney's expressions were developed using a continuous flow system with low levels of substrate, expressed as BOD:

$$\frac{dX^O}{d\theta} = k^O X^N \qquad (11)$$

and

$$\frac{dX^N}{d\theta} = - k^N X^N \qquad (12)$$

where:

X^O = concentration of biomass, (mg/l)

X^N = concentration of substrate, (mg/l)

k^O = production constant = $\frac{\text{biomass produced, (mg/l)}}{\text{substrate utilized, (mg/l)}}$, (time $^{-1}$)

k^N = substrate utilization rate constant, (time $^{-1}$)

θ = time

Eckenfelder and O'Connor used batch reactor systems to develop separate models for high and low substrate BOD_5. The transition point was reported to be somewhere between 200 and 300 milligrams per liter (mg/l):

For high BOD_5:

$$\frac{dX^O}{d\theta} = (k - k^D)X^O \tag{13}$$

For low BOD_5:

$$\frac{dX^N}{d\theta} = - k_2 X_a^O X^N \tag{14}$$

where:

k = biomass growth rate constant, (time $^{-1}$)

k^D = biomass auto-oxidation rate constant, (time $^{-1}$)

k_2 = substrate utilization rate constant, (mg/l) $^{-1}$ (time $^{-1}$)

X_a^O = average concentration of biomass, (mg/l)

When the two rate expressions developed by Eckenfelder and O'Connor were combined, they were shown to be an approximation of the continuous functions of Monod, which have no constraints:

$$\frac{dX^O}{d\theta} = \left[\frac{k^m X^N}{K + X^N}\right] X^O \tag{15}$$

and:

$$\frac{dX^N}{d\theta} = \frac{1}{Y^o}\left[\frac{k^m X^N}{K + X^N}\right] X^o \qquad (16)$$

where:

k = biomass growth rate constant, (time $^{-1}$)

k^m = maximum growth rate constant, (time $^{-1}$)

K = a constant, system dependent, (mg BOD/l)

Y^o = yield = $\frac{\text{biomass produced, (mg)}}{\text{BOD removed, (mg)}}$

Using the basic Monod model, Gates (20) developed the following relationships for continuous flow systems, to which the data from this investigation apply. The physical system is shown in Figure 3. Assumptions and conditions are stated as follows:

Q = volumetric flow rate, (ml) (time $^{-1}$)

V = volume of reactor, (ml)

X_0^N = substrate concentration in influent, (mg/l)

X_1^N = substrate concentration in effluent, (mg/l)

X_0^o = organism concentration in influent, (mg/l)

X_1^o = organism concentration in reactor, (mg/l)

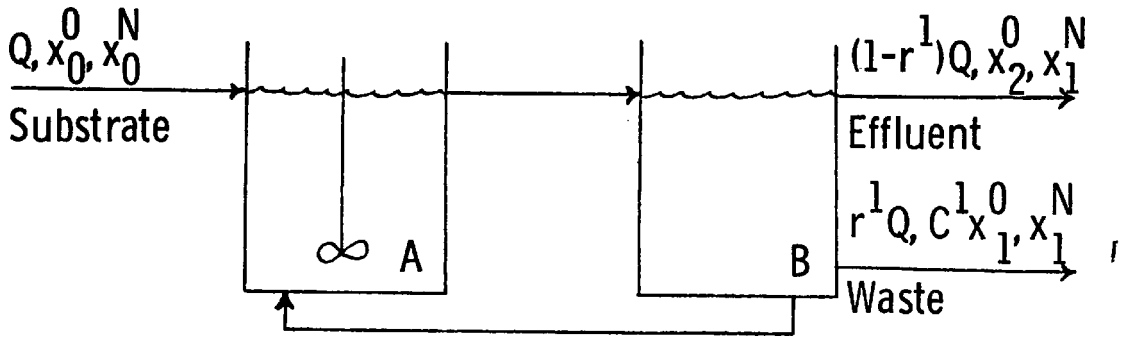
X_2^o = organism concentration in effluent, (mg/l)

r' + fraction of influent going to waste

C' = degree of concentration of organisms attained in settler

where:

$$C' = \frac{X^o \text{ in underflow}}{X^o \text{ in settler influent}} \qquad (17)$$



A Reactor

B Settler

Figure 3. Schematic illustration of the physical system used by Gates (20) for developing a kinetic model.

Assume:

1. There is no reaction in the settler.
2. The reactor is completely mixed.
3. The intermix flow model is applicable.

Let:

X_W^O = average concentration of organisms leaving the system,

then:

$$X_W^O = \frac{(1 - r')Q\, X_2^O + r'\, Q\, C'\, X_1^O}{Q} \tag{18}$$

Also let:

$$C^O = \text{recycle factor} = \frac{X_W^O}{X_1^O} \tag{19}$$

Materials balance for organisms:

$$V \frac{dX_1^O}{d\theta} = QX_0^O + kX_1^O V - k^D X_1 V - QX_W^O \tag{20}$$

For steady state conditions:

$$\frac{dX_1^O}{d\theta} = 0 \tag{21}$$

$$X_0^O \approx 0 \tag{22}$$

Substituting in (20):

$$0 = kX_1^O - k^D X_1^O - \frac{X_W^O}{\theta_T} \tag{23}$$

where:

k = organism growth rate constant, (time $^{-1}$)

k^D = auto-oxidation rate constant, (time $^{-1}$)

θ_T = theoretical hydraulic detention time, (time $^{-1}$)

$$\theta_T = \frac{V}{Q} \tag{24}$$

Also:

$$0 = k - k^D - \frac{C^o}{\theta_T} \tag{25}$$

Rearranging (25):

$$k = k^D + \frac{C^o}{\theta_T} \tag{26}$$

From the Monod Model:

$$k = \frac{k^m X_1^N}{K + X_1^N} \tag{27}$$

Substituting (27) in (26) and solving for X_1^N

$$\boxed{X_1^N = \frac{K(k^D\theta_T + C^o)}{k^m\theta_T - (k^D\theta_T + C^o)}} \tag{28}$$

Materials balance for substrate:

$$V\frac{dX_1^N}{d\theta} = QX_O^N - \frac{kX_1^O V}{Y^o} - QX_1^N \tag{29}$$

From the Monod Model:

$$\frac{dX^N}{d\theta} = \frac{1}{Y^o}\frac{dX^O}{d\theta} = \frac{1}{Y^o}kX^O \tag{30}$$

For steady state conditions:

$$\frac{dX_1^N}{d\theta} = 0 \tag{31}$$

Therefore:

$$0 = \frac{X_O^N}{\theta_T} - \frac{kX_1^O}{Y^o} - \frac{X_1^N}{\theta_T} \tag{32}$$

Substituting (26) in (32):

$$0 = \frac{X_0^N}{\theta_T} - \left[k^D + \frac{C^o}{\theta_T}\right] \frac{X_1^o}{Y^o} - \frac{X_1^N}{\theta_T} \tag{33}$$

Solving for X_1^o:

$$\boxed{X_1^o = \frac{Y^o (X_0^N - X_1^N)}{k^D \theta_T + C^o}} \tag{34}$$

The unoxidized substrate concentration is defined in Equation (28) as a function of K, a constant which is system dependent, the hydraulic detention time, and a recycle factor, C^o. As shown in Equation (34) the concentration of biomass in the reactor is a function of yield, substrate utilized, auto-oxidation rate, residence time, and recycle factor.

In any gas transfer reaction, phase compositions are altered by the transfer of a gas solute from one phase to another. Mass flux, which is defined as the rate of gas transfer per unit of gas-liquid interfacial area, is expressed as follows:

$$J = \frac{dN}{dtA} \tag{35}$$

where:

J = mass flux

N = gas transferred per unit time, (g-moles)

t = time, (min.)

A = interfacial area, (cm^2)

The rate of gas absorption by a liquid is directly proportional to its saturation deficit:

$$J = K_L(C_s - C_t) \tag{36}$$

where:

C_s = equilibrium gas concentration

C_t = actual gas concentration in the body of the liquid

$(C_s - C_t)$ = driving force, ($g\text{-moles}/cm^3$)

K_L = gas transfer coefficient, (cm/min.)

Adding the volume of liquid to Equation (36), the rate of gas transfer is shown to be a first order reaction:

$$\frac{(dN/V)_t}{dt} = \frac{dC_t}{dt} = K_L(A/V)(C_s - C_t) \tag{37}$$

which integrates to:

$$\ln \frac{(C_s - C_o)}{(C_s - C_t)} = K_L(A/V)t \tag{38}$$

Solving for K_L:

$$K_L = \frac{2.303}{t} \ (V/A) \log \frac{(C_s - C_o)}{(C_s - C_t)} \tag{39}$$

A number of theories have been proposed to explain the mechanism of gas transfer. Each may be correct for a specific process. No single theory is applicable to all gas transfer reactions, since the rate determining steps may differ. The main controlling factor is the turbulence regime maintained in the liquid phase.

According to the Two Film Theory (23), a liquid film exists in the liquid phase and a gas film exists in the gas phase at the interface. Gas transfer occurs solely by molecular diffusion through the two films, and the rate of transfer is directly proportional to the diffusivity coefficient, D. In the case of a sparingly soluble gas like oxygen, the major resistance is the liquid film.

Continuous renewal of the liquid surface by fresh eddies from the liquid bulk is proposed in the Penetration Theory (24). The rate of gas transfer is proportional to $D^{\frac{1}{2}}$; therefore, the effect of turbulence is considered. One of the limitations of this theory is that the rate of surface renewal is assumed to be constant. Dankwerts (25) proposed a modification which rendered this assumption unnecessary by postulating a random renewal of the surface film.

According to the Boundary Layer Theory (26), the rate of gas transfer is proportional to $D^{2/3}$. Convection in the form of a liquid

velocity gradient is considered to exist along the interface in the direction parallel to the phase boundary. As indicated by the comparatively low value of D, the forces of turbulence and convection are considered to be more significant than those of diffusion in gas transfer reactions. These theories and the corresponding values for K_L are summarized in Table I (28).

Bennett and Kempe (28) reported that the transfer of oxygen from air to a bacterial cell can proceed along either of two independent pathways. When the gas is dissolved in and transported through the bulk of the liquid to the surface of the cell, six stages, each with its own resistance, are involved. The resistances in the bulk of the liquid and through the liquid film around the cell are eliminated when the cell is adsorbed onto the gas bubble. When the liquid films around the cell and around the bubble are united, the gas is reported to be transferred directly across the common film.

In an activated sludge system, oxygen must be transferred to the biomass at a rate sufficiently high to ensure that metabolic activity is not limited by its availability. The overall transfer mechanism involves: (1) dissolution of oxygen from the gas phase to the liquid medium, (2) diffusion and convection transport through the bulk of the liquid to the cells, and (3) chemical reaction with oxidizable substrates through the respiratory enzyme systems of the cells. Bubble size and velocity, temperature, and the chemical composition of the waste affect the transfer mechanism. Solubility of the gas in the liquid phase is reduced by ionic impurities. Mancy and Okun (29) studied the effects of surface active agents on aeration and found that they varied with the hydrodynamic properties of the aqueous phase. In

TABLE I

SUMMARY OF THEORIES FOR GAS TRANSFER MECHANISM

Name of Theory	Definition of K_L	Principle	Reference
Two Film Theory	$K_L = \frac{D}{l}$	Steady state diffusion in a static surface layer	23
Penetration Theory	$K_L = (\frac{D}{\pi t_D})^{0.5}$	Non-steady state diffusion in a static renewable surface film	24
Dankwerts modification of penetration theory	$K_L = (fD)^{0.5}$	Random renewal of surface film	25
Boundary layer theory	$K_L = 0.8\ (D^{2/3}\nu^{1/16})\ (\frac{\omega}{l})^{0.5}$	Non-steady state diffusion in a dynamic surface film	26
Kishinevski moderation of penetration theory	$K_L = 2\left[\frac{(D+D_{turb})f}{\pi}\right]^{0.5}$	Combination of both the penetration theory and Dankwerts modification	27

turbulent systems, oxygen transfer depends on surface renewal, and the presence of surface active agents has no apparent effect. In quiescent systems, however, trace levels of surfactants cause marked changes in the physiochemical properties of the water surface and increase the resistance to gas transfer.

SURFACE ACTIVE AGENTS

By definition, surface active agents (SAA) are compounds which alter the energy relationships at interfaces. Structurally, they are composed of a hydrophilic (water soluble) group and a hydrophobic (water insoluble) group. In aqueous systems, they are adsorbed as a monolayer at the surface of the liquid with their hydrophilic groups immersed in the liquid phase. When the liquid surface is saturated (critical micelle concentration), they begin to form aggregates in the bulk of the solution. A viscous hydration layer composed of immobilized water molecules is formed under the surfactant monolayer and extends some distance (10-20 $\mathring{A}$) into the liquid bulk (Figure 4). Each of these layers provides a resistance to the transfer of oxygen from the atmosphere. The area of the liquid surface in contact with the vapor phase is reduced, and the active sites through which oxygen can be transferred at the gas-liquid interface are partially blocked by the monolayer. As the thickness and viscosity of the hydration layer are increased, the penetrating oxygen molecule encounters still greater resistance and has a longer diffusion path.

Mancy and Okun (29) defined the resistance to gas transfer as the reciprocal of the rate constant, K_L:

$$R_T = \frac{1}{K_L} \tag{37}$$

where:

R_T = total resistance to gas transfer, (min/cm)

K_L = the absolute transfer coefficient, (cm/min)

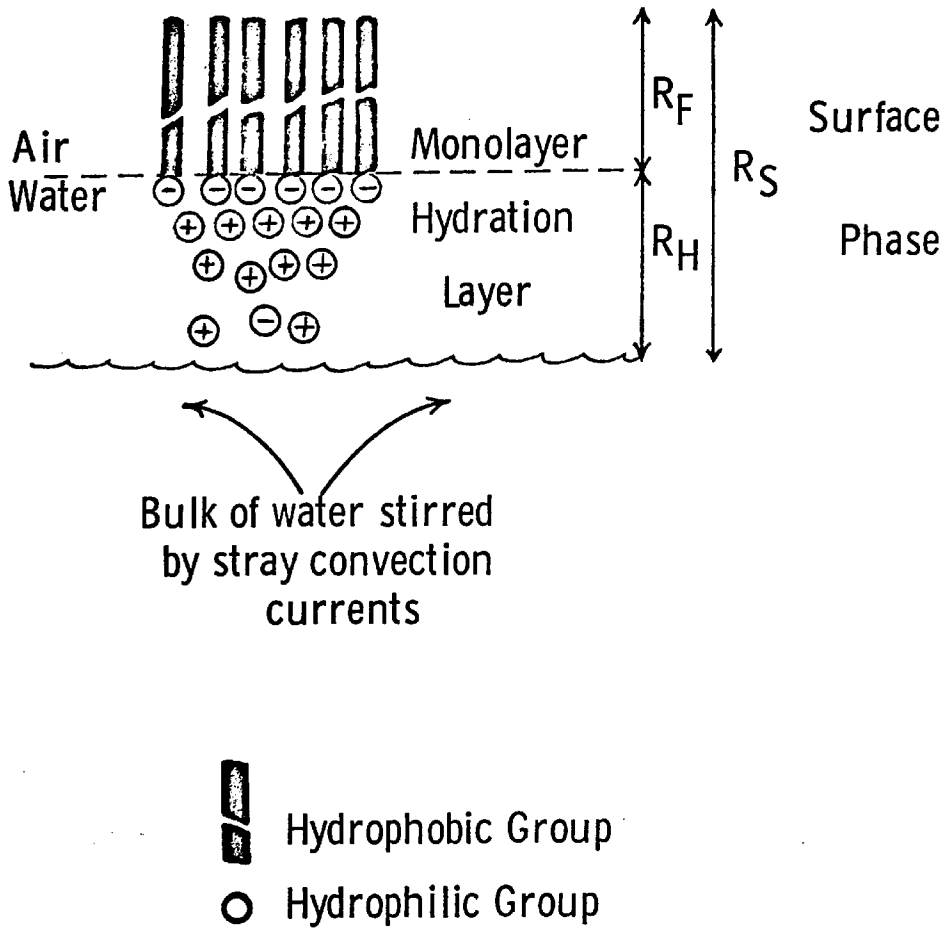


Figure 4. Structure of the air-water interface of a solution which contains SAA.

R_T was also expressed as the sum of the two main resistances, the liquid surface and liquid bulk, in series:

$$R_T = R_S + R_B \tag{38}$$

where:

R_S = surface resistance, (min/cm)

R_B = bulk resistance, (min/cm)

When surface active agents are present:

$$R_S = R_F + R_H \tag{39}$$

where:

R_F = resistance of the SAA adsorbed film, (min/cm)

R_H = resistance of the hydration layer, (min/cm)

and the net resistance caused by their presence (R_T) is the difference between the R_T of the solution containing SAA and the R_T of distilled water:

$$\Delta R_T = (R_F + R_H + R_B) - (R_S + R_B) \tag{40}$$

$$\Delta R_T = R_F + (R_H - R_S) \tag{41}$$

$$\Delta R_T = R_F + R_{HS} \tag{42}$$

where:

R_{HS} = resistance due to the increase in viscosity of the water surface, (min/cm)

In an activated sludge system, the presence of surface active agents may affect mass transport at the air-water and floc-water interfaces. As shown in Equation (39), the surface resistance due to an adsorbed film of SAA is a result of the increase in thickness and viscosity of the boundary layer.

Synthetic Surface Active Agents

The active ingredients in packaged household detergents are synthetic

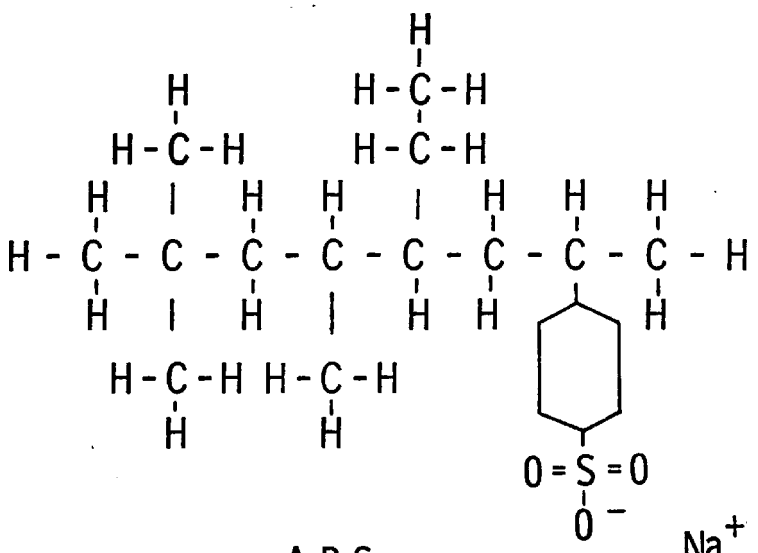
surface active agents, compounds which have been specially synthesized to provide surface active properties. Since World War II, the popularity of detergents has increased steadily. One billion pounds were produced for household use in 1963. Current demand is increasing at an estimated rate of from 3.8 to 5.0 percent per year (30).

Although surface active agents may be anionic, cationic, or nonionic in character, anionic surfactants are by far the most important in the field of waste treatment. Their concentration in untreated domestic waste water is reported to average 10 mg/l (32).

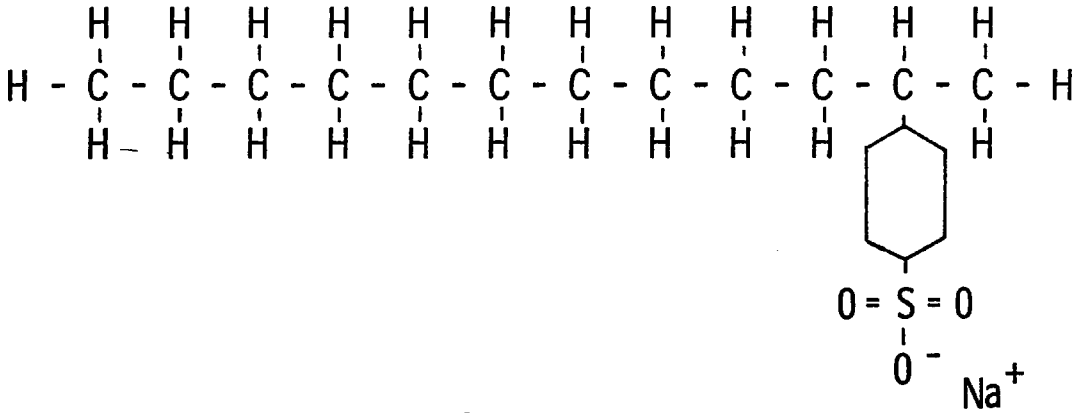
ABS is only about fifty percent degraded in conventional aerobic biological treatment systems (33). Its slow rate of biodegradation was shown by McKinney and Symons (34) to be due to structural branching. Quaternary carbon atoms in the chain block further metabolism at that point. With its tendency to produce foam in concentrations as low as 0.5 mg/l (35), ABS was reported to be causing aesthetically objectionable conditions on receiving rivers and streams (36). Although it was not toxic at levels found in waste treatment plants (37), it was thought to be associated with a variety of operational problems, including reduced settleability (38), emulsification of undesirable fats and oils (39), and foaming on aeration units (40), as well as decreased oxygen transfer rates (41, 42, 43, 44).

Public concern over problems attributed to ABS was spread rapidly through the mass communications media; and legislation was introduced on federal, state, and local levels to ban the sale of such detergents (35). At a cost of more than $100 million, the detergent industry responded to the public demands by developing LAS, a surfactant which is reported to be up to ninety percent biodegradable in conventional aerobic waste treatment processes (30). Pre-sale testing of LAS was

apparently limited to biodegradation studies. Swisher (45, 46, 47) used gas chromatography to show that it was degraded by beta-oxidation, the enzymatic process by which fatty acids are reduced to two-carbon fragments, acetyl-coA, before being completely oxidized to carbon dioxide and water. Typical structures of ABS and LAS are shown in Figure 5.



ABS



LAS

Figure 5. Structures of typical molecules of ABS and LAS.

A SIMPLIFIED MODEL

A simplified model of the experimental system may be expressed as follows:

$$(B) + (S) \xrightarrow[O_2]{} (B) + (S) + (P) \qquad (43)$$

$$(B) + (S) + (SAA) \xrightarrow[O_2]{} (B) + (S) + (P) + (SAA) \qquad (44)$$

Equation (43) describes the control. In either system, (3) is expected to increase and (s), to decrease, as Equations (1) through (3) indicate. The extent to which these conditions exist, however, may vary when SAA is added. In Equation (44), (P) includes products of substrate and of surfactant biodegradation.

It is logical to assume that the presence of SAA could interfere with substrate oxidation in any or all of the following ways: (1) toxicity, (2) inhibition, or (3) mass transport.

MATERIALS AND METHODS

This investigation was designed to include two separate phases: substrate oxidation and oxygen utilization. Characteristic changes in the biomass were observed during the first phase of the study.

SUBSTRATE OXIDATION

Experimental Apparatus

Six continuous flow activated sludge units similar in design to those developed in Germany for testing surfactant biodegradability (48) were used for measuring substrate oxidation. They were donated to the project by the Procter & Gamble Company. Each unit was constructed of glass and consisted of an aeration chamber connected to a sedimentation vessel. Twelve, five-gallon Pyrex wide mouth bottles served as substrate storage and effluent collection reservoirs. Substrate was fed by a Durrum twelve-channel liquid metering pump, Model 12AP. Neptune Dyna pressure-vacuum pumps, Model No. 3, were used to supply air to each of the aerators. Settled biomass was returned to the aeration units by air lifts operated from a laboratory line compressor. All air flows were regulated and metered by Gelman rotometers. A schematic diagram and a photograph of the experimental units are shown in Figures 6 and 7 respectively.

Initial Inoculum

A mixed population of microorganisms, activated sludge from a small, municipal, extended aeration waste treatment plant, was used as the initial inoculum for the experimental system. Although the characteristics of this material could be expected to vary somewhat over the course of the study, every effort was made to minimize these effects.

1 - Aeration chamber
2 - Sedimentation vessel
3 - Substrate
4 - Feed pump
5 - Air gauge
6 - Air lift for return sludge
7 - Effluent

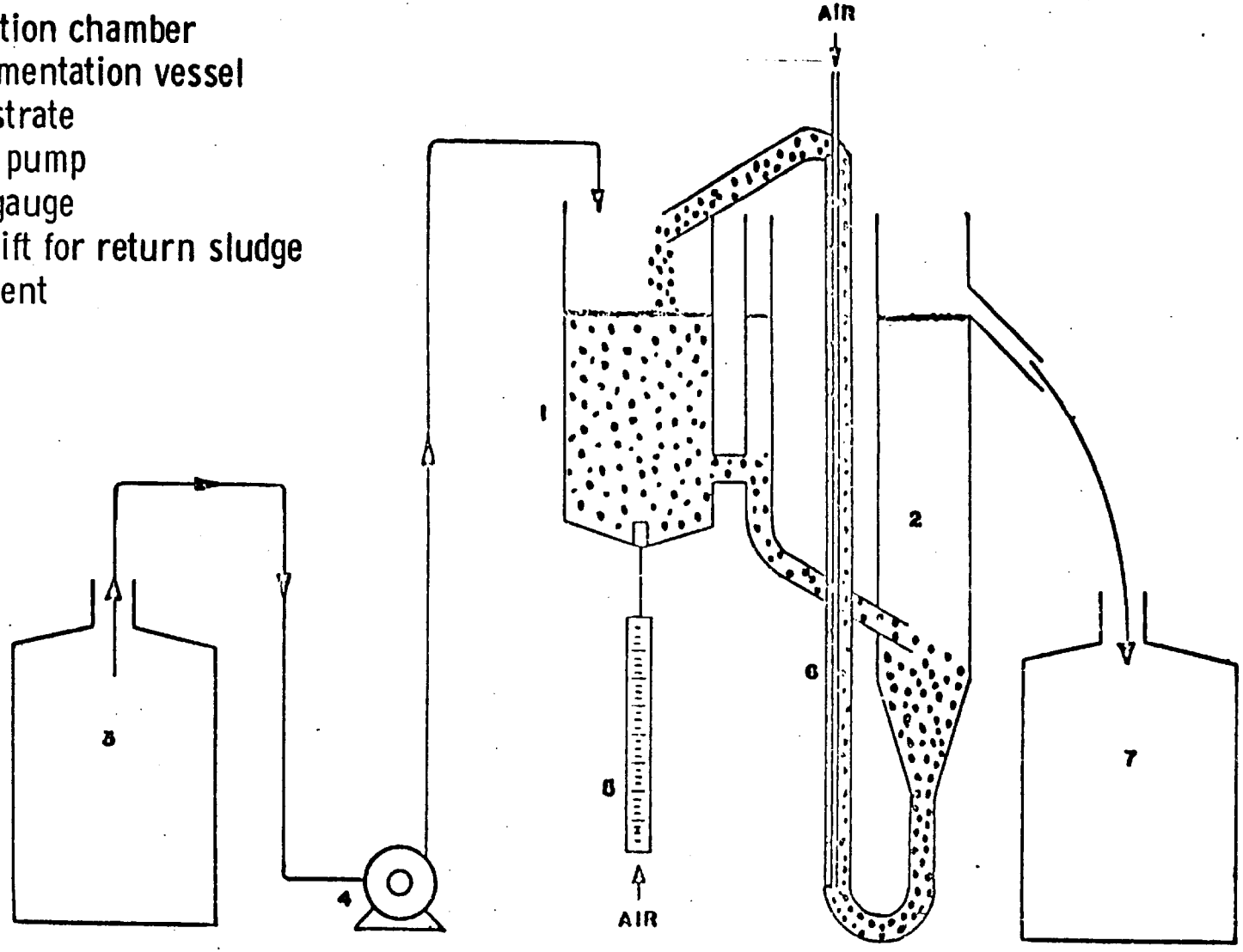


Figure 6. Reactor assembly.

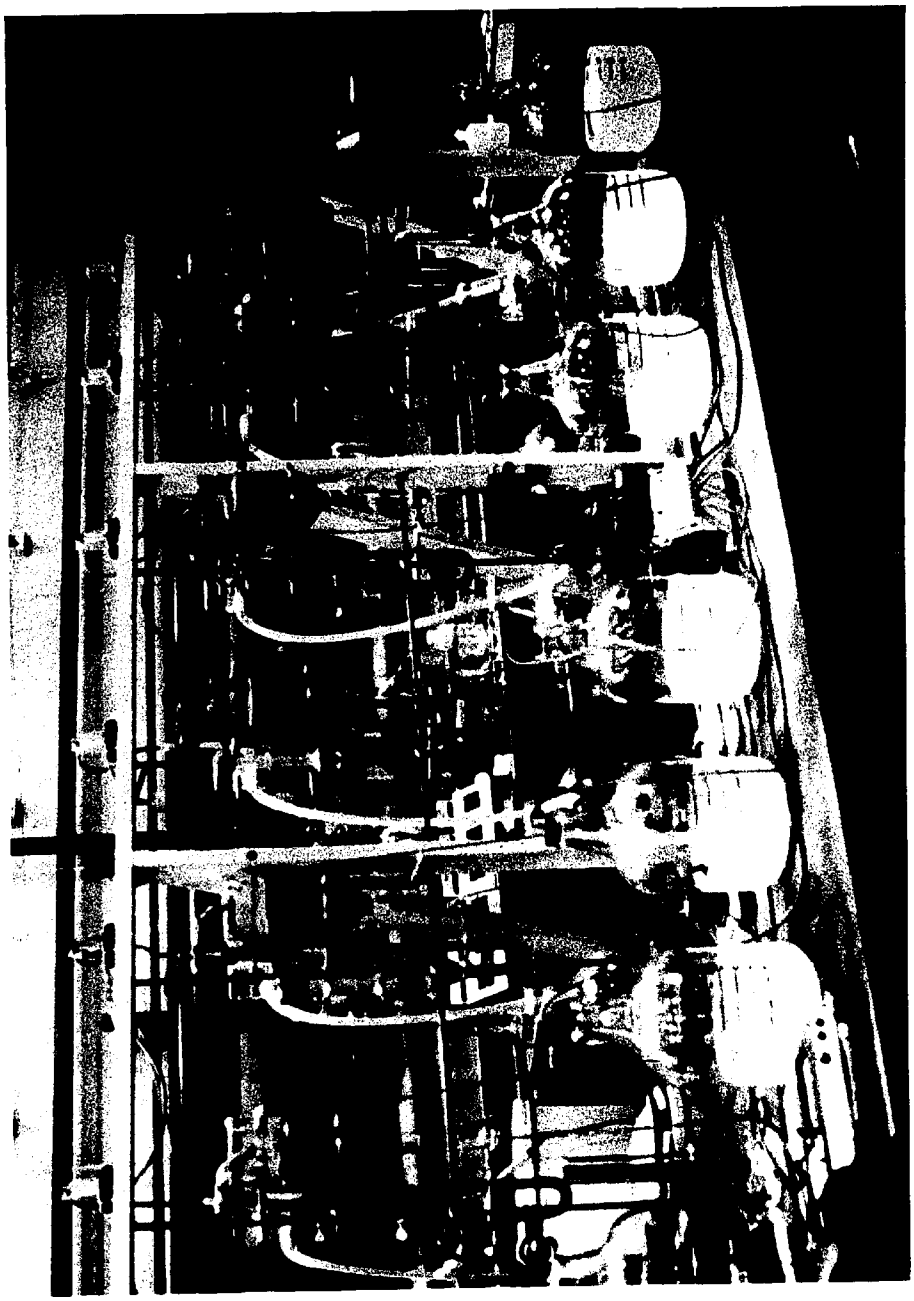


Figure 7. Experimental continuous flow activated sludge units.

All mixed liquor was obtained from the same source; namely, the National Sanitation Foundation's Research Site in Ann Arbor, where criteria for testing "package" waste treatment plants of the extended aeration type have recently been developed. Cultures brought from the field were aerated until virtually all unoxidized substrate was exhausted. They were acclimated to the experimental substrate before test data were collected.

Substrate

An extensive preliminary investigation was undertaken to select an appropriate substrate for use in this system. Synthetic wastes with glucose as the primary carbon source could not be used. Glucose encourages filamentous forms to predominate in activated sludge, creating a biomass with poor settling characteristics. In these small scale reactors, the usefulness of glucose-fed cultures was limited to two or three days.

In studying population dynamics in continuous mixed cultures, Cassell et al. (49) described a solution of skim milk which was said to be an "easily reproducible, soluble, model waste". A similar substrate was selected for this investigation. A stock solution containing 200 grams per liter of White House Brand nonfat, powdered dry milk was prepared every five days, autoclaved immediately for 15 minutes at 15 psi, and stored in a refrigerator at 5°C. Fresh substrate for each reactor was prepared daily by diluting 50 milliliters of the stock solution to 20 liters with Ann Arbor tap water. This was pumped directly into each aerator at the rate of 800 ± 100 milliliters per hour.

The manufacturer's approximate analysis of White House Brand powdered dry milk is shown in Table II. Based on these values, the theoretical composition of the diluted substrate is shown in Table III. Assuming

TABLE II

APPROXIMATE ANALYSIS OF WHITE HOUSE BRAND INSTANT NONFAT DRY MILK

		Percent
Lactose		51.0
Protein		36.8
Minerals		8.1
Calcium	1.3	
Phosphorus	1.0	
Others	5.8	
Milk Fat		0.9
Others		3.2

TABLE III

THEORETICAL COMPOSITION OF EXPERIMENTAL SUBSTRATE CONTAINING 0.025 PERCENT WHITE HOUSE BRAND NONFAT DRY MILK

	milligrams per liter
Theoretical Oxygen Demand	269.84
Organic Solids	221.75
Lactose	127.50
Protein	92.00
Ash	20.25
Total Solids	242.00

that the total oxygen demand was due to carbohydrate as lactose, protein as casein, and fat as tristearate, and that the concentration of the commercial powdered milk was 250 milligrams per liter, the total theoretical oxygen demand (TOD_S) of the experimental substrate feed was calculated to be 269.84 milligrams per liter.

Porges et al. (4) compared the assimilation equations for lactose and casein, and found that one mole of cell substance was produced from 240 units of the sugar and from 184 units of the protein, a ratio of 1.3:1. The oxygen equivalents are also similar, as indicated in the following expressions:

For lactose (from Equation 3):

$$\underset{240}{8\ CH_2O} + \underset{96}{3\ O_2} + NH_3 \longrightarrow \underset{124\ \text{(with ash content)}}{C_5H_7NO_2} + 3\ CO_2 + 6\ H_2O \qquad (45)$$

For casein:

$$\underset{184}{C_8H_{12}N_2O_3} + \underset{96}{3\ O_2} \longrightarrow \underset{124}{C_5H_7NO_2} + NH_3 + 3\ CO_2 + H_2O \qquad (46)$$

Lactose and casein are present in skim milk in similar proportions, approximately 1.3:1; therefore, the following expression for skim milk conversion to cellular material was obtained by adding the two expressions above:

For skim milk:

$$\underset{240}{8\ CH_2O} + \underset{184}{C_8H_{12}N_2O_3} + \underset{192}{6\ O_2} \longrightarrow \underset{248}{2\ C_5H_7NO_2} + 6\ CO_2 + 7\ H_2O \qquad (47)$$

$$\text{Theoretical Yield} = \frac{248}{240 + 184} \times 100 = 58.5\% \text{ by weight.}$$

In a continuous flow system treating a synthetic skim milk waste, the observed yield was 52 percent by weight. The reported difference, theoretical vs. observed, was thought to be due to endogenous respiration. Ludzack (50) related yield to reduction of BOD_5 or COD. For a synthetic milk substrate, 0.175 milligrams of solids are produced per milligram of COD removed. Theoretically, up to 10 percent of the yield can be auto-oxidized per day.

Nutritional Requirements

It is generally accepted that organic substrates biodegrade most rapidly when synthesis is unrestricted and a minimum amount of substrate is required for energy. Synthesis occurs when essential nutrients are available to active microorganisms living in a favorable environment. Measurable quantities of nitrogen as NH_4^+, phosphorus as PO_4^{3-}, and sulfur as SO_4^{2-} as well as trace amounts of magnesium, potassium, calcium, iron, manganese, zinc, copper, and cobalt must be available.

Nitrogen and phosphorus requirements are related to the organic strength of the waste and are reported conventionally in terms of BOD to N and BOD to P ratios. Nitrogen is required for synthesis, and is released back to the system during endogenous respiration (auto-oxidation), as shown in Figure 8. BOD:N should be in the order of 17:1 (22). When nitrogen deficiencies exist, the biomass develops poor settling characteristics. Sawyer (51) states that minimum nitrogen requirements can be determined from the following expression:

$$N_{min} = (BOD_5 \times 0.5) \times 0.07 \times \text{fraction of } V_m \text{ in biomass} \qquad (49)$$

where:

N_{min} = minimum nitrogen required

BOD_5 = 5 day BOD

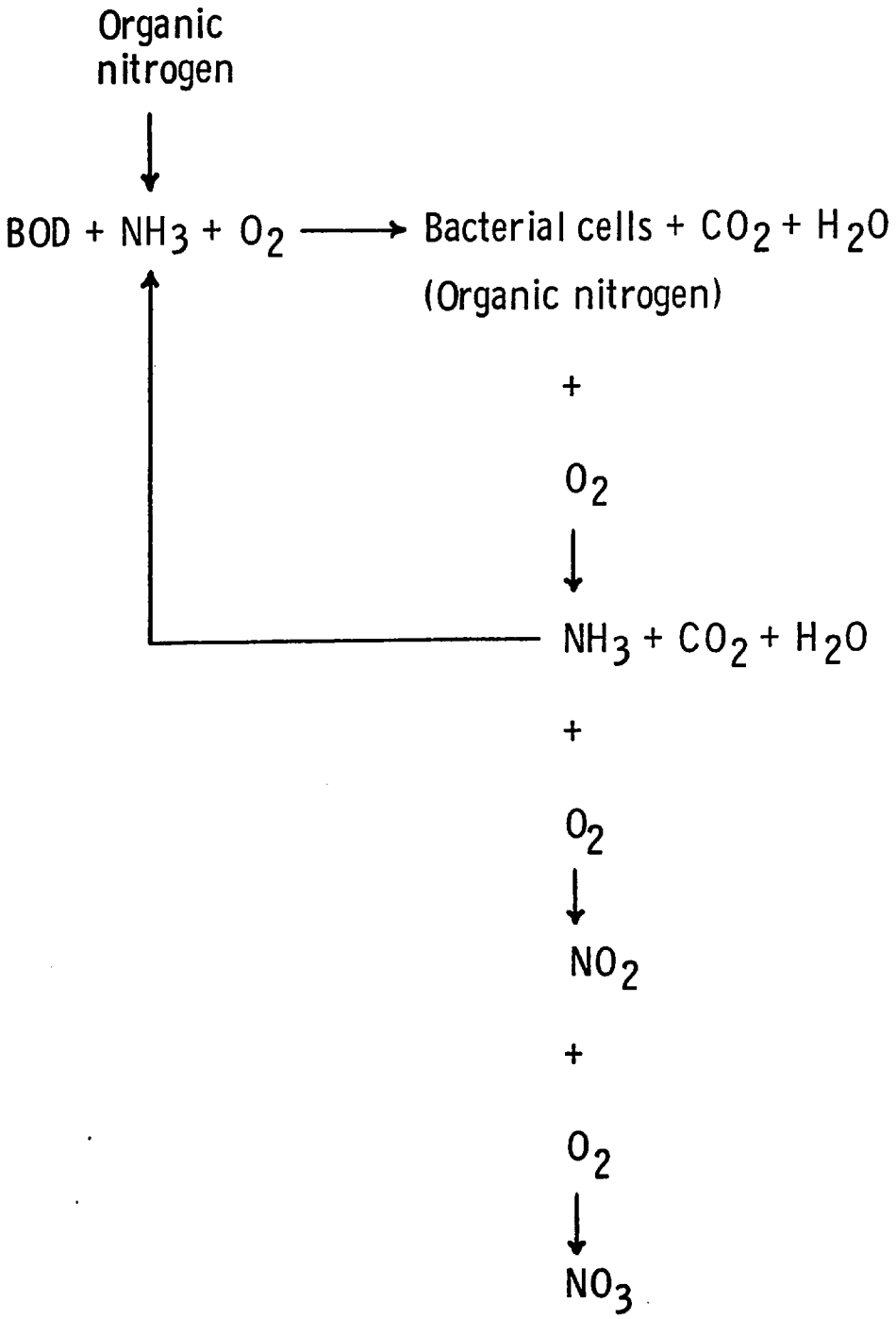


Figure 8. The role of nitrogen in aerobic biological waste treatment processes (22).

V_m = volatile solids in the biomass.

Use of the value 0.07 in Equation (49) is based on the assumption that a minimum of seven percent nitrogen should be present in the volatile portion of the sludge, as reported by Helmers (13). Based on this relationship, the experimental substrate used in this investigation should contain a minimum nitrogen concentration of 4.564 mg/l. The actual nitrogen content was approximately 14.0 mg/l and the BOD:N ratio was 14.3:1.

Phosphorus plays an important role in various enzyme systems. The BOD:P ratio should be approximately 100:1 (22). The test substrate contained a phosphorus concentration of 2.5 milligrams per liter and a BOD:P ratio of 80.0:1.

Surfactants

Standard ABS, 48.7 percent active, was supplied by the Soap and Detergent Association and used as the hard SAA. The soft test surfactant was 41.0 percent active LAS furnished by the Procter & Gamble Company. Both were mixtures of isomers with the hydrocarbon chains ranging from 8 to 16 carbons in length. The average length was 12. A concentration of 20 milligrams per liter SAA was found to exert a total theoretical oxygen demand (TOD_{SAA}) of 13.05 milligrams per liter.

Measurement Parameters

When applicable, the analytical techniques used in this investigation were performed in accordance with the current edition of Standard Methods for the Examination of Water and Wastewater (52). COD, BOD, and TOC removals were used to measure substrate utilization in the experimental system. COD was determined by dichromate oxidation, and BOD by the

Alsterberg-azide modification of the Winkler technique (52).

A Beckman Total Carbonaceous Analyzer was used to determine TOC (53, 54, 55). With this instrument, small amounts of carbon in aqueous solutions can be accurately measured. A micro sample is injected into a combustion tube heated to 950°C. It is instantly vaporized and swept in a stream of oxygen through a packing of cobalt oxide impregnated asbestos. The packing disperses the sample and catalyzes the oxidation of any carbon present to CO_2. Passage of the gas stream through a non-dispersive infra red analyzer (Beckman Model IR-315) sensitized to CO_2 produces a signal which is registered as a peak by a strip chart recorder. The height of the peak is directly proportional to the CO_2 in the gas stream, and, when referred to a standard calibration curve, can be read directly in terms of milligrams per liter carbon (or organic carbon) in the sample.

To determine total organic carbon, the sample must either be treated with barium hydroxide, precipitating inorganic carbon as barium carbonate, or acidified and bubbled with an inert gas prior to combustion, or it may be subjected to separate high and low combustion temperatures. The precipitation technique was used in this study.

The Total Carbon Analyzer is useful only for samples in solution, or for those which contain suspended solids with a particle size less than that of the 170 micron opening in the hypodermic needle used for injection. This was not considered to be a problem in this study, where only filtered samples were analyzed. Perhaps the biggest limitation was in developing the necessary precision for measuring such small sample volumes. Once this was accomplished, however, a large number of samples could be processed quickly, economically, and with a precision of ± 2 percent. TOC can be measured in two minutes after the sample has been

pretreated. When compared with an incubation period of five days for a BOD, and a reflux time of two hours for a COD, this is a major advantage. Although the initial cost of the instrument is relatively high, chemical consumption is negligible. In addition, this technique is virtually free from interferences, and it provides a more direct means for indicating the biodegradability of organic wastes and the efficiency of waste treatment plant processes.

In selecting the optimum level at which dissolved oxygen (DC) concentrations should be maintained in the aerators, several criteria were considered. It had to be kept: (1) above the level at which it would be limiting to substrate utilization reactions, (2) sufficiently high to ensure complete mixing of the biomass suspensions, and (3) low enough to avoid undue physical shearing of the floc. These conditions were best satisfied by a DO level of 3.0 to 5.0 milligrams per liter. Measurements were made twice daily with a Precision Galvanic Cell Oxygen Analyzer (GCOA), calibrated against the standard Winkler determination.

Surfactant concentrations are reported in terms of methylene blue active substance (MBAS). Methylene blue reacts quantitatively with anionic surfactants to form a blue colored complex (56). Photometric measurements were made on a Bausch & Lomb Spectronic 20 spectrophotometer at a wavelength of 652 millimicrons. Emulsification problems were minimized by analyzing only filtered effluent samples and by filtering solvent extracts through mats of sodium sulfate suspended on glass wool.

Experimental Procedure

In a typical run, each of the reactors was filled with six liters of mixed liquor, adjusted to a suspended solids concentration of

3,500 ± 500 milligrams per liter, and aerated without the addition of fresh substrate for the next two to seven days to allow the organisms to acclimate to their new food source. When COD removals were approximately uniform in all reactors for at least two consecutive days, surfactants in concentrations from 5 to 20 milligrams per liter were added to the substrates for four of the six units. Two reactors served as controls and remained surfactant free. Zero time on the plotted data represents the point at which SAA was added to the system.

Effluent samples were collected for one hour each day, on a grab sampling basis. These, along with corresponding samples of the influents, were analyzed to determine the effect of surface active agents on substrate utilization in this system. This procedure was continued for as long as thirty days after the addition of surfactants. It was discontinued when operational problems developed from a filamentous biomass, or when no further changes in reactor efficiencies could be observed.

The pH of the mixed liquor remained at 7.45 ± 0.15. This was expected since, as shown in Reactions (45) and (46), the ammonia liberated in the oxidation of casein is utilized in carbohydrate assimilation.

As a part of the routine daily maintenance schedules, solids were scraped from the aerators and the settling units and flushed from the return sludge line immediately after the samples were collected. Substrate and effluent reservoirs were thoroughly rinsed with hot tap water each day and cleaned twice weekly, along with the influent lines through the dosing pump, with strong chlorine solution.

Biomass Characteristics

Physical changes in the biomass characteristics were noted as a

function of the type and the concentration of surfactants being fed into the system. Changes in population dynamics were observed microscopically at magnifications of 470 x and 970 x. Identification of predominant species in the biomass was made from Ward and Whipple (57).

OXYGEN UTILIZATION

Experimental Apparatus

Oxygen utilization measurements were made in a closed vessel, 8 inches x 8 inches x 12 inches, constructed from ¼ inch plexiglass. Baffles 6 inches x 1 inch, were placed at the midpoint of each side to provide for a uniform pattern of mixing. A 2 inch teflon coated stirring bar was driven by a Labline, king size, pyro magnetic mixer at a speed of 400 rpm. A Precision Galvanic Cell Oxygen Analyzer was suspended into the liquid through a tight fitting port in the center of the top of the reactor. Heat transfer to the bulk of the liquid was controlled by a sheet of asbestos placed between the reactor and the mixer. The experimental assembly is shown in Figure 9.

Initial Inoculum

During the course of this investigation, operational problems developed at the local treatment plant. These problems were traced to a change in characteristics of the raw waste. Oxygen uptake rates in the mixed liquor were significantly lower than normal, and the floc was predominantly filamentous in nature. For this reason, the source of the initial inoculum for this phase of the study was changed. A fresh stock culture of Escherichia coli was suspended in filtered raw sewage and aerated for a period of seven days. At the end of this time, the culture was split and each half was diluted to eight liters with buffered BOD dilution water. Each of these cultures contained a biomass

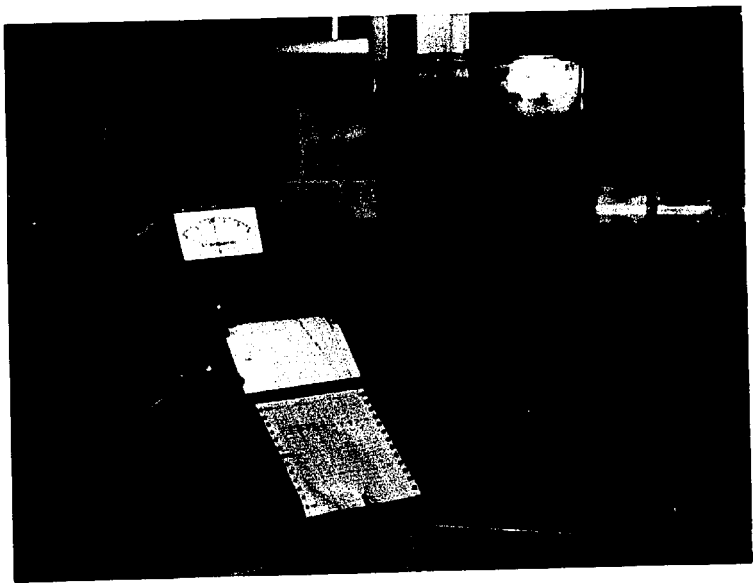


Figure 9. Experimental apparatus used for measuring oxygen utilization.

concentration of 500 ± 50 milligrams per liter. One was used as the initial inoculum for measurements made with ABS; the other was used with LAS.

Substrate and Surfactants

A synthetic substrate of pure Difco Bacto lactose, 250 milligrams per liter, was fed to this system on a batch basis. The surfactants used were the same as those described for studying substrate utilization.

Measurement Parameters

Substrate utilization was measured directly by the anthrone test (58), a colorimetric determination specific for carbohydrates. Oxygen utilization was monitored with the GCOA, read out on a Simpson DC microammeter, and recorded on an Esterline Angus servo recorder.

Experimental Procedure

Pure oxygen was bubbled into the system through a capillary tube until the saturation level was reached. Excess bubbles were allowed to escape, and the reactor cover was rested on the baffles. Approximately one liter of the suspension served as a seal to prevent atmospheric reaeration at the surface of the test solution. Substrate was added to the system, and oxygen utilization was recorded with respect to time. As the oxygen was nearly depleted, the system was reoxygenated and the oxygen utilization rate was again determined. When several identical rate curves were available, 20 milligrams per liter of standard ABS were added, and new rate curves were recorded for oxygen utilization in the presence of SAA. This entire procedure was repeated using LAS in place of ABS.

Samples were removed from the reactor at various intervals of time and analyzed for carbohydrate content. Solids in the system were measured gravimetrically at the beginning and at the end of each run. No significant yield was observed during the relatively brief experimental period.

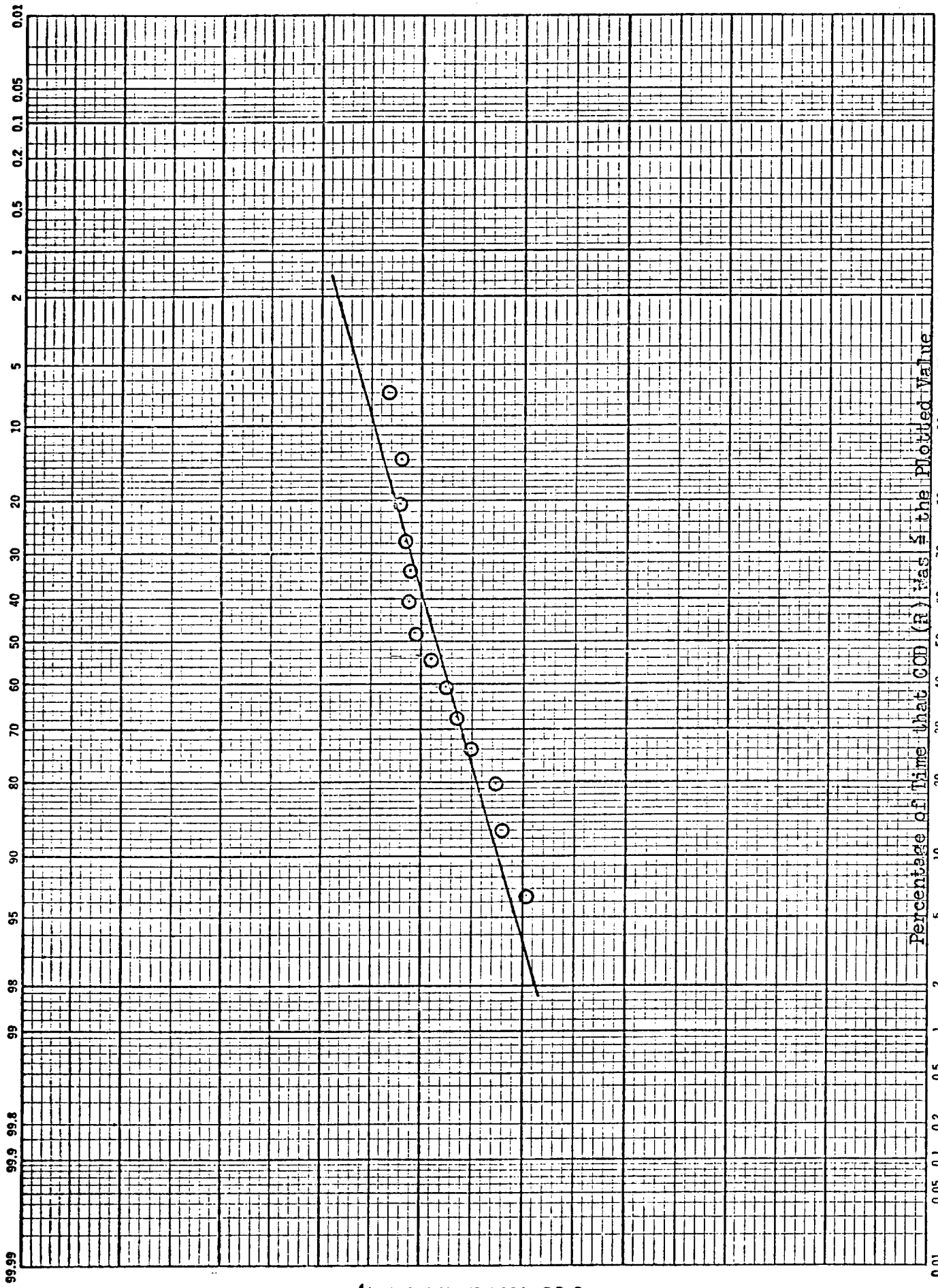
STATISTICAL INTERPRETATION OF RESULTS

The statistical significance of the data presented in Figures 12 through 17 was determined by the graphical method developed by Hazen (53) and described by Velz (54) and Gannon (55). Plotting positions were determined by arranging the data in order of ascending magnitude, assigning a number (m) to each, and calculating the values for the X coordinate from the formula $m/n+1 \times 100$, where n is the total number of observations in the series. The Y coordinate refers to calculated percent reductions of COD in the experimental system. Standard deviation (σ) is related to the slope of the line and can be determined graphically by this method. The graphical mean ($\bar{x}$) is read from the point at which the plotted line intersects the mid-point (50%) on the X scale. To determine the statistical significance of the differences, the standard error of the mean (S.E.) was calculated for each series of observations, according to the following relationship:

$$S.E. = \frac{\sigma}{\sqrt{n}} \qquad (50)$$

At the 95 percent confidence level, the true mean falls within the range of $x \pm 2$ S.E. This range of values was calculated for each level of SAA. When no overlapping occurs between two ranges of values, it is 95 percent certain that the apparent effect is significant.

As an example of the application of this method, a series of data for COD reduction in the control system is plotted in Figure 10.



PROBABILITY 46 8003
X 90 DIVISIONS MADE IN U.S.A.
KEUFFEL & ESSER CO.

Figure 10. Example of statistical plot of control data.

RESULTS

In all cases, the data which are reported in this section represent arithmetic mean values for a number of replicate runs. Their significance is determined in accordance with the statistical method previously described.

SUBSTRATE UTILIZATION

Biochemical Oxygen Demand

Because of the relatively rapid rate at which milk is oxidized, BOD reduction is not an effective parameter for indicating the effect of surface active agents on substrate utilization under the conditions of this study. BOD_5 reductions in the presence of 10 and 20 milligrams per liter LAS, 10 and 20 milligrams per liter ABS, and in the control system are shown in Table IV and in Figure 11. After 21 days of operation, 98.1 percent of the influent BOD_5 was being removed in the control system, 97.6 percent, in the presence of 10 milligrams per liter LAS, and 96.1 percent, in the presence of 20 milligrams per liter LAS. After the same period of time, the reactor efficiency was somewhat reduced in the presence of the hard surfactant. With 10 milligrams per liter ABS, reduction of BOD_5 was 93.5 percent, 4.1 percent less than the system with a similar concentration of LAS. At 20 milligrams per liter ABS, 90.3 percent of the BOD_5 was being removed. Although this was 5.9 percent less than the system with 20 milligrams per liter LAS, an activated sludge unit which is removing more than 90.0 percent of its organic loading, expressed as BOD_5, would in practice be considered to be an effective treatment device. The significant effects which SAA are shown to have on substrate utilization when COD and TOC removals are

TABLE IV

THE EFFECT OF 10 AND 20 MILLIGRAMS PER LITER ABS AND 10 AND 20 MILLIGRAMS PER LITER LAS ON SUBSTRATE UTILIZATION, EXPRESSED IN TERMS OF BOD

	Control	ABS, mg/l		LAS, mg/l	
		10	20	10	20
Substrate	163	166.8	170.5	166.5	170.0
Effluent t, days					
7	1.0	2.6	4.2	1.5	1.2
14	1.9	5.7	8.6	2.5	3.0
21	3.0	10.8	16.3	4.0	5.9
Reduction (R),% t = 22 days	98.1	93.5	90.3	97.6	96.1

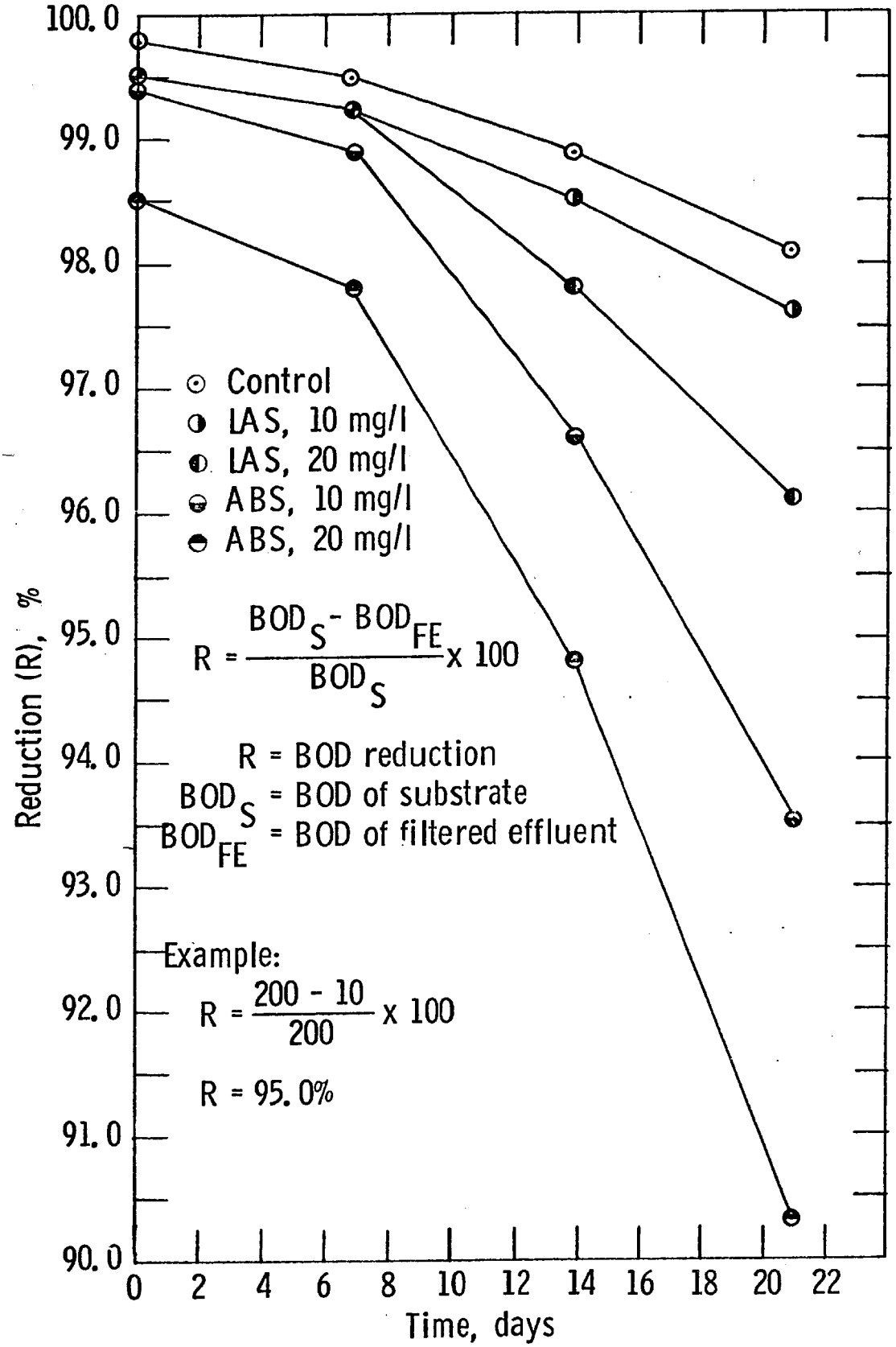


Figure 11. The effect of hard vs. soft surfactants on substrate utilization, expressed as percentage reduction of BOD.

considered is masked in the measurement of this parameter.

Total Organic Carbon

Total organic carbon measurement provides a meaningful parameter for expressing substrate utilization. In this study, the use of a well defined synthetic substrate provided a basis for calculating its theoretical TOC. The theoretical TOC content of the control substrate was 124.1 milligrams per liter. The arithmetic mean of 100 observed values was 122.0 milligrams per liter; therefore, the observed values varied from the theoretical by 1.7 percent. This is within the stated precision of the analytical method (± 2 percent). For each milligram of SAA added to the substrate, the theoretical TOC was increased by 0.68 milligrams. Theoretical vs. observed values for substrate TOC in each of the test systems are shown in Table V.

After 22 days of operation, TOC reduction in the control reactor was 90.1 percent. Correspondingly, in the presence of 5, 10, 15, and 20 milligrams per liter LAS, TOC removals averaged 88.5, 86.7, 85.2, and 83.6 percent, respectively. Reactor efficiency was reduced to a greater degree with similar concentrations of ABS when TOC reductions averaged 84.7, 78.3, 75.7, and 73.2 percent respectively for the same period of operation.

Chemical Oxygen Demand

The effect of various concentrations of ABS - 5, 10, 15, and 20 milligrams per liter - on COD reduction in the experimental system is shown in Figure 12. Effluent samples were filtered through Whatman No. 1 filter paper to remove the suspended solids, and the COD of each filtrate was measured. At the two highest levels of ABS, there was an

TABLE V

THEORETICAL TOTAL ORGANIC CARBON IN THE SUBSTRATES AND OBSERVED TOTAL ORGANIC CARBON CONTENT OF THE SUBSTRATES AND EFFLUENTS FOR TWENTY-TWO DAYS OF OPERATION IN THE CONTROL SYSTEM AND IN THE PRESENCE OF SAA

	Control	ABS, mg/l				LAS, mg/l			
		5	10	15	20	5	10	15	20
Substrate									
Theoretical	124.1	127.5	131.0	134.4	137.8	127.5	131.0	134.4	137.8
Observed	122.0	125.5	131.5	133.5	139.6	125.5	131.3	133.7	139.3
Effluent t, days									
1	9.7	11.4	14.0	18.5	24.5	10.4	11.0	12.3	19.4
2	9.8	11.5	14.5	17.4	19.6	10.5	11.1	11.6	12.3
3	9.7	11.7	14.7	17.5	19.8	10.6	11.2	11.7	12.4
4	9.7	11.7	15.0	17.6	19.9	10.7	11.2	12.0	13.2
6	9.8	12.0	15.3	17.7	20.1	10.8	11.4	12.2	13.3
8	10.0	12.5	15.4	18.0	21.6	10.9	11.5	12.5	14.1
10	10.0	13.0	15.5	19.3	21.8	11.1	11.8	12.6	14.2
12	10.1	13.4	17.5	21.0	24.4	11.6	13.2	14.9	16.7
14	10.1	14.5	20.3	22.5	27.4	12.0	13.8	15.5	17.3
16	10.5	14.8	21.6	25.5	29.7	12.9	15.3	16.7	18.0
18	11.5	15.6	25.5	28.5	33.5	13.8	15.7	18.0	20.3
20	11.7	17.4	27.0	31.0	34.6	14.1	16.5	18.5	21.1
22	12.0	19.5	28.5	32.5	37.6	14.5	17.5	19.7	23.1
Reduction (R), %	90.1	84.7	78.3	75.7	73.2	88.5	86.7	85.2	83.6
t = 22 days									

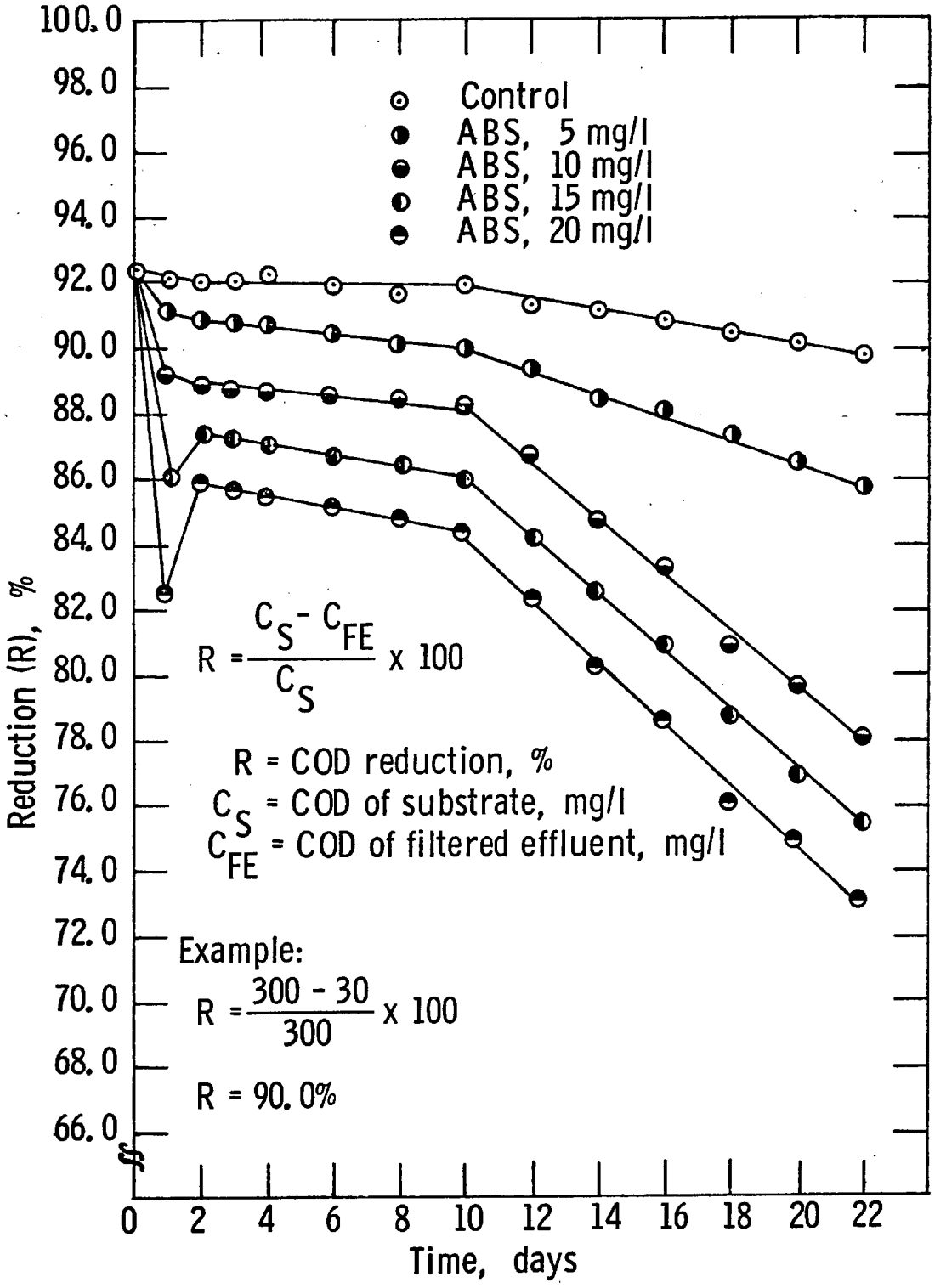


Figure 12. The effect of ABS on substrate utilization, expressed as percentage reduction of COD.

immediate, temporary effect of apparent toxicity. After ten days of operation, the efficiency dropped in all units. This drop became significantly greater as the concentration of surfactant was increased. After 22 days of operation, COD reduction in the presence of 5 milligrams per liter ABS was 4.2 percent less than it was in the control reactor. In the presence of 10, 15, and 20 milligrams per liter of the hard surfactant, this difference was 11.8, 14.4, and 16.6 percent, respectively. In the control system, the drop in efficiency could be attributed to a change in population dynamics which developed from the use of synthetic milk substrate.

In the presence of LAS, again the reactor performance was reduced as the concentration of surfactant was increased. The effect of 10 and 20 milligrams per liter LAS on COD reduction is shown in Figure 13. Although the same characteristic change in performance occurred after 10 days of operation, it was considerably less drastic than it was with ABS. At the end of the test interval, COD reduction in the reactor with 10 milligrams per liter LAS was only 2.8 percent less than it was in the control system. With 20 milligrams per liter of the soft surfactant, this difference was 5.6 percent.

The effect of hard vs. soft surfactants on substrate utilization is shown in Table VI and Figures 14 and 15. COD reductions in the presence of 10 milligrams per liter ABS and 10 milligrams per liter LAS are plotted with the control system. After 10 days, the efficiency of the reactor with the soft SAA was only 1.2 percent less than that of the control. In the presence of ABS, this difference was 3.8 percent. At the end of the test interval, COD reduction in the control system was 3.0 percent better than the reactor with LAS and 11.8 percent better than the reactor with ABS.

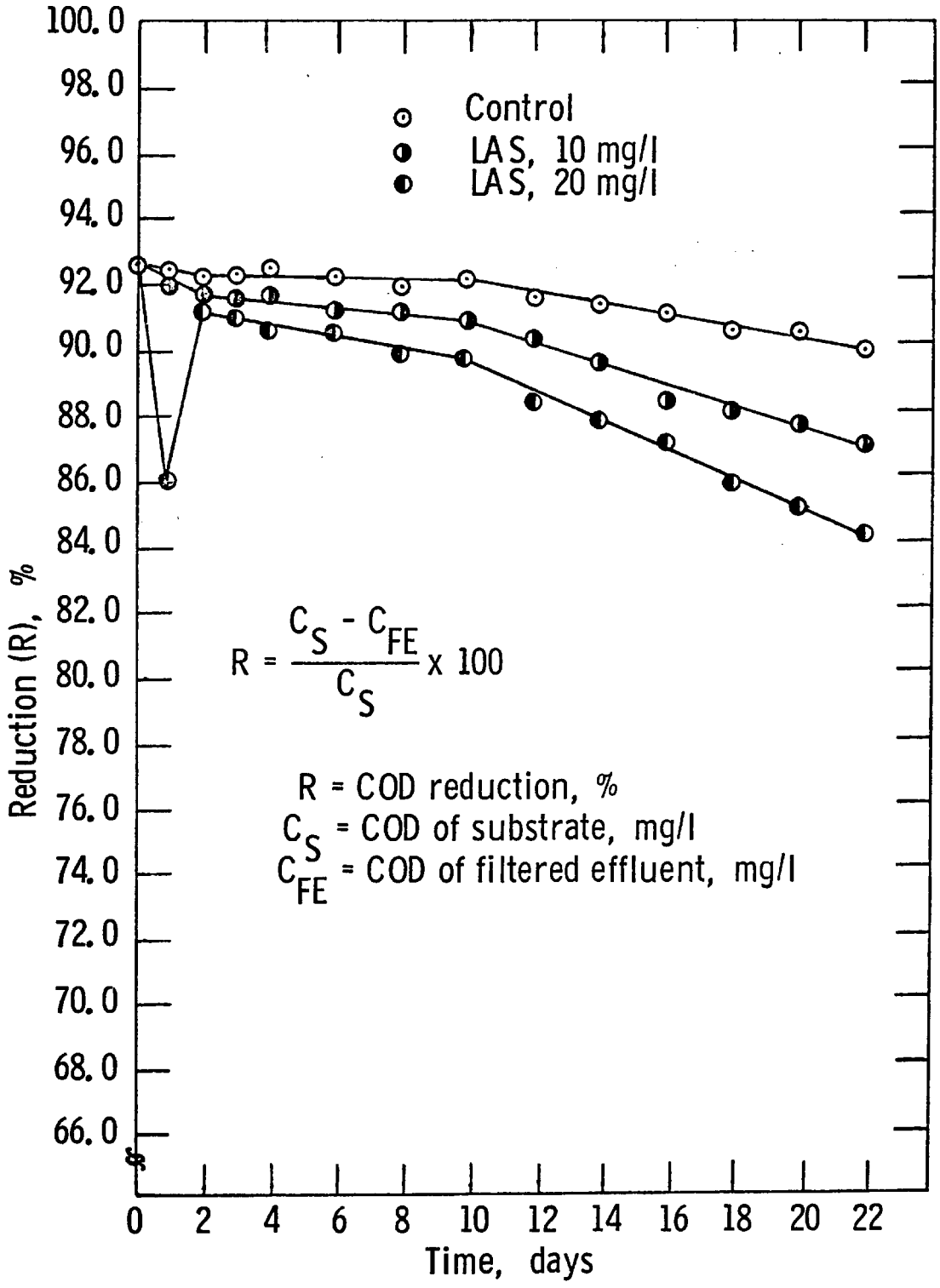


Figure 13. The effect of LAS on substrate utilization, expressed as percentage reduction of COD.

TABLE VI

THE EFFECT OF ABS AND LAS ON SUBSTRATE UTILIZATION, EXPRESSED IN TERMS OF COD

	Control	ABS, mg/l		LAS, mg/l	
		10	20	10	20
Substrate	288.0	295.0	305.0	295.0	304.0
Effluent t, days					
0	21.0	21.0	21.0	21.0	21.0
1	22.7	32.0	53.7	24.2	43.9
2	22.7	33.1	43.0	25.1	28.0
3	23.0	33.1	44.1	25.1	28.2
4	22.7	34.0	44.8	25.2	29.3
6	23.0	34.5	45.0	26.3	29.3
8	23.2	34.7	47.0	26.5	32.7
10	23.0	35.0	47.5	27.6	32.9
12	25.0	40.2	54.0	29.8	36.5
14	25.3	46.0	60.1	30.4	39.1
16	26.0	49.7	65.2	35.0	40.2
18	27.9	57.0	73.5	35.2	44.3
20	28.1	60.3	76.3	37.5	46.5
22	30.0	65.0	82.0	39.7	48.9
Reduction (R), % t=22 days	89.8	78.0	73.2	86.8	84.2

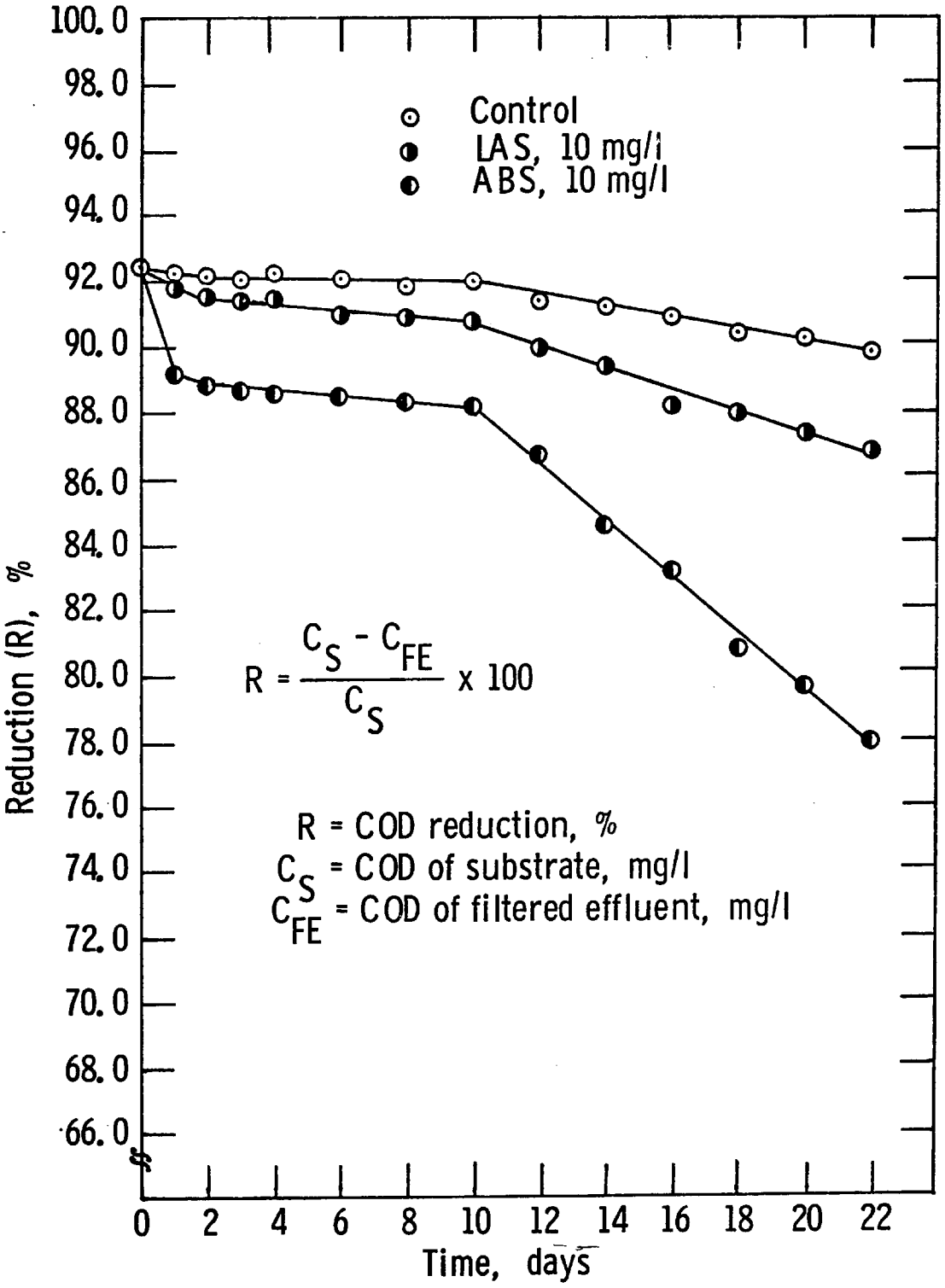


Figure 14. The effect of 10 mg/l of hard vs. soft surfactants on substrate utilization, expressed as percentage reduction of COD.

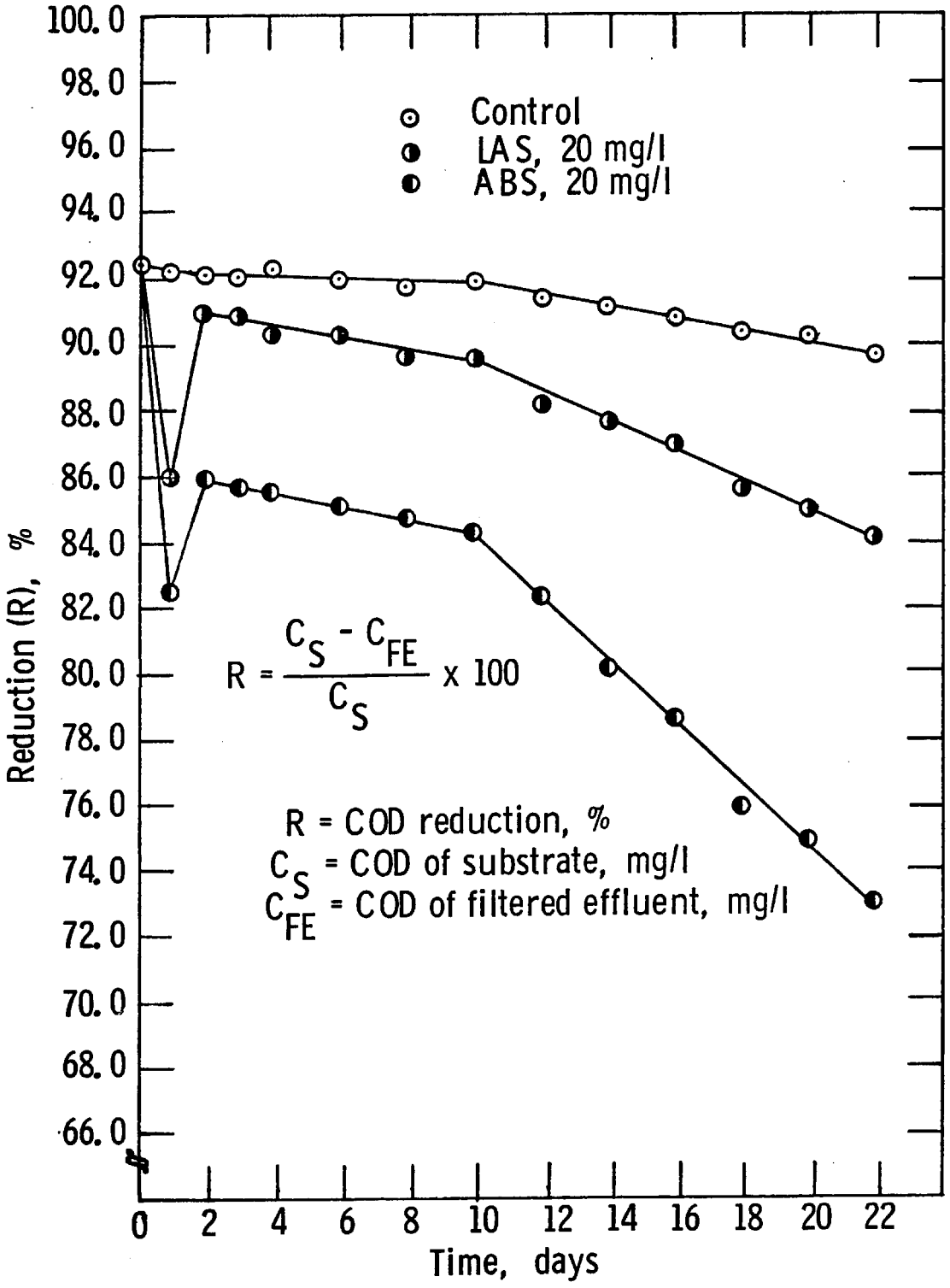


Figure 15. The effect of 20mg/l of hard vs. soft surfactants on substrate utilization, expressed as percentage reduction of COD.

In Figure 15, data obtained from units with 20 milligrams per liter of each surfactant are plotted with data from the control system. At these concentrations, both LAS and ABS had an initial shocking effect on the system, but partial recovery occurred relatively rapidly. After 2 days, the performance of the control reactor was 6.2 percent better than the reactor with ABS. In the presence of either SAA, the efficiency decreased significantly throughout the entire period of observation. At the end of 22 days, the performance of the control system was 5.6 percent better than the reactor with LAS, and 16.6 percent better than the unit with ABS.

"Retardation effect", which is defined as the difference between substrate utilization in a control system and one in which SAA is added, is an effective means for illustrating the effects of hard vs. soft surfactants on substrate utilization. Data from Figure 14 were used to prepare Figure 16, which shows the relative retardation of 10 milligrams per liter LAS and 10 milligrams per liter ABS. Figure 17 is a similar plot for 20 milligrams of both SAA.

A summary of the statistical significance of the effect of ABS and LAS on substrate utilization, expressed as reduction of COD, is shown in Table VII. All concentrations of ABS tested, and LAS at levels of 10 milligrams per liter and greater, were found to interfere with substrate utilization in the experimental system.

Surface Active Agents

Table VIII indicates the extent to which surface active agents were removed in the experimental units. Surfactant concentrations in the substrates are theoretical values. Because of the tendency to form emulsions in the presence of colloidal solids, direct measurements using methylene

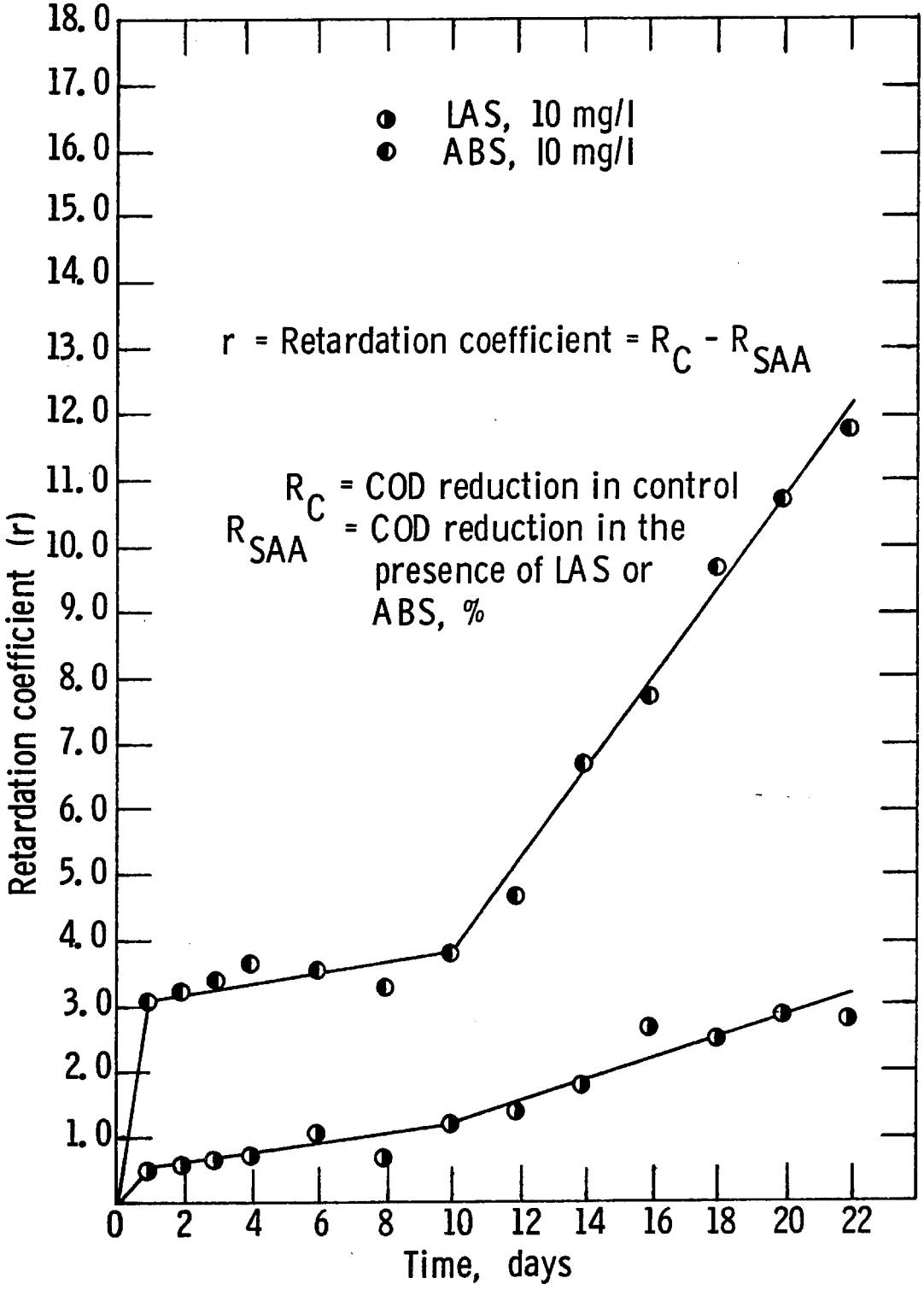


Figure 16. Relative effect of retardation of substrate utilization of 10 mg/l of LAS and ABS.

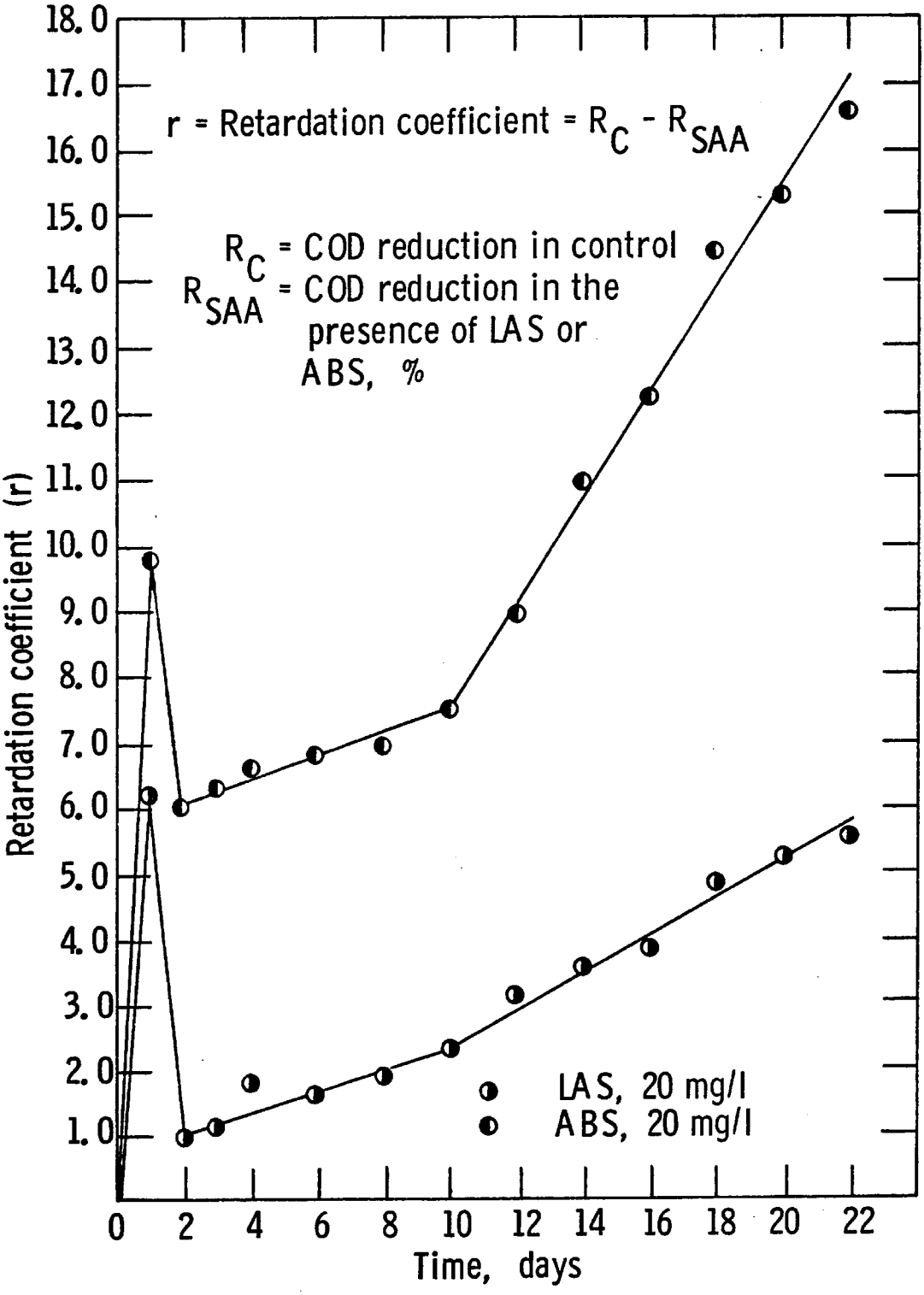


Figure 17. Relative effect of retardation of substrate utilization of 20 mg/l of LAS and ABS.

TABLE VII

SUMMARY OF STATISTICAL SIGNIFICANCE OF THE EFFECT OF ABS AND LAS ON SUBSTRATE UTILIZATION

	Control	ABS, mg/l				LAS, mg/l	
		5	10	15	20	10	20
$\bar{X}$	91.5	89.4	86.1	84.0	82.3	90.0	88.5
	0.9	2.1	4.1	5.7	6.6	1.8	2.7
S.E.	0.24	0.56	1.10	1.52	1.77	0.48	0.72
$\bar{X} \pm$ 2 S.E.	91.02-91.98	88.28-90.52	83.90-88.30	80.96-87.04	78.76-85.84	89.04-90.96	87.06-89.94

TABLE VIII

MEAN PERCENT REDUCTION OF 10 AND 20 MILLIGRAMS PER LITER OF ABS AND LAS IN THE EXPERIMENTAL SYSTEM

	S mg/l	FE mg/l		R,%
ABS	10	2.9		71.0
ABS	20	7.5		62.5
LAS	10	0.55		94.5
LAS	20	1.85		90.8

S = MBAS in substrate, mg/l

FE = MBAS in filtered effluent, mg/l

R = reduction of MBAS, % = $\frac{S-FE}{S} \times 100$

blue were not practical. The filtered effluent and percent reduction are arithmetic mean values for 21 days of operation. After the first 3 days, MBAS reduction in any given unit varied by less than 2 percent.

Biomass Characteristics

In a mixed culture system, the nature of the substrate has a profound effect on population dynamics. On several occasions, experiments had to be terminated early when atypical organisms became predominant in the system. A series of photomicrographs is presented to illustrate this problem.

The apparent good health of the sludge when it was first obtained from the field is shown in Figure 18. The floc is well agglommerated, and protozoa are present. On two occasions, the paramecia shown in Figure 19 became so abundant after two weeks of testing that both the sludge and the effluent had a pink hue. Nematodes, identified as Aeolosoma quaternarium, became predominant in several runs (Figures 20 and 21). These organisms ingested the biomass and congregated at liquid-solid interfaces. Although they were microscopic in size, patches of brilliant orange appeared as a result of the characteristic bright globules of oil which they contain.

The soluble substrate selected for this study tended to develop a light, flocculent biomass. In each run, the physical condition of the test system determined the period over which data were collected. When filamentous forms became predominant (Figure 22), bulking occurred (Figure 23) and testing was terminated. Because of the nature of the feed and the hydrodynamic design of the test apparatus, the maximum length of any run was 30 days after the introduction of surfactants. The minimum test period for which data were considered valid was 21 days. It is

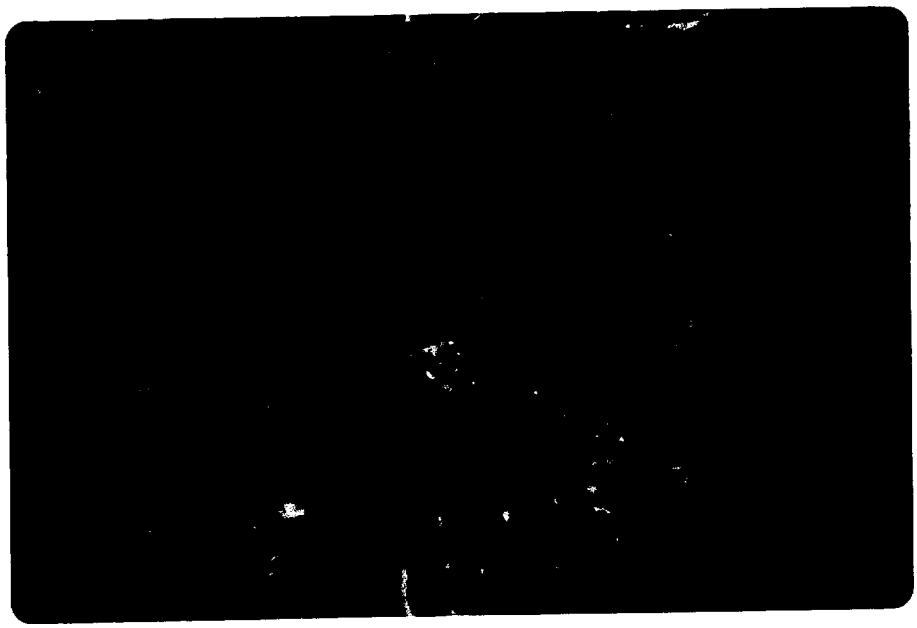


Figure 18. Photomicrograph of fresh initial inoculum, 970x.

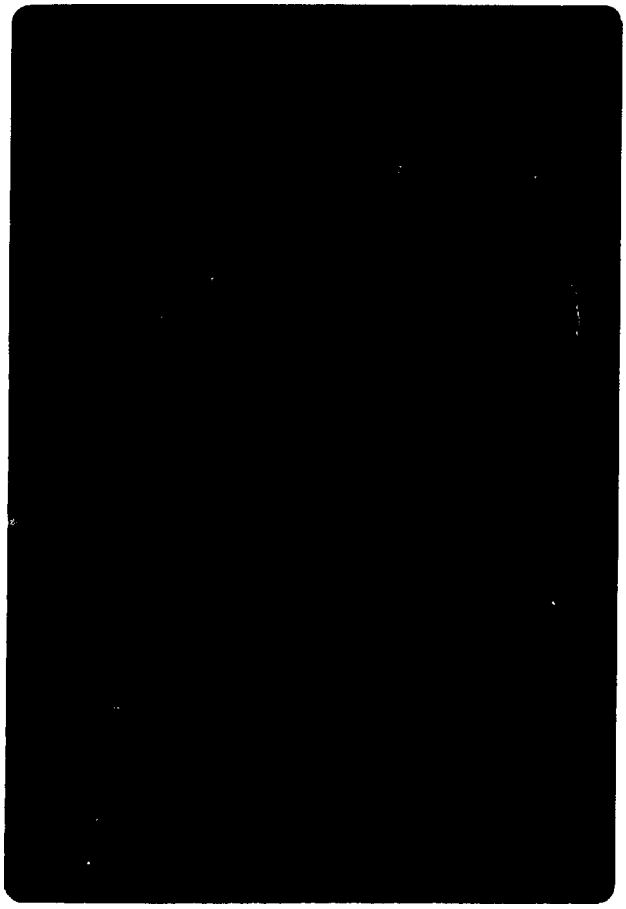


Figure 19. Photomicrograph of biomass with paramecia predominating, 470x.

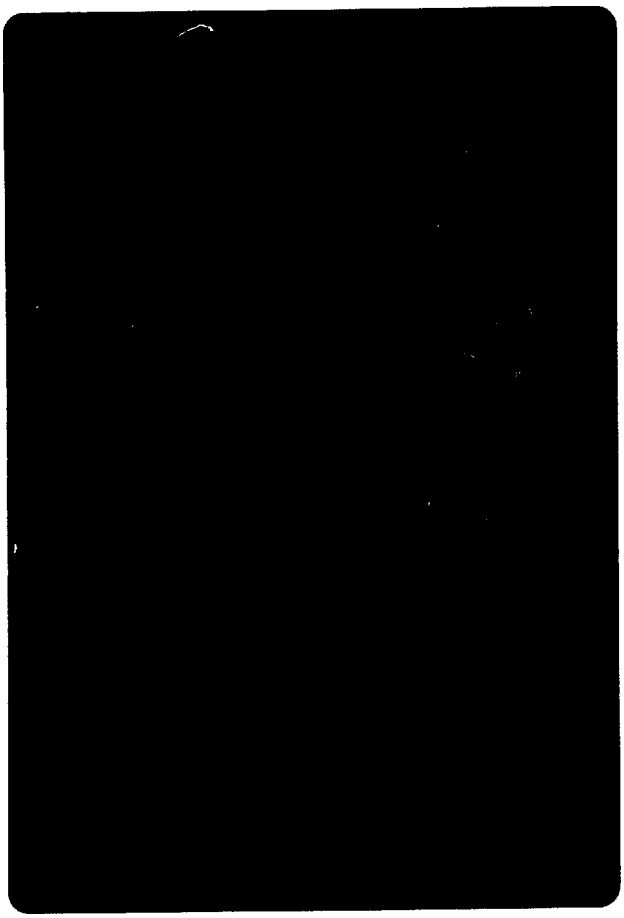


Figure 20. Aeolosoma quaternarium in the biomass, 470x.

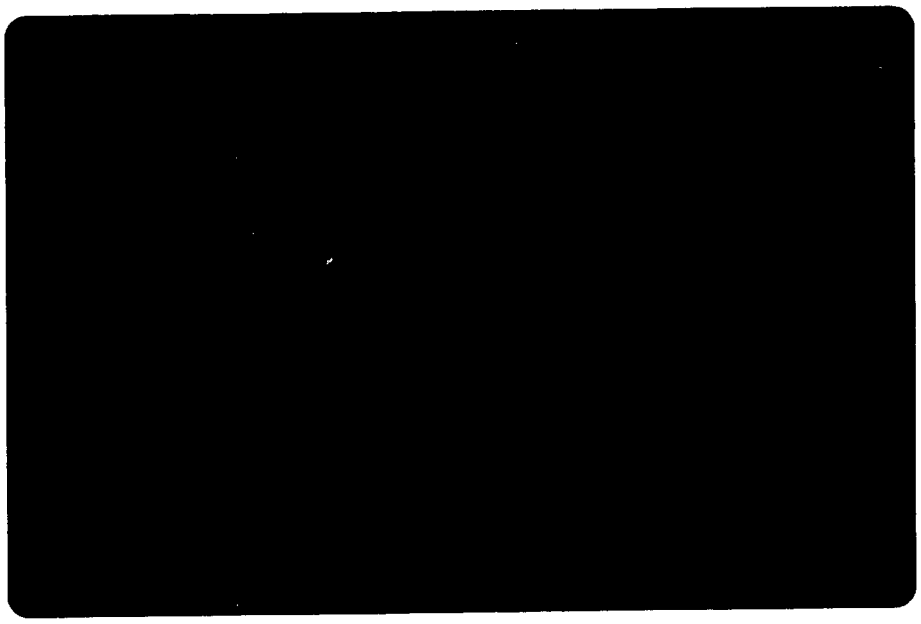


Figure 21. Aeolosoma quaternarium, 970x.

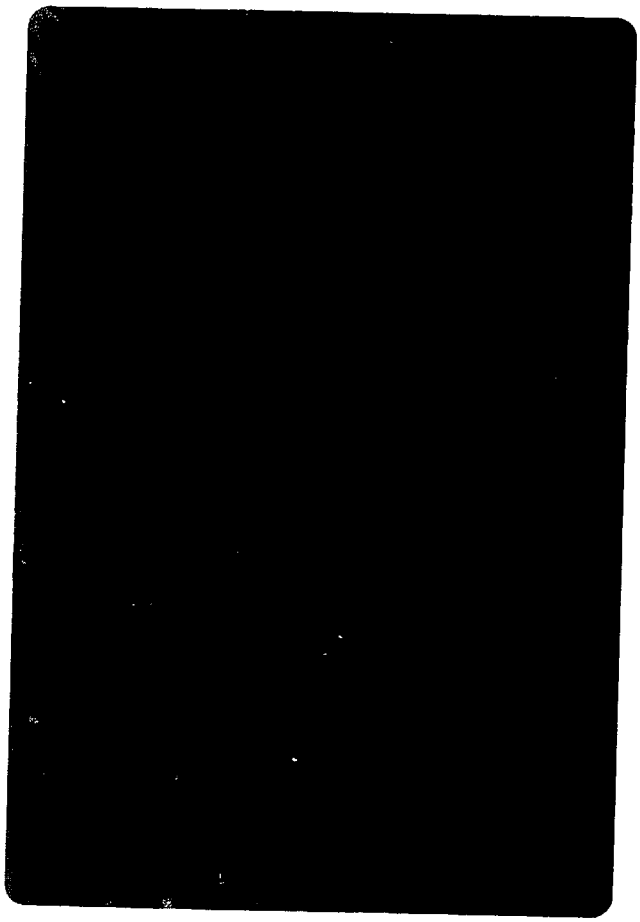


Figure 22. Filamentous biomass, 970x.



Figure 23. Bulking in the experimental system.

felt that longer term data might have been available if a substrate which contained suspended solids had been used. The fish meal described by Ludzack (62) is an example of this type of synthetic waste.

Although the temperature in the test area varied only ± 2°C. during any test period, it was difficult to bring activated sludge in from the field during the winter months and acclimate it to a warmer environment. Performance was generally better and test periods were longer when the temperature change from field to laboratory was not drastic.

A significant change in the settleability of the biomass could be observed within 48 hours after it was fed the synthetic substrate. When brought from the field, it commonly settled to 35 ± 10 percent in a one liter graduate in 30 minutes. After 48 hours with the milk substrate, it changed to 80 ± 10 percent under similar test conditions. In the control system, however, the effluent remained clear as shown in Figure 24. A typical reactor with 20 milligrams per liter ABS in the substrate is shown in Figure 25. When ABS was present, solids were held in suspension and washed from the system in the effluent. LAS did not appear to have this effect.

The high foaming tendency of ABS is also apparent in Figure 25. During test periods, a small gauze bag impregnated with an inert stopcock lubricant was suspended from the tube returning solids to the aerators. This broke the foam before it could escape from the system.

OXYGEN UTILIZATION

The effect of 20 milligrams per liter ABS on oxygen utilization is shown in Table IX and Figure 26. Before ABS was added to the system, oxygen was consumed at a rate of 1.1 milligrams per liter per hour. After the addition of ABS, the oxygen utilization rate was reduced to 0.3

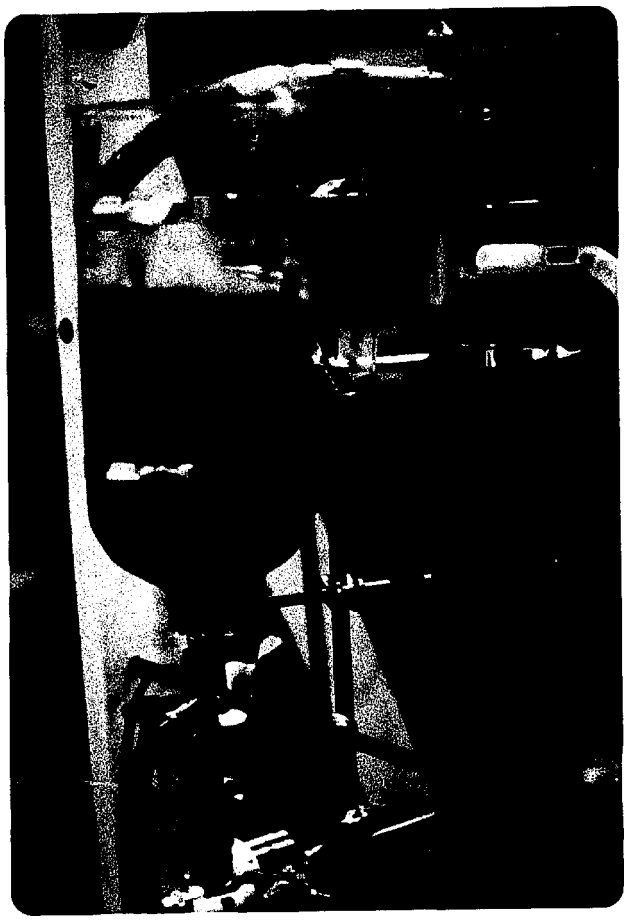


Figure 24. A typical control reactor with clear supernatant in the settling unit.

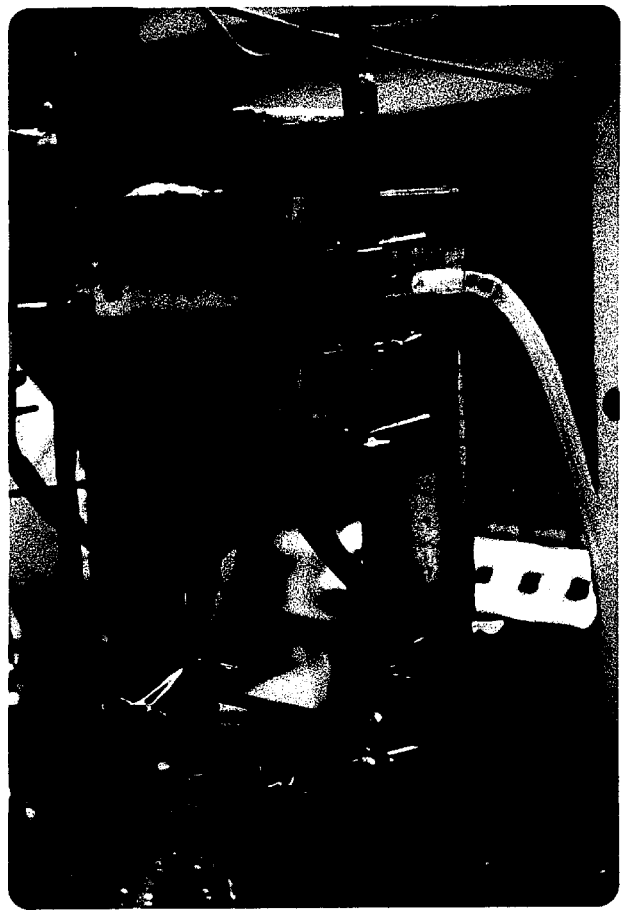


Figure 25. A typical reactor with 20 mg/l ABS in the substrate. Supernatant in the settler contains a high concentration of suspended solids, and foaming in the aerator is appreciable.

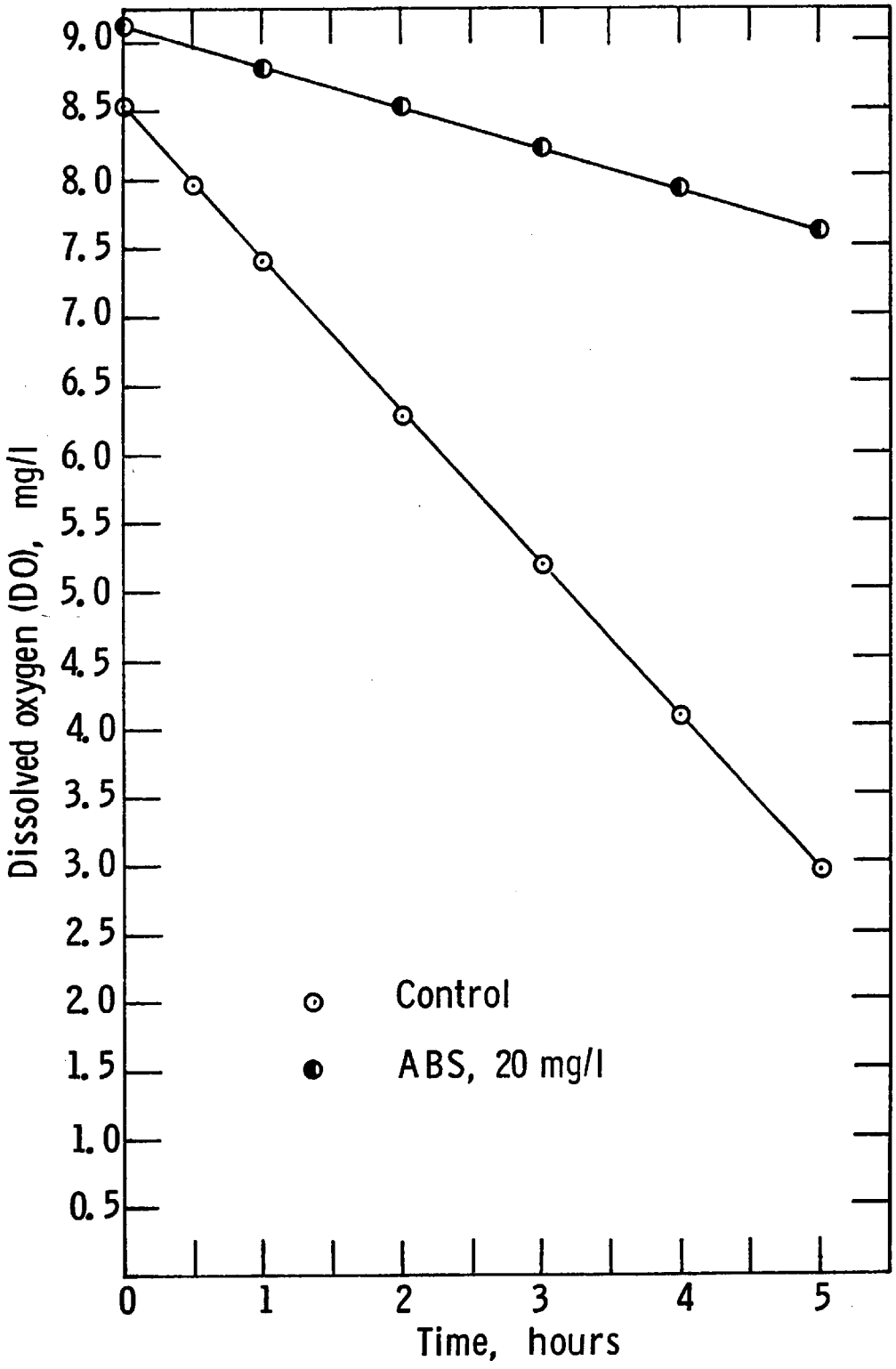


Figure 26. The effect of 20 mg/l ABS on oxygen utilization in a mixed culture of microorganisms, measured with a GCOA.

milligram per liter per hour. In the presence of 20 milligrams per liter LAS, oxygen consumption changed from 1.2 milligrams per liter per hour of 0.8 milligram per liter per hour (Figure 27). The effect of either surfactant on this parameter was significant. Substrate utilization, determined by direct measurement of lactose concentration, followed a similar pattern, as shown in Figure 28.

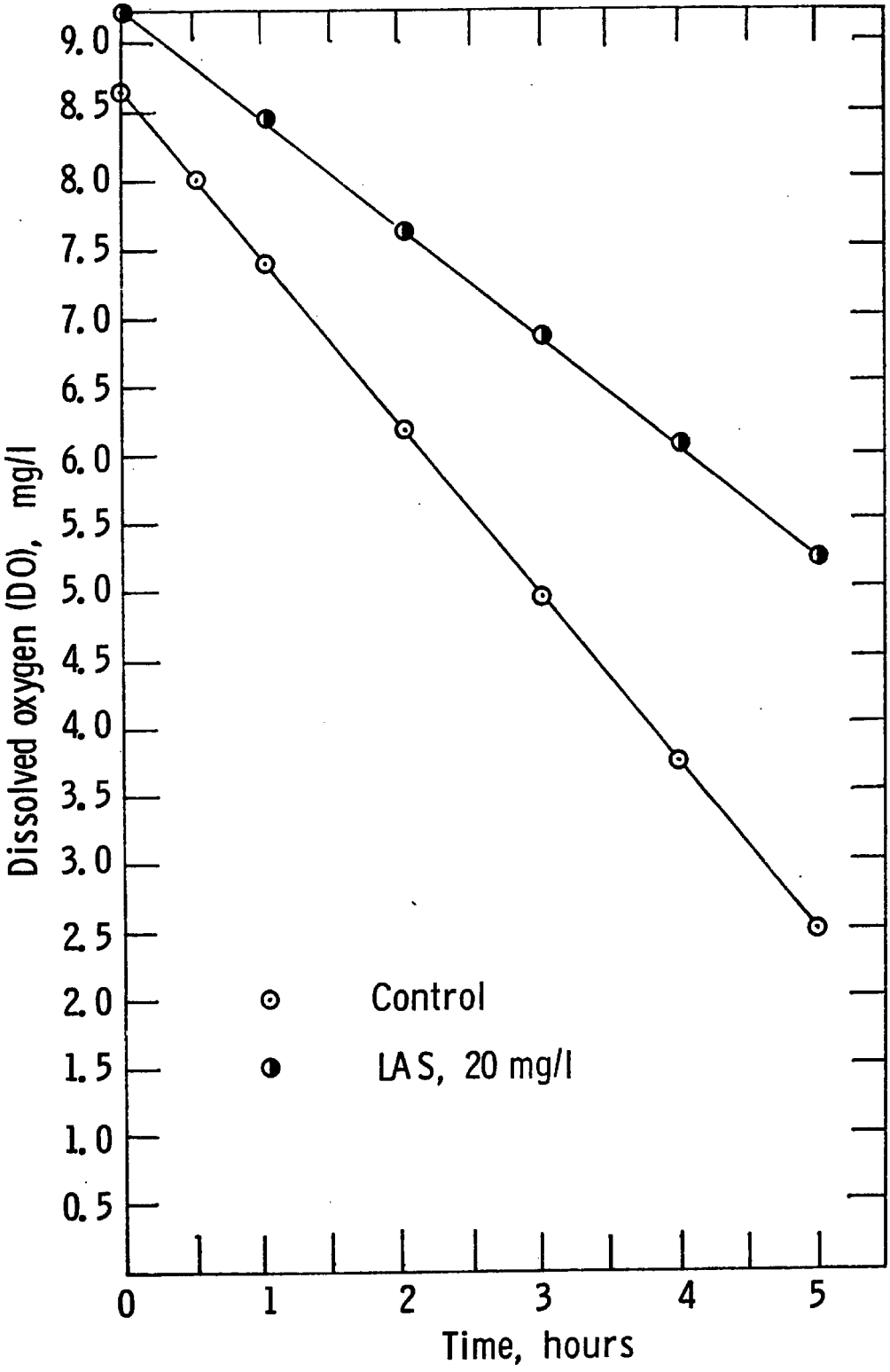


Figure 27. The effect of 20 mg/l LAS on oxygen utilization in a mixed culture of microorganisms, measured with a GCOA.

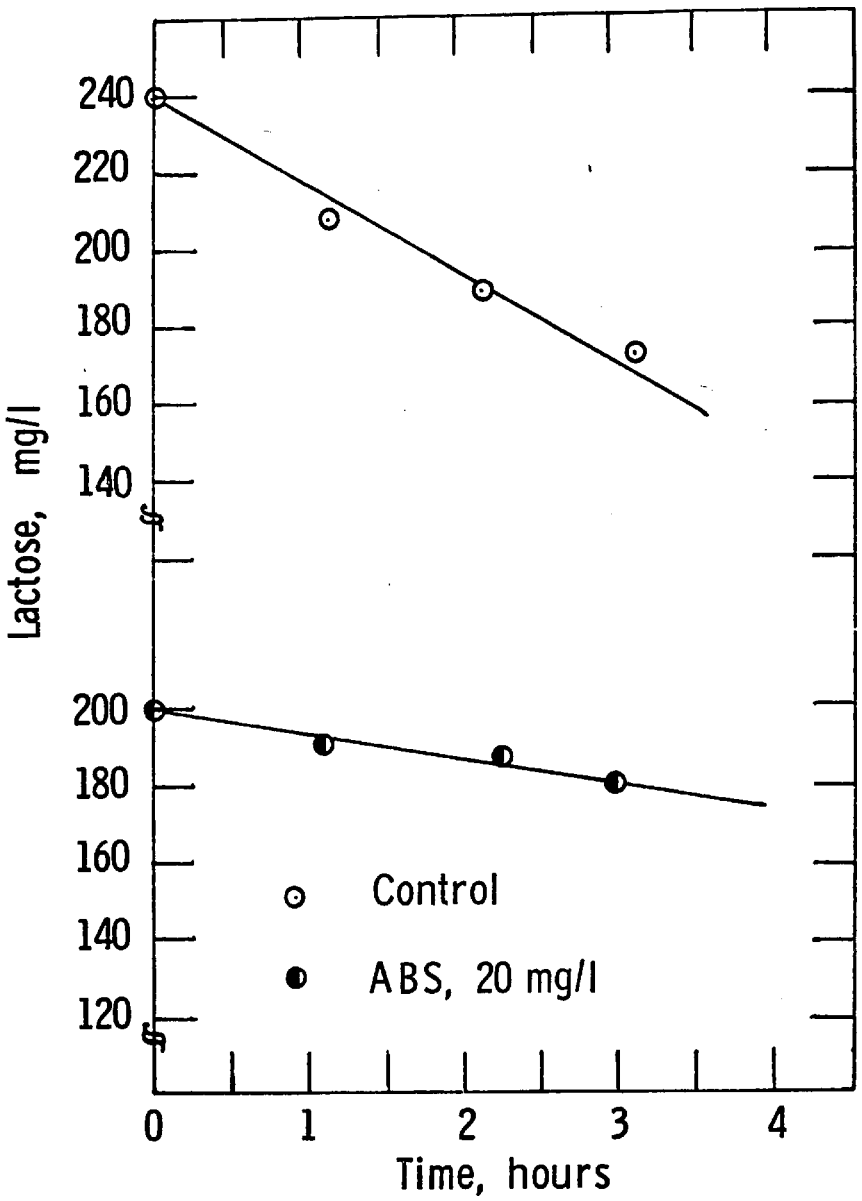


Figure 28. The effect of 20 mg/l ABS on lactose utilization in a mixed culture of microorganisms.

DISCUSSION

Experimental results obtained during this investigation indicate that substrate utilization in an activated sludge waste treatment system is reduced when surface active agents are present. Both ABS and LAS affected the rate of substrate removal in the experimental units. The extent of this effect was related to the structure and the concentration of the SAA present. It is postulated that any or all of the following mechanisms could have been responsible for the observed effect: (1) toxicity, (2) enzyme inhibitions, or (3) reduced mass transport between the biomass and the bulk of the solution.

The bactericidal and bacteriostatic action of a number of surface active agents has been reported (63). Quaternary ammonium compounds, which are cationic surfactants, are known for this effect; however, in the concentrations used in this investigation, it is extremely doubtful that either of the anionic compounds, ABS or LAS, could exert a direct bactericidal action on the biological floc. To support this theory, biomass characteristics were carefully observed. Shifts in the microbial population were essentially the same in the absence of surfactants as they were in their presence. These changes are attributed to the response of the organisms to the powdered dry milk substrate. In a mixed culture, shifts in predominance may be the result of selective enrichment, relative differences in individual growth rates, or interactions which occur among the individual species in the microbial community. When a mixed population is brought from the field and maintained under laboratory conditions, shifts in its predominant structure must be expected.

In an active culture, the rate of substrate utilization increases exponentially with time. The number of organisms, and thus the concentration of enzymes, increase at a similar rate. Stumm (64) related substrate depletion to enzyme concentration and bacterial mass, as shown in the following expressions:

$$\frac{dX^N}{d\theta} = K_1 E \tag{51}$$

where:

E = enzyme concentration at time "θ"

X^N = substrate concentration

K_1 = rate constant

In growing bacterial cultures, maintained under steady state conditions, any enzyme constitutes a constant function of the total bacterial mass:

$$E = K_2 X^O \tag{52}$$

where:

K_2 = a proportionality constant

X^O = concentration of biomass

Combining (51) and (52), and adding the equation for exponential growth ($dX^O/d\theta = \mu X^O$, or $X^O = X_0^O e^{\mu\theta}$, where μ = the specific growth rate constant and X_0^O = initial concentration of biomass):

$$-\frac{dX^N}{d\theta} = K_1 K_2 X_0^O e^{\mu\theta} \tag{53}$$

As shown in these equations, the rate of substrate utilization will increase when new enzyme is being formed and no inhibition occurs.

Equation (53) can be reduced to the following simple form:

$$-\frac{dX^N}{d\theta} = R = Ke^{\mu\theta} \tag{54}$$

where:

K = an overall proportionality constant

R = rate of substrate utilization

This relationship can be linearized and plotted semilogarithmically as follows:

$$\ln R = \ln K + \mu\theta \qquad (55)$$

Substrate utilization rates obtained experimentally in this study do not fit the relationship expressed in Equation (56). This may be the result of: (1) the complexity of the experimental system, or (2) the hypothetical nature of Equations (51) through (56). It is more logical to assume, however, that the biological activity of the floc is affected adversely by the presence of surfactants in the system.

SAA adsorbed on the surface of the biomass may result in suppressing the extracellular hydrolysis of substrate particles. An adsorbed SAA film at the floc-solution interface may interfere with the production, and the availability, of extracellular hydrolytic enzymes and act as a barrier between the substrate molecule and its respective enzyme site. The degree of enzyme inhibition, and, consequently the substrate utilization rate, is a function of the type and concentration of inhibitor (SAA) present. The higher the level of SAA in the bulk of the solution, the greater the amount of adsorbed film at the floc-solution interface; therefore, the lower the rate of substrate utilization. Because LAS is more easily oxidized than ABS, less suppression of substrate utilization occurs in the presence of the soft surfactant than in systems with the hard compound.

SAA may also suppress the rate of substrate utilization by reducing the rates of mass transport. Biological nutrient uptake occurs through a series of consecutive steps: (1) mass transport in the bulk of the aqueous phase, (2) mass transport across a boundary layer at the floc-solution interface, and (3) enzyme catalyzed reactions on the surface of the floc. Because adequate mixing conditions were maintained in the aqueous phase of the experimental system, mass transfer rates in the bulk were greater than those across the boundary layer. It is also logical to assume that chemical reactions at the floc surface occurred at a greater rate than that of mass transport across the boundary layer; therefore, the rate limiting step in this model is that of mass transport across the boundary layer at the floc-solution interface.

The viscosity and the hydrodynamic activity of the floc may be altered significantly by SAA adsorbed at the floc-solution interface. Levitch (65) reported that the hydrodynamic activity of bubbles was significantly retarded in the presence of SAA. SAA adsorbed on the surface of the bubble increased the diameter of the bubble and decreased its velocity. The hydrodynamic activity of the biomass in an activated sludge system is affected similarly when its boundary layer is increased by a SAA adsorbed film.

In an activated sludge system, adsorbed SAA may also reduce the elasticity of the floc. As it acquires a higher viscosity, the floc becomes more rigid and does not yield to the inertial forces of liquid eddies, driven by turbulance in the bulk. As a result, the floc is fragmented, becomes more dispersed, less settleable, and it may be washed out of the system. As shown in Figure 25, this effect was observed in the experimental system, particularly in the presence of high concentrations of ABS.

CONCLUSIONS

Regardless of the mechanism involved, under the specific conditions of this investigation, ABS in concentrations equal to or greater than 5 milligrams per liter, and LAS in concentrations equal to or greater than 10 milligrams per liter interfered significantly with substrate utilization in the experimental activated sludge system. The extent of interference was related to the structure and concentration of SAA in the system. Because of their surface active characteristics, both ABS and LAS tend to concentrate at interfaces. At the floc-solution interface, they form adsorbed films which are readily available to the enzyme systems in the biomass. LAS, with its linear configuration, was oxidized more rapidly than ABS; therefore, it had less effect on substrate utilization in the system.

At the specific levels tested, neither ABS nor LAS was toxic to the system. Shifts in population dynamics were observed frequently; however, these changes occurred as a direct result of adapting field cultures to a laboratory environment and to a synthetic substrate. They were not related to the presence of either surfactant.

The results of this study can be attributed to the tendency of surface active agents adsorbed at the floc-solution interface to increase the thickness and viscosity of the boundary layer, reduce mass transport across the phase boundary, and retard the hydrodynamic activity of the biological floc. A loss of surface elasticity would cause the floc to fragment more easily and settle less readily when surface active agents are present in the system.

REFERENCES

1. Bolotina, V.L.B., Malyusova, M.M., and Novozhilov, P.G. "The Effect of the Synthetic Detergent Prepared from NP-1 According to the BMS-8 Recipe on the Performance of Purification Installation of a Municipal Sewage System," Akademiia Kommunal Nogo Khoziastva Nauchnye Trudy (USSR), 37, 44, (1965).

2. Hanna, G.P., Weaver, P.J., Sheets, W.D., and Gerhold, R.M. "A Field Study of LAS Biodegradation," Water and Sewage Works, 111, 478, (1964).

3. Renn, C.E., Kline, W.A., and Orgel, G. "Destruction of Linear Alkylate Sulfonates in Biological Waste Treatment by Field Test," Journal Water Pollution Control Federation, 36, 864, (1964).

4. Porges, N., Jasewicz, L., and Hoover, S.R. "Principles of Biological Oxidation," Biological Treatment of Sewage and Industrial Wastes, Vol. I, ed. by Eckenfelder, W.W., Jr., and McCabe, Br.J. New York: Reinhold Publishing Corp., 1956.

5. Eckenfelder, W.W., Jr., and Weston, R.F. "Kinetics of Biological Oxidation," Biological Treatment of Sewage and Industrial Wastes, Vol. I, ed. by Eckenfelder, W.W., Jr., and McCabe, Dr.J. New York: Reinhold Publishing Corp., 1956.

6. Hellums, J.D. "Transport Phenomena," Water Technology, A Multidisciplinary Approach, ed. Busch, A.W. Houston, Texas: Rice University Press, 1966.

7. Swilley, E.L., Bryant, J.W., and Busch, S.W. "Significance of Transport Phenomena in Biological Oxidation Processes," Proceedings 19th Industrial Wastes Conference, Vol. I. Lafayette, Indiana: Purdue University, 1964.

8. Lehninger, A.L. Bioenergetics. New York: W.A. Benjamin, Inc., 1965.

9. Kountz, R.R., and Forney, C. "Metabolic Energy Balances in a Total Oxidation Activated Sludge System," Sewage and Industrial Wastes, 31, 819, (1959).

10. Monod, J. "The Growth of Bacterial Cultures," Annual Review of Microbiology, 3, 371, (1965).

11. Hinshelwood, C.N. Chemical Kinetics of the Bacterial Cell. London: Clarendon Press, 1947.

12. Placak, O.R., and Ruchhoft, C.C. "Studies of Sewage Purification, XVII: The Utilization of Organic Substrates by Activated Sludge," Sewage Works Journal, XIX, 423, (1947).

13. Helmers, E.N., Frame, J.E., Greenberg, A.E., and Sawyer, C.N. "Nutritional Requirements in the Biological Stabilization of Industrial Wastes, II: Treatment with Domestic Sewage," Sewage and Industrial Wastes, 23, 884, (1951).

14. Heukelekian, H., Orford, H.E., and Manganelli, R. "Factors Affecting the Quantity of Sludge Production in the Activated Sludge Process," Sewage and Industrial Wastes, 23, 945, (1951).

15. Garrett, M.T., Jr., and Sawyer, C.N. "Kinetics of Removal of Soluble BOD by Activated Sludge," Proceedings 7th Industrial Wastes Conference. Lafayette, Indiana: Purdue University, 1952.

16. McCarty, P.L. "Thermodynamics of Biological Synthesis and Growth," Proceedings 2nd International Conference on Water Pollution Research, Vol. II. Oxford, England: Pergamon Press, 1965.

17. Servizi, J.A., and Bogan, R.H. "Thermodynamic Aspects of Biological Oxidation and Synthesis," Journal Water Pollution Control Federation, 36, 607, (1964).

18. Pipes, W.O., Miholits, E.M., and Boyle, O.W. "Aerobic Cell Yield and Theoretical Oxygen Demand," Proceedings 18th Industrial Wastes Conference. Lafayette, Indiana: Purdue University, 1963.

19. Bauchop, T., and Elsden, S.R. "The Growth of Microorganisms in Relation to Their Energy Supply," Journal General Microbiology, 23, 457, (1960).

20. Gates, W.E. "The Kinetics of Activated Sludge." (Unpublished)

21. McKinney, R.E. "Mathematics of Complete Mixing Activated Sludge," Journal Sanitary Engineering Division American Society Civil Engineers, 88, 1, (1962).

22. Eckenfelder, W.W., Jr., and O'Connor, D.J. Biological Waste Treatment. Oxford: Permagon Press, 1961.

23. Whitman, W.G. "Two Film Theory of Gas Absorption," Chemical and Metallurgical Engineering, 29, 146, (1923).

24. Higbie, R. "The Rate of Absorption of a Pure Gas in a Still Liquid During Short Periods of Exposure," Transactions American Institute Chemical Engineers, 31, 365, (1935).

25. Danckwerts, P.V. "Significance of Liquid Film Coefficient in Gas Absorption," Industrial and Engineering Chemistry, 43, 1460, (1951).

26. Schlichting, H. Boundary Layer Theory, 4th Ed. New York: McGraw-Hill Book Co., Inc., 1960.

27. Kishinevski, M. "Two Approaches to the Theoretical Aspects of Gas Absorption," Journal Applied Chemistry (USSR), 28, 881, (1955).

28. Bennett, G., and Kempe, L.L. "Oxygen Transfer Mechanisms in the Gluconic Acid Fermentation by Pseudomonas ovalis," Biotechnology and Bioengineering, VI, 347, (1964).

29. Mancy, K.H., and Okun, D.A. "The Effects of Surface Active Agents on Aeration," Journal Water Pollution Control Federation, 37, 212, (1965).

30. State of California Water Quality Control Board, Department of Water Resources, and Department of Public Health. Detergent Report: A Study of Detergents in California. A Report Prepared for the California State Legislature, 1965.

31. Moilliet, F.L., Collie, B., and Black, W. Surface Activity, 2nd Edition. London: E. and F.N. Spon, Ltd., 1961.

32. Coughlin, F.J. "Detergents and Water Pollution Abatement," Soap and Detergent Association Technical Report No. 1, 1965.

33. McGauhey, P.H., and Klein, S.A. "Removal of ABS by Sewage Treatment," Sewage and Industrial Wastes, 31, 877, (1959).

34. McKinney, R.E., and Symons, J.M. "Bacterial Degradation of ABS, I: Fundamental Biochemistry," Sewage and Industrial Wastes, 31, 549, (1959).

35. Task Force. Detergent Problems in Perspective. A Report by Sanitary Group, Los Angeles, California Section American Society Civil Engineers, 1965.

36. Pitillo, R.F. "Yesterday's Waste Water," Bulletin Southern Research Institute, 16, 1, (1963).

37. Swisher, R.D. "Surfactant Effects on Humans and Other Mammals," Soap and Detergent Association Technical Report No. 4, 1966.

38. McKinney, R.E. "Syndets and Waste Disposal," Journal Water Pollution Control Federation, 29, 654, (1957).

39. Rudolfs, W., Manganelli, R., and Gellamn, I. "Effect of Certain Detergents on Sewage Treatment," Journal Water Pollution Control Federation, 21, 605, (1949).

40. Deglus, P.N. "Review of Experience with Froth Foundation in Sewage Treatment Plants," Journal Water Pollution Control Federation, 26, 1494, (1954).

41. Mancy, K.H., and Okun, D.A. "Effects of Surface Active Agents on Bubble Aeration," Journal Water Pollution Control Federation, 32, 351, (1960).

42. Eckenfelder, W.W., Jr., and Barnhardt, E.C. "The Effect of Organic Substances on the Transfer of Oxygen from Air Bubbles in Water," Journal American Institute Chemical Engineering, 7, 631, (1961).

43. Lynch, W.O., and Sawyer, C.N. "Effects of Detergents on Oxygen Transfer in Bubble Aeration," Journal Water Pollution Control Federation, 32, 25, (1960).

44. Carver, C.E. "Absorption of Oxygen in Bubble Aeration," Biological Treatment of Sewage and Industrial Wastes, Vol. I, ed. by Eckenfelder, W.W., Jr., and McCabe, Dr.J. New York: Reinhold Publishing Corp., 1956.

45. Swisher, R.D. "Transient Intermediates in the Degradation of Straight Chain ABS," Journal Water Pollution Control Federation, 35, 1557, (1963).

46. Swisher, R.D. "Chemical Mechanism of Straight Chain ABS Biodegradation," Soap and Chemical Specialties, 39, 47, (1963).

47. Ibid., 57, (1963).

48. Germany, Ministers of Science and Health. Regulation Concerning the Degradability of Detergents in Washing and Cleaning Compounds. Bonn, Germany: Federal Record Publication 253/62, 1962.

49. Cassell, E.A., Sulyer, F.T., and Lamb, J.C., III. "Population Dynamics and Selection in Continuous Mixed Cultures." (Unpublished).

50. Ludzack, J.J. Personal Communication.

51. Sawyer, C.N. "Bacterial Nutrition and Synthesis," Biological Treatment of Sewage and Industrial Wastes, Vol. I, ed. by Eckenfelder, W.W., Jr., and McCabe, Br.J. New York: Reinhold Publishing Corp., 1956.

52. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. Standard Methods for the Examination of Water and Wastewater, 12th Edition. New York: American Public Health Association, 1966.

53. Schaffer, R.B., VanHall, C.E., McDermott, G.N., Barth, D., Stenger, V.A., Sebesta, S.J., and Griggs, S.H. "Application of a Carbon Analyzer in Waste Treatment," Journal Water Pollution Control Federation, 37, 1545, (1965).

54. Williams, R. . "The Carbonaceous Analyzer As a Water Pollution Research Tool," Instrument Society of America, Preprint No. 5.2-4-66, (1966).

55. Busch, A.W. "Carbon Analysic As a Concept and Procedure in Water Pollution Technology," The Analyzer, 8, 3, (1967).

56. Task Force. "Determination of Synthetic Detergent Content of Raw Water Supplies," Journal American Water Works Association, 50, 1343, (1958).

57. Chitwood, B.G., and Allen, M.W. "Nemata," Ward and Whipple Fresh Water Biology, 2nd Edition, ed. by Edmondson, W.T. New York: John Wiley and Sons, Inc., 1966.

58. Zipf, R.E. and Waldo, A.L. "Spectrophotometric Analysis of Carbohydrates and Study of Anthrone Reagent," Journal of Laboratory and Clinical Medicine, 39, 497, (1952).

59. Hazen, A. "Storage to be Provided in Impounding Reservoirs for Municipal Water Supply," Transactions American Society Civil Engineers, 77, 1539, (1914).

60. Velz, C.J. "Graphical Approach to Statistics," Water and Sewage Works, 99, R-106, (1952).

61. Gannon, J.J. "Statistical Basis for Interpretation of Data." (Unpublished).

62. Ludzack, F.J. "Observations on Bench-Scale, Extended Aeration Sewage Treatment," Journal Water Pollution Control Federation, 37, 1092, (1965).

63. Sykes, G. Disinfection and Sterilization. Princeton, New Jersey: D. Van Nostrand Co., 1958

64. Stumm, E. "Substrate Utilization in Heterogeneous Bacterial Communities," Journal Water Pollution Control Federation, 40, R213, (1968).

65. Levitch, V.G. Physicochemical Hydrodynamics. Englewood Cliffs, New Jersey: Prentice Hall, Inc., 1962.