THE APPLICATION OF ALGAL GROWTH POTENTIAL TECHNIQUES TO SURFACTANT AND ZINC TOXICITY STUDIES

by

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INTRODUCTION

Statement of Purpose

The need for rigid control of environmental stresses (both actual and potential) has generated many new approaches to monitoring the physical, chemical, and biological fluxes in aquatic ecosystems. One of these approaches that is currently being used is the algal bioassay. This technique has been used primarily in predicting the algal growth potentials of waters from a variety of sources. It also has been used to identify the algal growth-limiting nutrient in these waters. The purpose of this investigation was to examine the suitability of algal growth potential techniques for toxicity studies. Studies of this type are very useful since they may establish criteria by which environmental standards may be set for many toxicants. The toxicants which I studied included two anionic surfactants, a nonionic surfactant, and zinc. The goals of the surfactant studies were different from those of the zinc study, but both studies were related in that algal bioassay techniques were used in each. The intent was to demonstrate the versatility and practicality of the techniques for toxicity studies.

More specifically, the purpose of the surfactant studies was to determine the effects of biodegradation on the toxicity of three surfactants to an algal test organism. The surfactants were biodegraded in bench-scale activated sludge units, and the effluents were bioassayed. Both static and continuous-flow bioassay techniques were used.

The purpose of the zinc studies was to determine the levels of zinc required to inhibit algal growth under defined experimental conditions

and to study the effects of temperature on the toxicity of these zinc levels. Static algal bioassay techniques were used. Standing crop levels and oxygen production rates were measured to study the toxic effects of zinc and its possible mechanism of action.

Literature Review of Algal Bioassay Theory and Use

General Considerations

Krock and Mason (1971) defined algal bioassays as tests of the ability of chemicals to affect the processing of energy by algae. This definition is applicable whether the bioassays are to test for toxicity or for algal growth potential. Mackenthun (1969) stated that the bioassay, in general, is an important tool for investigating toxic wastes because results can indicate the degree of hazard to aquatic life of a particular discharge. Recommendations can then be made concerning the level of discharge that can be tolerated by aquatic organisms. Hutchinson and Stokes (1973) stressed the importance of bioassays in that living organisms provide a sensitive index of their environment, and their responses can be intelligently interpreted. Voluminous literature exists on many types of bioassay studies, and much of it describes work done with various species of fish. However, a justifiable need exists for the use of algal bioassay data in evaluating environmental stresses. Hutchinson (1973) stated that the phytoplankton are important to study since they are the major food source for grazing zooplankton (and ultimately for fish). Kauss et al. (1973) agreed with the importance of studying algae since any deleterious effects on algal species are likely to be detrimental to higher trophic levels. Krock and Mason (1971) stated that fish are

usually the aquatic organisms of greatest economic importance to man, but fish must depend on the energy processing action of the producers, decomposers, and herbivores. Thus, we are justified in studying the lower trophic level organisms because the stability of the entire system is important for the livelihood of any single component of the system (e.g. fish).

This need for standard algal bioassay procedures resulted in an attempt by industry and government (Joint Industry/Government Task Force on Eutrophication 1969) to develop a standardized algal assay procedure (primarily for nutrient level assessment). Toerien et al. (1971) made several comments on this procedure and its potential for practical use. He said that the Provisional Algal Assay Procedures (PAAP) could be used in the solution of eutrophication problems by identifying growth-limiting nutrients in receiving water and wastewater. Only then can one determine which nutrients (if any) need to be reduced in concentration. The amount of needed reduction can only be determined from algal growth kinetics information. He also indicated that the procedures could be used with practical applicability in studies of toxicity evaluation. The criteria used by the task force in selecting algal assay procedures were as follows: 1) methods should be simple, convenient, and capable of being carried out by technicians, 2) equipment requirements should be simple and economical, 3) results should be accurate and precise, 4) geographic variation in the methods should be minimal, and 5) results should be applicable (with judgment) to the natural environment. These procedures (with a few modifications) have been widely used for algal growth potential and toxicological assessment. Krock and Mason (1971) said that the main

advantages of using algae instead of fish as bioassay organisms were 1) short generation time, 2) fast metabolic rate, and 3) no known detoxification mechanisms.

Most advocates of using algal bioassays have stressed that assays should not be substitutes but supplements for chemical data (e.g. Maloney et al. 1973). Aubert (1972) pointed out that mere analysis of the chemical composition of water is inadequate for evaluating pollution. The bioassay technique provides a more comprehensive account of the dangers to different levels of the food chain. In discussing the control of sewage effluents, Forsberg and Forsberg (1972) stated that the use of algal assays to test chemical treatment efficiency gives a more comprehensive picture of effluent quality than chemical tests alone. Johnson et al. (1970) said that chemical analyses do not show the relation of chemical concentration to phytoplankton growth. Certain combinations of these chemicals may react synergistically or antagonistically. Skulberg (1964, 1966) felt that physical and chemical data alone are inadequate to characterize the biology of a lake. Chemical analyses give information on plant nutrient concentrations but give no indication of their availability to algae. Assays do not duplicate environmental conditions, thus they must supplement rather than substitute for physical and chemical data. In algal growth potential studies, algal assays may be better than chemical tests in determining levels of assimilable nitrogen and phosphorus (Cullimore and McCann 1972). Wang et al. (1973) generalized that the most direct evaluation of eutrophication is to study living algal organisms. They are the most immediate biotic reflection of the nutrient status of their environment, and their short life cycle and facility for rapid adaptation make them ideal organisms for laboratory examination.

The results of algal bioassay studies should be applicable, to some extent, to the natural environment. However, this must be done with a degree of caution. Lee and Veith (1971) stated that although bioassays provide essential information for evaluating the significance of hazardous chemicals in the environment and for establishing control measures for their use, the accuracy with which bioassay results can be extrapolated to environmental conditions depends largely on the knowledge of the state of the chemical in the environment and the ability to reproduce this chemical state under test conditions of a bioassay procedure. Because few studies successfully do this, Lee and Veith were relatively critical of the PAAP as an algal growth potential test. However, many researchers believe that laboratory data can be successfully applied to the natural environment if it is done with care (Foree and Wade 1972, Provasoli 1958 as cited by Clesceri 1973, Ukeles 1965). Kolata (1974) said that by isolating the key components of a system and treating them as much as possible in isolation, ecologists attempt to produce models that describe broad classes of phenomena. In discussing algal bioassay static tests, Middlebrooks et al. (1971) stated that results can never be interpreted directly in terms of outdoor ecosystems involved in eutrophication, but, like the BOD test, algal assays can be useful as basic water quality parameters. Warnick and Bell (1969) did toxicity studies with aquatic insects, but their justification for the applicability of acute bioassay results also applies to algal tests. They said that acute toxicity tests indicate relative species sensitivity and lethal concentrations. This information can be used for long-term tests to establish necessary requirements for healthy aquatic life. The discussion by Toerien et al.

(1971) concerning the application of algal assay results to the environment is very constructive. They pointed out that the standing crop attained in static assays is far removed from that attained in nature. Static assays always have higher standing crops since maximum standing crops are rarely attained in nature. This is due to environmental limiting factors such as predation, settling of cells, etc. As soon as a water sample is removed from its normal environment, factors have been introduced which make it nonrepresentative. Thus, one can only analyze for those properties that are intrinsically part of the sample (i.e. chemical content, organisms, and detritus). Further treatment such as filtration only makes extrapolation of the results to nature more difficult.

As was mentioned earlier, algal bioassays have been used primarily in two ways: for algal growth potential tests and for toxicity tests. Much more literature is available concerning algal growth potential tests than toxicity tests. The following researchers have used algal assays for algal growth potential purposes: Berland et al. (1972), Brown 1972a, Clasen and Bernhardt 1974, Clesceri 1973, Cullimore and McCann 1972, Eyster 1958, Forsberg and Forsberg 1972, Forsberg and Hokervall 1972, Johnson et al. 1970, Krock and Mason 1971, Lange 1971, Lindahl 1973, Maloney et al. 1972, Miller and Maloney 1971, Moss 1973, Skulberg 1964, Skulberg 1966, and Toerien et al. 1971. Only a few authors have published papers in which algal assay techniques were used to test for toxicity. Some of those include Brown 1972, Hall 1973, Krock and Mason 1971, and Toerien et al. 1971. Several others have indicated that algal growth potential techniques could be used in toxicity studies (Forsberg and Forsberg 1972, Johnson et al. 1970, and Weber 1973).

I have stated that algal bioassays can be used for two basic purposes. There are also several types of bioassay techniques from which to choose for a given purpose (i.e. static, continuous-flow, and in situ tests). Each has its advantages and disadvantages which will be discussed in more detail. Very little work has been done with standard procedures for in situ assays; therefore, they will not be considered here. The static and continuous-flow techniques are the most commonly encountered, and they were the ones utilized in this study.

Static Algal Bioassays

In outlining the procedures for the static algal bioassay, Weber (1973) listed the following uses for the test: 1) identification of algal growth-limiting nutrients, 2) biological determination of the availability of algal growth-limiting nutrients, and 3) determination of the toxic or inhibitory effects to algae of various compounds or water samples. As is the case in most discussions of the uses of algal assays, its primary purpose was presented as an algal growth potential test. However, more recently, authors have made provisions for the techniques to be used for toxicity tests. Even though the procedures are essentially identical regardless of the purpose of the test, the basic theory is quite different for each of the intended purposes. If the purpose of the bioassay is a nutrient study, then the limiting nutrient concept is the most important consideration in the test. Weber (1973) described this concept by stating that algal growth is limited by the nutrient present in shortest supply with respect to the needs of the organism. Miller and Maloney (1971) said that a nutrient is limiting if growth is stimulated by adding it.

If, however, the bioassay test is intended for toxicity purposes, the limiting nutrient concept is not the most important consideration. The concentration of the toxicant, the time of exposure, and the mechanism of response are the most important factors to consider (Krock and Mason 1971). Even if the purpose of the test is a nutrient study, the presence of toxic substances in natural water samples to be bioassayed may severely alter results.

The static algal bioassay is basically a closed system (except for gas exchange) in which the chemical composition of the medium is controlled only until the time of inoculation. Then the metabolic activities of the inoculated algae continuously alter the environmental conditions in the enclosed system. The system of dynamic equlibrium which exists in nature is certainly abbreviated in a culture vessel. It is for this reason that many researchers are critical of results generated by this method. However, as was stated earlier, there are many proponents of the test and its potential for useful results.

Many authors have presented detailed discussions of the kinetic theory of the algal bioassay static test (Joint Industry/Government Task Force on Eutrophication 1969, McGauhey 1969, McGauhey 1970b, Toerien et al. 1971, Tunzi 1972, Weiss and Helms 1971). I will not discuss the details of kinetic theory since extensive monographs are available on the topic and since most of the mathematical relationships are not applicable to toxicity studies (but rather to nutrient studies).

The static algal bioassay test, whether used for toxicity or nutrient study purposes, is not a standard procedure (Environmental Protection Agency 1971, Weber 1973). Extensive testing and evaluation of the static

methods have yielded acceptable levels of precision and accuracy for practical use (Weiss and Helms 1971). Some of the uses of the static test will now be discussed.

When the static algal bioassay has been used as an algal growth potential test, the source of the water to be tested has been either natural waters from lakes, reservoirs, and streams, or wastewaters from industries and domestic sewage facilities. Allen and Nelson (1910), Maloney et al. (1973), McGauhey (1969), McGauhey (1970a), McGauhey (1970b), Toerien et al. (1971), and Wang et al. (1973) tested natural water samples for algal growth potential using the static test. Wastewaters have been tested by Clesceri (1973), Forsberg (1972), Goldman et al. (1974), and Middlebrooks et al. (1971). All algal bioassays for algal growth potential purposes are conducted in one of two basic ways (Cullimore and McCann 1972). The water or waste sample may be diluted to a series of strengths, and assays may be run on each dilution, or the sample may be spiked with one or more chemicals, and assays may be run for each different spike. Spiking studies are usually run to determine tha algal growth-limiting nutrient. Dilution studies are usually run to assess the algal growth potential of the sample. For both types of tests, the parameters of interest are the maximum standing crop and/or the maximum specific growth rate for the test period. The measurements of these parameters are then used to evaluate the limiting nutrient or algal growth potential characteristics of the sample.

The static test has been used much less frequently for toxicity studies. Those who have used it for toxicity test purposes include Bartlett et al. (1974), the Environmental Protection Agency (1971),

and Toerien et al. (1971). There are several reasons that the test has not been used extensively for toxicity purposes. First, toxicity tests have classically been run primarily with fish. Secondly, interest in the lower trophic level organisms for water quality monitoring purposes is a relatively recent development. Thirdly, the static test procedures have been publicized primarily as algal growth potential tests. The static test procedures are certainly not more difficult to use for toxicity tests. As was true for algal growth potential studies, toxicity studies are usually run on either natural water or wastewater samples. Samples may be spiked with toxicants before bioassaying, or the concentration of toxicants present in the sample may be reduced by dilution before testing. Standing crop and/or growth rate are also the parameters of importance in toxicity tests. These measurements are used to evaluate either the toxicity of a number of chemicals to the test organism (spiking studies) or the strength of toxicants already present in the sample (dilution studies).

Continuous-Flow Algal Bioassays

Like the static test, the continuous-flow test was developed primarily for measuring algal growth potential but can be used for toxicity studies. Continuous-flow techniques are more complicated than static test procedures, and they require more time, expense, and trained personnel for operation (Tunzi 1972). The theory of chemostat operation has been discussed extensively by Foree and Wade (1972), Joint Industry/Government Task Force on Eutrophication (1969), McGauhey (1969), Toerien et al. (1971), and Tunzi (1972). The mathematical expressions describing continuous-flow growth kinetics will not be described here. The equations are basically

those outlined by Michaelis and Menten (1913) (as cited by Toerien et al. 1971) in describing enzyme kinteics, but they are applicable only for limiting nutrient studies. The continuous-flow bioassay is an open system in which fresh sample to be bioassayed is constantly being fed into the chemostat. This continuous addition of sample allows the maintenance of relatively constant chemical conditions in the medium. As the algae grow, their metabolic activities do tend to change the chemical constituency of the medium, but the constant flow of fresh feed tends to hold the conditions constant. Assuming that algal growth is not inhibited by a toxicant in the sample, the steady state level of growth attained by the algae is a function of the limiting nutrient concentration and the rate at which the sample is fed into the chemostat. These two parameters can be varied at will while other parameters such as lighting, agitation, and temperature are held constant. If toxicants are present in the sample, the limiting nutrient concentration is no longer a primary factor in determining the steady state level of growth. A certain concentration of toxicant might reduce growth, halt growth altogether, or kill the cells regardless of the limiting nutrient concentration. If growth is completely halted or if the cells are killed, the chemostat will eventually be flushed of all algal cells, and steady state levels of growth are impossible to attain. If growth is only reduced by the toxicant, then steady state growth may be achieved but at a lower level than if no toxicant were present. Thus, for a nutrient study, higher steady state levels of growth indicate greater algal growth potential due to a greater concentration of limiting nutrient. For toxicity studies, reduced steady state levels of growth indicate the presence of toxicants in sufficient

concentrations to inhibit (to some degree) the normal metabolic growth rate of the algae under the specified conditions. Like the static tests, continuous-flow algal bioassays may be spiking tests or dilution tests depending on the purpose of the assay. In any case, it is vitally important that the following parameters be held constant: flow rate, medium composition, culture volume, pH, temperature, aeration, and adequate mixing (Hamilton and Preslan 1970).

Unlike the static test, the continuous-flow assay has not yet been recommended as a standard procedure. It has not been tested as extensively as the static test because it is a complicated procedure to perfect, and many more variables must be controlled. There have been very few examples of successful chemostat studies (Tunzi 1972).

The continuous-flow algal bioassay was used for algal growth potential studies by Dunstan and Menzel (1971), Foree and Wade (1972), Foree and Scroggin (1972), Fuhs (1972), McGauhey (1969), McGauhey (1970b), Scherfig and Dixon (1973), and Toerien et al. (1971). Most of these studies involved evaluation of the algal growth potential of wastewaters. They were carried out essentially by diluting the samples or by determining the limiting nutrient and spiking with a range of concentrations of that nutrient. For a given flow rate, the steady state levels of growth for each set of dilutions or for each series of spikes was compared to controls. This indicated the capacity of the samples to support various levels of algal growth under the experimental conditions used. The results were primarily used to indicate the efficiency of tertiary sewage treatment (in removing nitrogen and/or phosphorus) or to evaluate the algal growth potential of the receiving water after mixing with the effluent. In most cases, these continuous-flow assays were carried out in addition to static assays.

Very little use has been made of the continuous-flow test for toxicity studies. Two toxicity studies in which these procedures have been used include Toerien et al. (1971) and Reynolds et al. (1973). Perhaps one of the main reasons that this test has not been used for toxicity purposes is the length of time required to achieve steady state and thus to obtain results. In most instances, results of toxicity tests are needed quickly so that corrective actions may be taken. Time however is not a factor in instances where the test is used in the laboratory to study the effects of various levels of different toxicants. Theoretically, these results could be used to determine critical levels of toxicants in the environment. Routine chemical monitoring could then be used regularly to insure that the critical levels (or standards) are not exceeded. Unfortunately, the lack of use of the continuous-flow test for toxicity studies has left many unanswered questions concerning its accuracy, precision, and its response to physical and chemical variables. Much more interlaboratory testing must be carried out to elevate continuous-flow techniques to standard procedures.

Advantages and Disadvantages of Static and Continuous-Flow Assays

Many of the assets and liabilities of the various algal bioassay test procedures have already been referenced. Forsberg (1972) said that it is necessary to develop algal assay methods that require small culture volumes, small samples, and short incubation periods if they are to become routine practices. This would certainly tend to support the use of static rather than continuous-flow assays. Tunzi (1972) stated that static assays are best for running a large number of samples very rapidly

with good statistical reliability. Chemostat assays are best when time, money, and trained personnel are ample. Toerien et al. (1971) defended the continuous-flow test, but they considered only the evaluation of algal growth potential. They said that static assays require less equipment and are simpler to conduct, but continuous-flow assays are more versatile and yield more valuable information with higher precision. Their studies indicated that limiting nutrients were more easily identified with continuous-flow tests. These tests allow for maintaining a culture for a long period of time with a constant population, constant growth rate, constant physiological state, and constant environmental conditions. In static tests, not all the cells are in the same physiological state. Toerien et al. (1971) recommended that static tests be used for crude screening and routine monitoring and that continuous-flow tests be used for quantitative assessment of algal growth-supporting properties of water, limiting nutrient determination, and kinetic description of nuisance algae. Tunzi's objections to continuous-flow procedures were mainly that cultures were difficult to keep clean if not pure over long periods of time and that good statistical reliability of results was difficult to attain since replication is usually low. Neither author addressed themselves specifically to toxicity tests in discussing advantages and disadvantages.

Selection of Assay Organisms

The algal bioassay procedures that have been developed by the Environmental Protection Agency recommend that single algal species be used as test organisms. There has been substantial disagreement with this recommendation from many researchers who argue that natural populations rather

than single species should be used. Dunstan and Menzel (1971), Krock and Mason (1971), and Wang et al. (1973) have carried out algal bioassays using natural populations. Wang et al. stated that the advantage of using a mixed population rather than a single species is the adaptability to different aquatic environments where more than one species is available for potential growth dominance. Skulberg (1964) agreed with this saying that single species grown in the laboratory differ to some degree in cell physiology from natural organisms. Single species may not respond in a manner representative of the natural organisms in question. Also, the effects of important biotic factors for the development of algae in nature are excluded when single species are used.

However, a strong case can be made for the use of single species. The key to the argument is the intended purpose of the test. If the purpose of the test is to describe quantitatively and qualitatively exactly what happens in nature under specified nutrient or toxicity conditions, then perhaps the use of natural populations is advisable. But single species can also provide valuable information concerning the behavior of natural organisms. No one species is a perfect representative of all other species and their individual characteristics, but organisms that are representative in many ways can indicate how many organisms will respond to various stimuli. Use of single species allows emphasis on the specific response of an organism to a nutrient or toxicant without having to consider the effect of community interactions. This is not to say that community interactions are not important, for certainly they are. But sometimes the individual components of a complex response must be analyzed in isolation before the significance of the overall response is understood.

Several authors have described some of the characteristics of an algal organism that make it desirable for bioassay use. Toerien et al. (1971) listed the following characteristics: 1) have a broad nutrient response, 2) have a distinct shape, 3) have a uniform size, 4) divide distinctly, 5) not attach to glass and surfaces, 6) stay in suspension with slight agitation, 7) should not clump, 8) grow at maximum rate in a medium that is simple to constitute, 9) be nonauxotrophic, 10) should not excrete toxic substances, and 11) should normally be associated with oligotrophic waters. Johnson et al. (1970) also listed many of these characteristics. Skulberg (1964) added that the organism should be sensitive enough to exhibit variations in growth response when the change in the growth conditions is small. The need for an organism with these characteristics lead to the selection of Selenastrum capricornutum Printz as a representative species. The organism was originally secured from O. Skulberg of Oslo, Norway (Toerien et al. 1971), and has since been extensively used by algal bioassay investigators as their test organism.

Literature Review of Surfactant Toxicity

Sawyer and McCarty (1967) stated that synthetic detergents have been widely accepted as substitutes for soap, especially since 1945. Use of synthetic detergents is desirable since they do not form insoluble precipitates with the ions causing hardness. Most detergents contain 20-30% surfactant and 70-80% builders. The surfactant is responsible for micelle formation with dirt and grease and is the actual cleansing agent in the synthetic detergent mixture. The builders enhance the cleansing properties of the surfactant by such actions as maintaining the proper

pH for maximum cleansing efficiency. The widespread use of synthetic detergents has resulted in substantial levels of methylene blue active substances (MBAS) in receiving waters. Examples of typical MBAS levels in raw domestic sewage and laundromat effluent were reported by the ORSANCO Detergent Subcommittee (1963) (as cited by the U.S. Dept. of the Interior 1967). MBAS levels ranged 3.1-13.8 mg/l in raw sewage for four cities and 50-90 mg/l for laundromat effluent. Environmental problems arise because the MBAS levels contributed by the surfactant can be toxic to aquatic organisms. Also, nutrients such as phosphates contributed by the builders can stimulate algal growth in receiving waters and ultimately cause troublesome blooms. It is for these reasons that synthetic detergents, their components, and their subsequent by-products have been more intensively studied in recent years. Although increased emphasis has been placed on the effects of surfactant components, very little research has been done concerning surfactant toxicity to algae. Most of the surfactant toxicity studies have been carried out using fish. As was previously indicated, the toxic effects of different substances should be evaluated at all trophic levels in the aquatic ecosystem since each level is in dynamic equilibrium with each other level.

All surfactants have rather large polar molecules. One end is readily soluble in water (hydrophilic) (Sawyer and McCarty 1967). Synthetic surfactants are of three major types: anionic, nonionic, and cationic. The classification of a specific surfactant depends on its ionization properties. Anionic surfactants are sodium salts and ionize to yield a Na^+ cation and a surface-active anion. Sulfates and sulfonates are the most common types. The principle sulfonates are derived from

esters, amides, and alkyl benzenes. The anionic surfactants derived from alkyl benzenes consist of a benzene ring with an attached alkyl group and a sulfonate group in the para position. In the past the alkyl groups were highly branched, and the surfactants were known as alkyl benzene sulfonates (ABS). The branching made these compounds resistant to biological degradation. Recently the branched groups have been replaced with straight-chain groups, and these surfactants are called linear alkyl sulfonates (LAS). LAS surfactants are much more biodegradable, and they are the most widely used surfactants in syntheitc detergents today. This makes them the greatest contributors of MBAS to wastes.

The nonionic surfactants do not ionize at all and depend upon groups in the molecule to make them soluble. They depend on polymers of ethylene oxide for this solubility. Nonionic surfactants are used in some liquid detergents and low sudsing products and are more expensive to produce than anionics.

The cationic surfactants are salts of quaternary ammonium hydroxide. Upon ionization, the molecule splits into a small anion, such as Cl^-, and a large surface-active cation. Cationics are used widely as sanitizers in laundering and dishwashing, but their overall commercial use is rather limited. Only anionic and nonionic surfactants were tested in this study.

There have been very few reports of microbial growth stimulation by surfactants. Kidder et al. (1954) reported that some surface-active agents stimulated the growth of the ciliate Tetrahymena pyriformis. Macleod et al. (1958) found that some surface-active agents stimulated bacterial growth at low concentrations, but this was due to the surface-active properties rather than use as a substrate. The wetting action of

the surfactants on the cell surfaces may have altered the permeability and consequently enhanced nutrient uptake across the membranes.

As for toxicity studies, Maloney (1966) stated that little attention has been given to the effects of synthetic detergents on algae in spite of their importance as primary producers in the aquatic environment. Detailed studies have not been made to determine the role of synthetic detergents and their individual ingredients on algal metabolism. Sililar thoughts were expressed by Forsberg et al. (1967) and Hall (1973). Yet several studies have been reported in which the effects of detergents on algae were tested (Whitton 1967, Wurtz-Arlet 1959). Forsberg et al. (1967) tested surfactant concentrations in the range of 0.13 to 10.0 mg/1. Most surfactant concentrations in this range inhibited algal growth. Maloney (1966) found that 3.6 mg/1 ABS slightly inhibited growth of Chlorella pyrenoidosa, while 10, 20, and 30 mg/1 ABS reduced growth 41, 68, and 100%, respectively. He pointed out that receiving waters rarely have ABS levels of this magnitude (usually it is less than 0.2 mg/1). Two of the best studies of surfactant toxicity to algae were done by Hall (1973) and Ukeles (1965). Hall used static assays to test the toxicity of an anionic surfactant (LAS) and a nonionic surfactant (linear alkylethoxylate) along with several other compounds to S. capricornutum and other species. He found that the concentration of LAS that slightly inhibited the growth of S. capricornutum was 10 mg/1. The nonionic surfactant inhibited the growth of S. capricornutum almost completely at 50 mg/1 and little to none at the next lowest concentration tested, 10 mg/1. Thus, threshold toxicity for the nonionic surfactant was between 10 and 50 mg/1. For all surfactants tested, the levels causing any toxic

effect at all were 10-100 times greater than the maximum predicted environmental level (0.2 mg/1). Hall concluded that the surfactant levels commonly found in the aquatic environment do not adversely affect aquatic organisms.

Ukeles reported the toxic responses of 12 marine phytoplankton species (all Chlorophyceae) to anionic, nonionic, and cationic surfactants. Static bioassay procedures were used. Results showed that responses varied widely among the species tested, but tolerances of species of the same family were similar. The surfactant concentrations tested ranged from 10^{-1} to 10^{-5}% (w/v) of whole product. ABS concentrations of 10 mg/1 were found to inhibit the growth of most of the species tested. Ukeles stressed that an important variable that might affect toxic responses to surfactants in the environment is the variety of impurities and fillers included in detergent products by various manufacturers.

The details of the mechanisms by which surfactants are toxic to algae are still speculation. Ukeles (1965) stated that the mechanisms of action of surfactants must be understood in the laboratory for valid analyses of natural situations. The early biological work with surface-active agents of synthetic detergents was primarily concerned with evaluating the detergents as bactericides (Hotchkiss 1946). One hypothesis which attempts to explain the inhibitory effect of surfactants on microbial cells involves protein denaturation. Jirgensons (1961) stated that the efficiency of detergents in unfolding protein molecules at interfaces is due primarily to the lipophilic hydrocarbon chain. The hydrocarbon tail of the surfactant penetrates into the hydrophobic interior of the protein where it remains bound by weak cohesive forces. Ukeles thought that ionic surfactants

(anionic and cationic) may interact with protein by an electrostatic attraction or repulsion of charged groups on the protein or lipoprotein of the cell surface ultrastructure.

The hypothesis of altered membrane permeability was previously mentioned. Maloney (1966) thought that an increase in Chlorella cell wall permeability by ABS may have increased the respiratory rate. Smith et al. (1961) found that various nonionic surfactants increased the wall permeability of the fungus Ashbya gossypii. Sokolski et al. (1962) found similar results in testing the flagellate Ochromonas danica with anionic detergents. Ukeles (1965) found few reports of growth stimulation or inhibition attributable directly to the surface-active properties of surfactants. Indications are, however, that growth inhibition by anionic surfactants probably depends on their surface-active properties. There is ample evidence that lowering the interfacial tension between outer cell walls and the watery medium so alters permeability as to increase the rate of nutrients entering or leaving the cell. Surfactant ions might enter more quickly in the same manner. Of the nonionic surfactants tested by Ukeles, the more hydrophobic agents were more inhibitory to algal growth. This tends to support the permeability change hypothesis.

Ukeles also discussed the cell wall structure as an important influence on surfactant toxicity to algae. Since surface-active agents accumulate at interfaces, the outer cell wall must be important in the response of of the organism to surfactants. Thus, organisms with similar cell wall characteristics respond similarly to specific surfactants. The thickness of the wall may help protect against cell destruction. Ukeles found that organisms with thick cell walls were more resistant to surfactants than

those with thinner walls. But the cell wall thickness is not the only important criterion in predicting tolerance. The chemical composition of the wall could be very important. The absence of cellulose could cause a toxic response even if the wall is thick. Also, high lipid and protein contents in the wall and membrane could cause inhibition even in the presence of thick walls. Lipids would allow penetration of hydrophobic surfactants, and proteins would contribute to the destruction of the cell wall. Protein molecules unfold at water-oil interfaces with consequent destruction of structural and metabolically active protein elements.

Since algal cells are microscopic, it is often difficult or impossible to determine if toxicants cause morphological changes. With fish, this is not as great a problem since detrimental morphological responses are usually observable. Schmid and Mann (1961) observed the action of the surfactant dodecyl benzene sulfonate (DBS) on the gills of trout. At 5 mg/l DBS the epithelium was reduced, there was a loss of mucous cells on the top of the gill lamina, and the respiratory folds of the lamina were attached. At 20 mg/l DBS (after a 1-hr exposure) the epithelium was so destroyed that the position of the respiratory folds was marked only by the presence of resistant pillar cells. Multiple haematomas marked the destruction. Oxygen uptake by the cells was impeded, and this ultimately caused death by suffocation. Possibly the types of chemical reactions that occured in fish gill cells in response to surfactants are similar to those that occur in algal cells. In any case, more studies need to be done on the mechanisms of surfactant toxicity to aquatic organisms, especially algae.

Literature Review of Heavy Metal Toxicity

Whitton (1970a) discussed the toxicity of heavy metals to freshwater algae in a review paper. He pointed out many anomalies that exist in the literature concerning this topic. Davis et al. (1958) (as cited by Williams and Mount 1965) reported that some metals were highly concentrated by algae and consequently were readily transferred upward through the food chain. Jones (1958) also stressed the persistence of heavy metal pollution and pointed out that while organic pollutants may be removed by oxidation (which facilitates recovery), heavy metals are not removed but only diluted. The importance of algae in the aquatic food chain was previously emphasized. Heavy metals represent another group of compounds which can have severe effects on the ability of lower trophic level organisms to process energy. Erickson (1972) pointed out the need for environmental studies of synergistic and antagonistic effects of mixed metals along with studies of the effects of nonmetallic pollutants on the toxicity of metals.

Passow et al. (1961) (as cited by Antonovics 1971) defined heavy metals as those metals which have a density greater than 5 g/cc. This encompasses about 38 elements. Hutchinson (1973) listed the following sources of metal pollutants in water: 1) domestic and municipal sewage, 2) industrial wastes, 3) organic wastes from food processing, 4) mineral wastes from metal mining, 5) metal smelting and processing, 6) ore extraction, 7) lumber processing, 8) soil erosion, 9) use of agricultural pesticides, and 10) use of algicides in water.

The presence of many heavy metals in trace amounts is desirable since all microbial organisms use a variety of them as micronutrients. Foree

and Tapp (1970) described micronutrients as metal constituents of enzymes which enter into biological reactions. Saunders (1957) stated that trace metals are necessary as cofactors of enzyme systems and components of biologically significant metallo-organic compounds. Eyster (1964) found that in studying Chlorella, micronutrients were needed for both autotrophic and heterotrophic growth as were macronutrients. But whereas the macronutrient quantities required for both were about equal, the micronutrient quantities required were much greater for autotrophic growth. This indicated the importance of micronutrients in the photosynthetic process. Important as micronutrients are, their presence in excess causes various degrees of morphological damage or metabolic inhibition of aquatic organisms.

Zinc was arbitrarily selected for this study primarily because it is a metal that has frequently caused problems in aquatic environments, and its effect on aquatic organisms other than algae (such as fish) has been well documented. Little (1973) described zinc as a very soluble metal, and in his studies of heavy metal contamination on leaf surfaces, zinc was found incorporated appreciably into leaf structure. Vallee (1959), in discussing the roles of zinc in metabolism, stated that zinc is essential to the mechanism of action of the enzyme carbonic anhydrase. This enzyme catalizes the dehydration of carbonic acid and participates in the elimination and incorporation of CO_2. Carbonic anhydrase activity is observed in both plants and animals. Vallee pointed out that zinc has been found in many enzymes, but progress has been slow in determining the occurrence and function of the metal. The primary reason for this is the difficulty involved in analyzing small concentrations of zinc in tissues and fluids.

Several researchers have used algal assays to study heavy metal toxicity. Studies by Whitton (1970b) on zinc, copper, and lead toxicity to Chlorophytes showed that different algal species were highly variable in their responses to different metals. This is one reason that finding an algal indicator species for heavy metal pollution is difficult. Brown (1972) and Krock and Mason (1971) studied metal toxicity to algae in San Francisco Bay-Delta waters. Erickson (1972) studied the toxic effects of copper on Thalassiosira pseudonana using static algal bioassays. Maloney and Palmer (1956) studied the toxicity of several metals to a variety of algal species. Mandelli (1969) studied the inhibitory effects of copper on marine phytoplankton. He was interested primarily in the nature of copper uptake by algal cells. Stokes et al. (1973) used the algal assay static test to study metal tolerance of algae isolated from contaminated lakes near Sudbury, Ontario. They found that elevated copper concentrations affected colony formation in Scenedesmus. Bartlett et al. (1974) used static algal bioassays in testing the effects of copper, zinc, and cadmium on S. capricornutum. They were interested primarily in the algistatic and algicidal effects of the toxicants. Algistatic effects are those which halt cell growth. Algicidal effects are those which result in cell fatality. Results showed that zinc first affected algal growth at 0.03 mg/1 Zn. Growth was completely inhibited by 0.12 mg/1 Zn, and the cells died at 0.7 mg/1 Zn. Results also indicated that increasing the metal concentration caused an extension of the lag phase growth period. This indicated that the inhibitory effects of the metals occurred quickly if the concentration was high enough. The cells apparently do not have to be in the log growth phase (at their greatest metabolic rate) for the

toxicants to exert an effect. Fitzgerald and Faust (1963) studied the toxicity of copper to Chlorella. They found that copper toxicity was independent of the copper source. They also indicated that precipitated copper was just as toxic to the algae as soluble copper. Other algal species tested had varying degrees of resistance to copper. Hutchinson (1973) did static algal assays on metal toxicity using a variety of algal species and metals. He found that copper and nickle acted synergistically in culture, while selenium and cadmium acted antagonistically. In some cases, initial inhibition by metals was followed by recovery. Hutchinson concluded that the determination of water quality criteria based on uni-metal experiments is extremely difficult due to the interactions which take place. He stressed, though, that uni-metal experiments are better than nothing at all.

Application of toxicity bioassay data to the environment must be approached with care. Hutchinson and Stokes (1973) pointed out that the results of lab assays cannot be simply extrapolated to field situations, because reduction of growth in the lab is not always an adequate indication of true danger levels in nature. Wilber (1969) said that acute metal toxicity studies are of little value in practical evaluation of stream pollution. He felt that acute assays should be used for 1) revealing sensitivity, 2) studying the comparative toxicity of metals, and 3) the evaluation of general biological responses. These uses do make acute toxicity studies valuable (to some degree) in the laboratory assessment of environmental stress conditions. However, results must not be over-extrapolated in predicting true environmental conditions. The metal toxicity studies reviewed here indicated that excessive care cannot be taken against

over-generalization. A variety of metals act in a variety of ways on a variety of organisms. Each combination of toxicants and algal species in a specific environmental setting (laboratory or field) results in a response that may be entirely different if any one of the components is altered. Since all aquatic ecosystems have a combination of physical, chemical, and biological characteristics that makes them unique, extrapolation of laboratory results to the natural environment is risky. We are justified, however, in looking at the individual components of a very complex dynamic system in attempting to discover the details of interrelationships between the various components. The success of attempts at modeling various aquatic ecological phenomena depends on the validity of this approach.

If laboratory bioassay results are to be useful in solving environmental metal toxicity problems, a workkng knowledge of the effects of various physical, chemical, and biological variables is essential. Hutchinson (1973) listed the following factors that affected the degree of metal toxicity to fish: 1) the species of fish, 2) age and physiological condition of the fish, 3) anions associated with the metal, 4) cations associated with the metal, 5) the form of the metal in water, 6) pH, 7) temperature, 8) dissolved oxygen concentration, 9) valence of the metal, 10) the volume of water within which the fish is found, and 11) the time of exposure to the metal. Lloyd (1960) listed many of these variables as important factors in the toxicity of zinc sulfate to rainbow trout. He found that survival times for trout were shorter at higher temperatures, but the threshold toxicity concentration was not affected by temperature. He also pointed out that pH is important since zinc precipitates out of solution at pH levels above 8, but insoluble zinc also was shown to be

toxic. Skidmore (1964) also discussed variables which affect metal toxicity to algae. Steeman-Nielson et al. (1969) listed the following factors other than concentration which affected the influence of copper on the rate of photosynthesis in Chlorella pyrenoidosa: 1) exposure time, 2) light intensity under which the bioassay is run, 4) changes in the metal concentration during the test, 5) algal cell concentration, and 6) chemical composition of the medium. Chelation is one of the most important factors in metal toxicity tests. Saunders (1957) stated that the chelation of trace metals may affect algae in four ways: 1) reduce the availability of the needed metal by lowering the concentration in the medium, 2) reduce the toxic concentrations of metals to sublethal levels, 3) remove a metal ion which is antagonistic to a metal toxicant thus increasing the toxicity of the metal, and 4) keep the metal in solution if it tends to precipitate out. Fitzgerald and Faust (1963) stated that chelators definitely make a difference in the results of algal toxicity tests. They found that with ethylenediaminetetraacetic acid (EDTA) as the chelating agent, copper toxicity was reduced. Whitton (1967) found the same results using 3.2 mg/1 EDTA in toxicity tests of a variety of metals on Cladophora. Morgan and Lackey (1958) reported that chelation definitely reduced metal toxicity to bacteria in BOD tests. A source of chelators that is often overlooked in toxicity studies is algal organic excretory products such as amino acids and peptides. Mandelli (1969) stated that the metabolic by-products of the algae tested reduced the toxicity of copper by chelation. It is practically impossible (and probably undesirable) to totally avoid the effects of chelation in metal toxicity tests on water samples. Almost every sample collected will

invariably contain some chelating agents. This factor must be taken into account, however, in evaluating the results of toxicity tests.

Hardness is a very important factor to consider in toxicity tests. Studies on fish (Skidmore 1964) indicated that increased hardness had an antagonistic effect on zinc toxicity. The details of how this happens are not yet known. Various hypotheses state that the hardness ions either reduce membrane permeability and consequently the rate of metal uptake, or they inhibit the coagulation of protoplasm by metals. Carter and Cameron (1973) reported that hardness reduced the toxicity of lead to the ciliated protozoan Tetrahymena pyriformis (antagonism) but intensified the toxicity of mercury (synergism). Whitton (1970a) stated that practically no work has been done on the effects of hardness on metal toxicity to algae.

Light intensity is a parameter that is usually held constant in algal bioassays, but the intensity selected may have a definite effect on the results of metal toxicity tests. Whitton (1968) found that light could act in decreasing the toxicity of metals to Anacystis nidulans. Greenfield (1942) found that zinc toxicity to Chlorella was increased as the light intensity was increased.

The time period selected for a metal toxicity test is very important because the rates of chemical reactions and biological responses are certainly not constant for a variety of toxicants and test species. The rate of diffusion of one toxicant through the cell membrane may be much less than that of another toxicant. That would often tend to make the former toxicant less toxic than the latter. Also, different species may respond quite differently to the same toxicant. One species might respond significantly to a given level of toxicant after 24 hours while

another species might take much longer to respond to the same level of toxicant (if it responds at all). Hutchinson and Stokes (1973) pointed out that the time period of an algal bioassay is important also because some organisms may be more sensitive to a toxin at an earlier age. Thus, the time of duration of a bioassay must always be specified in reporting toxicity test results.

The pH and alkalinity of the toxicity test medium also are very important parameters. Maloney and Palmer (1956) reported that copper sulfate precipitates or complexes into insoluble copper compounds under conditions of high pH and alkalinity. In their studies, this resulted in a reduced toxicity of copper to the algae tested. Other studies in which precipitated metals were as toxic as soluble metals were discussed previously.

Adaptation and resistant strain development are problems that must be considered in the experimental design of algal toxicity tests and the evaluation of results. Hutchinson and Stokes (1973), Stokes et al. (1973), and Kellner (1955) (as cited by Mandelli 1969) all reported results indicating that certain algal species could adapt themselves to higher concentrations of metal toxicants. Tests of metal toxicity to algal species isolated from waters of high metal content and species that had been maintained in the laboratory indicated that the algae from the field were much more resistant to metal toxicants than were laboratory strains of the same species. It is therefore important to know the history of algal species to be used as test organisms.

The search for details of the mechanism(s) of metal toxicity to algae has yielded about as many hypotheses as there are possibilities.

Mandelli (1969) stated that when algal cultures are exposed to heavy metals in solution at concentrations above the cellular metabolic needs, two main processes generally take place: 1) binding of the ions to the cell surface and 2) interference with metabolic functions. Rothstein (1959) said that toxic chemicals act through chemical reactions with biochemical substances in the cell, and this disturbs cellular functions. He also pointed out that the chain of events between the first chemical contact and the subsequent observed response may be so complicated as to be impossible to trace. However, a number of studies have been done that narrow the possibilities. Rothstein felt that the main problems involved in analyzing toxic effects at the molecular level in the cell were: 1) metals are generally not highly selective, and they may inhibit a variety of enzymes and 2) a metal that has the capability of acting at certain cellular sites may never reach those sites in the cells. Bartlett et al. (1974) thought that the great variability among various metals in their toxic effects to algae indicated that the toxicity of the specific metals was governed by their metabolic roles in algal species. It is apparent that generalizations concerning metal toxicity to algae are very difficult (if not impossible) to formulate. Heavy metals cannot be considered as a group in characterizing their toxic actions just as algal species cannot be considered as a group in characterizing their responses to toxicants.

Rothstein (1959) investigated the cell membrane as the site of toxic action of heavy metals. He reasoned that the outer surfaces of cells were logically the first sites affected by metal toxicants. The cell membrane protects the cytoplasmic system to some extent by allowing only certain metal ions to pass through, but once past the membrane the metals encounter

many chemically reactive sites. Many of these sites may be physiologically inert, thus the metal may accumulate in the cell without causing a noticeable physiological response. To a certain degree, the inert sites protect the active sites from the metal ions. These reactions indicate the importance of membrane permeability.

The cell wall as well as the plasmalemma also has been recognized as an important site in metal toxicity to algae. Steeman-Nielsen et al. (1969) thought that copper toxicity to Chlorella pyrenoidosa was due to the inhibition of autospore liberation through the attachment of copper to the plasmalemma and primarily to the cell wall. If cleavage of the wall was inhibited, autospores could not be released, and the accumulation of their metabolic by-products depressed the photosynthetic rate. Whitton (1967) noticed that both zinc and copper caused a change in the cell wall structure of Cladophora. The affected wall appeared as two layers with frequent spaces between the layers. Sometimes the outer layer developed protruding bulges.

Lisk (1972) said that heavy metal ion uptake may be by an active metabolic process or by simple diffusion and that most algae absorb by diffusion. The toxicity of various metals is very dependent on the rate at which this diffusion occurs. Mandelli (1969) found that the concentration of copper which caused toxicity was inversely proportional to the rate of copper uptake by the algal cells. Knauss and Porter (1954), in testing Chlorella, found that the absorption rates of almost all elements tested were directly proportional to the concentration of the elements in the nutrient solution. Hutchinson and Stokes (1973) emphasized the importance of membrane permeability and metal diffusion rates in stating that metals

which are not readily taken up by cells are not as toxic and that the higher the initial metal concentration, the greater the amount of metal taken up.

The most frequently encountered hypothesis concerning the mechanism of metal toxicity to algae deals with the reaction of the metal toxicant with proteins and enzymes. Morgan and Lackey (1958) discussed this subject in detail stating that heavy metals are known to coagulate and precipitate proteins (many of which are denatured by this action). Rothstein (1959) listed two types of heavy metal reactions with proteins: 1) reactions with carboxyl, imidazole, and sulfhydryl groups and 2) reactions associated with special arrangements of amino acid residues. Albert (1950) stated that ions of heavy metals form very stable complexes with amino acids, proteins, and porphyrins. Morgan and Lackey's results indicated that copper and chromium were toxic to bacteria by neutralizing the SH group on proteins. This inhibition was reversible for proteins at low metal concentrations, but enzymes were usually permanently denatured. The peptide bonds of proteins also may act as reactive sites for heavy metals. Lisk (1972) said that enzyme poisoning was the probable mode of toxic action of heavy metals. Electronegative metals have a strong affinity for the reactive groups previously mentioned.

Whitton (1968) stated that if toxic metals acted only on the cell walls of algal cells, then the toxic responses should be equal in the light and the dark. But, in fact, many studies have indicated that various metals severely affect the photosynthetic reactions in algal cells rather than the respiratory reactions. Steeman-Nielsen (1969) found copper to inhibit both the light and the dark reactions of photosynthesis in Chlorella.

Greenfield (1942) found that copper inhibited both reactions (but primarily the dark reaction), while zinc inhibited only the dark reaction in Chlorella vulgaris. Greenfield stated that the inhibition of the dark stage of the photosynthetic process may be caused by interferences with enzyme systems. Since salts of heavy metals combine with proteins, retardation of the dark reaction, the light reaction, or both may have been caused through combinations with the enzymes or other proteins involved. The light reactions in eucaryotes occur within the chloroplast lamellae (thylakoids) (Keeton 1972), and each electron transfer is catalyzed by an enzyme. The dark reactions in eucaryotes occur in the chloroplast stroma (Conn and Stumpf 1967). The reduction of CO_2 to carbohydrate is a multistep process with an enzyme catalyzing each step. The process is known as the Calvin cycle. Metal toxicants such as zinc which have been shown to inhibit the Calvin cycle in certain algae probably act by blocking one or more of the enzymes which catalyze the steps of the process.

Hassall (1963) found that zinc would inhibit respiration in Chlorella vulgaris depending on the agitation of the culture. Shaken cultures were slightly stimulated. Greenfield (1942) reported that all algae tested with various compounds yielded similar respiratory rates. Although it appeared that respiration was not affected by metal toxicity, the respiratory rates were quite lower than the photosynthetic rates and, consequently, were less precise. If the substances tested were affecting respiration, the effect was not significant enough to show up in the low respiration readings. Also, the common practice of estimating gross photosynthesis by adding net photosynthesis to respiration is not entirely valid in light and dark studies. Black (1973) found that light respiration

in plants is quite different in rate and carbon pathway from dark respiration. Respiration in the light (photorespiration) is not mitochondrial respiration and is much less efficient than dark respiration (Lehninger 1970). This certainly must be considered in metal toxicity tests on algae where photosynthetic rates are measured as critical parameters.

The distinction between algistatic and algicidal effects of toxicants was drawn previously. The studies of Bartlett et al. (1974) indicated that the algicidal concentrations of several metals were many times greater than the algistatic concentrations. This suggested that algal cells could reach a point where enough enzymes or proteins were complexed by metals to cause a halt in cell growth but not cell death. The inhibitory reactions, in most cases, were reversible by resuspending the cells in fresh medium with no toxicant present. Thus, the inhibitory effect of an algistatic concentration was, to some degree, a function of the medium toxicant concentration. Only when the toxicant concentrations were greatly increased above the algistatic levels did the cells actually die. The actual mechanism of death was probably the irreversible deactivation, denaturation, coagulation, or precipitation of enzymes and proteins in the particular metabolic pathway affected. If algae in natural aquatic environments respond in a similar manner, the reduction of metal levels in these ecosystems may reduce algicidal concentrations to algistatic or, preferably, less inhibitory levels.

Literature Review of Temperature Effects

Temperature was listed previously as one of the factors which affected metal toxicity to aquatic organisms. It is an extremely important factor

and has been studied extensively in various types of experiments. Reynolds et al. (1973) stressed that the combination of thermal and toxic waste discharges is a current problem, and electrical power generating facilities have been singled out as the major sources of future thermal pollution. It is necessary to develop relationships which will evaluate and predict the effects of increased temperatures on the toxicity of various wastes. Warren (1971) said that thermal pollution exists when man's activities lead to changes in temperature that decrease the distribution and abundance of aquatic organisms. The combined effects of toxins and thermal pollution may be very complex (Wilber 1969).

Mackenthun (1969) stated that temperature is a prime regulator of natural processes in the aquatic environment which governs physiological functions in organisms and affects aquatic life with each change. Temperature affects chemical reaction rates, enzymatic functions, molecular movements, and molecular exchanges between membranes. Since temperature regulates molecular movement, it largely determines the rate of metabolism and activity of all organisms. Because of this capacity to determine metabolic rate, temperature may be the most important single environmental entity to life and life processes.

The Q_{10} expression has frequently been used to characterize the effects of temperature on aquatic organisms. If the metabolic rate doubles with a 10^{o} C rise in temperature, the Q_{10} is 2. The Q_{10} values for most aquatic organisms are around 2. However, Wilber (1969) criticized the use of Q_{10} values saying that there is no theoretical basis to support the Q_{10} rule (that metabolic rate doubles with a 10^{o} C rise in temperature). The action of temperature in increasing the metabolic rates of aquatic

organisms is well documented. In studies on fish, Black et al. (1973) found that temperature controlled the metabolic rate as well as physical and chemical properties of the system such as equilibrium, adsorption, and kinetics of chemical reactions. Rodgers and Dillon (1974) found that phytoplankton in a power plant thermal plume had significantly elevated C^{14} uptake rates. Fogg (1965) stated that the temperature at which an organism is most abundant in nature does not necessarily correspond to its optimum temperature as determined in the laboratory. This is important to remember in extrapolating laboratory results to the natural environment.

Several researchers have investigated the effects of temperature on heavy metal toxicity to fish. Cairns and Scheier (1957) found no differences in the survival times of bluegill sunfish exposed to zinc concentrations at 18 and 30^{o} C. Lloyd (1960) found reduced survival times of trout exposed to zinc at warmer temperatures. Wilber (1969) reported that many fish show less tolerance to toxicants as the temperature rises.

Very little work has been done to investigate the effects of temperature on the toxicity of metals to algae. Mandelli (1969) reported that higher levels of copper were taken up into algal biomass at elevated temperatures. However, algae have the capacity to take up metals without exhibiting toxic effects (as was previously discussed). Brock (1967a) mentioned three categories in which algae may fall based on their temperature preferences: thermophiles, mesophiles, and psychrophiles. The temperature limits of the mesophilic range (10-40^{o} C) generally encompass the temperatures found in the aquatic environment. Borchardt and Azad (1968) stated that the temperature range most practical for studying the effects of temperature on algae is 15-30^{o} C. Blue-green algae generally have higher optimum

temperatures than do the greens (Brock 1967b), but these temperatures are usually in the mesophilic range. Temperature increases are not tolerated by algae over a wide range. Brown (1972b) found that after algae reached the temperature giving maximum growth, slight temperature increases were very inhibitory. The temperature at which the algae are acclimated has significant effects on how the algae respond in laboratory assays.

The toxicity of various compounds to algae has only recently become an intensively investigated field. The effects of various parameters in algal toxicity studies has been even less studied. The effects of temperature on metal toxicity to algae is one of the most deficient areas. The present magnitude of metal and thermal pollution problems coupled with the importance of primary producers certainly calls for more concentrated research efforts in this area.

MATERIALS AND METHODS

Surfactant Toxicity Studies

Studies were undertaken to evaluate the effects of biodegradation on the toxicity of surfactants to algae. The Soap and Detergent Association (SDA) provided three surfactants for study: two anionic mixtures and one nonionic surfactant. The anionic surfactants tested were linear tridecyl benzene sulfonate (LTBS) and linear dodecyl benzene sulfonate (LDBS). These surfactant mixtures were 43.5% and 41.6% MBAS, respectively. The nonionic surfactant was a primary alcohol ethoxylate that was not a mixture but 100% active compound. Chemical descriptions of the surfactants were provided by the SDA and are presented in Table I. The surfactants were separately biodegraded in bench-scale activated sludge units which were operated and described by Dolan (1974). Effluents were produced for both 4- and 12-hr residence times. The effluents from both control (no surfactant added) and experimental units were tested regularly to determine levels of orthophosphate, total phosphate, nitrate nitrogen, ammonia nitrogen, pH, dissolved oxygen (DO), temperature, total alkalinity, total hardness, MBAS, chemical oxygen demand (COD), and suspended solids. Standard methods were used for these determinations (American Public Health Association 1971). Nonionic surfactant levels were analyzed by the cobaltothiocyanate method of Greff et al. (1965).

The experimental procedures used for static and continuous-flow algal bioassays were essentially those outlined by the Environmental Protection Agency (EPA) (Joint Industry/Government Task Force on Eutrophication 1969, Toerien et al. 1971, Environmental Protection Agency 1971).

Table I

Analyses of Surfactants as Provided by the Soap and Detergent Association

Mixture Component	% of Mixture	Carbon Chain Distribution in Active Ingredient		Phenyl Isomer Distribution in Active Ingredient	
		Chain Length	% of Total	Phenyl Position	% of Total
		Linear Tridecyl Benzene Sulfonate			
solids	49.1	C_{10}	1.2	2 phenyl	15.6
free oil	0.4	C_{11}	4.0	3 phenyl	17.0
pH	11.8	C_{12}	16.5	4 phenyl	16.5
alcohol insoluble	3.6	C_{13}	48.0	5 phenyl	19.8
active ingredient	45.3	C_{14}	30.2	6 & 7 phenyl	31.0
		Linear Dodecyl Benzene Sulfonate			
solids	44.7	C_{10}	13.2	2 phenyl	19.6
free oil	0.8	C_{11}	32.7	3 phenyl	19.2
pH	8.8	C_{12}	37.9	4 phenyl	18.5
alcohol insoluble	2.2	C_{13}	13.2	5 phenyl	25.9
active ingredient	41.6	C_{14}	3.0	6 & 7 phenyl	16.8

Table I, Continued

Nonionic Surfactant

A blend of primary alcohol ethoxylates, 100% active ingredient, carbon chain lengths C_{12}-C_{15}, 60% ethoxylation by weight.

The test organism, Selenastrum capricornutum Printz, was secured from available stocks at the Pacific Northwest Water Laboratory, EPA, Corvallis, Oregon. Cultures of the test organism were maintained in New Algal Assay Medium (NAAM) (Environmental Protection Agency 1971) and were transferred to fresh medium weekly. The cultures were maintained under continuous lighting conditions of 400 foot-candles (ft-c) (4300 lux) cool-white flourescent illumination (20 watt) at 24 $\pm$ 1° C.

The algal inoculum for static and continuous-flow bioassays was prepared using cell counts so that the initial cell concentration of assays could be precisely controlled. Inoculum was prepared by centrifuging seven-day old cells out of suspension at 2100 rpm for 20 min. The cells were then resuspended in fresh NAAM. Cell counts were made with a Sedgewick-Rafter counting cell and Whipple disk at a magnification of 210 x. Five Whipple fields were counted.

Algal growth levels were measured with a double-beam spectrophotometer at 600 nm. The wavelength of maximum absorbance for chlorophyll (660 nm) was not used since the relationship between chlorophyll content and cell numbers varies with changes in environmental and physiological factors (Johnson et al. 1970). Cell counts were not used to measure growth because the size of a cell varies with physical and chemical variations in the medium (Toerien et al. 1971). Thus, algal biomass may be quite different for a given number of cells under a variety of environmental conditions. Absorbance data can be used to quantitate algal biomass provided that the measurements are in the linear range (i.e. an increase in absorbance should represent a proportional increase in algal biomass) (Miller 1973). Absorbance measurements of algal growth on the double-beam

spectrophotometer were linear in the range of 0.05-1.0 absorbance units at 600 nm.

Static algal bioassays were carried out in 250-ml Erlenmeyer flasks containing a medium volume of 60 ml. The initial concentration of S. capricornutum cells after inoculation was 10^3 cells/ml. At least three replicate flasks were prepared for each treatment. The flasks were incubated at 24 $\pm$ 1° C on a shaker providing 100 oscillations/min (with a stroke of approximately 2.5 cm). Lighting was as described for the algal stock cultures. Growth measurements were recorded daily for each flask over a period ranging 7-10 days. This incubation period provided control cultures ample time to reach stationary phase growth.

Continuous-flow algal bioassays were conducted using 12 chemostats with individual capacities of approximately 1100 ml. The chemostats were constructed of glass tubing that had an outside diameter of 57 mm, wall width of 3 mm, and height of 70 cm. One end was sealed by attaching a watchglass with clear silicone rubber sealer. An L-shaped piece of glass tubing was constructed into the wall of each chemostat to act as an overflow port. Three wooden frames were constructed to hold the chemostats with each frame having a 4-chemostat capacity. The frames were equipped with cool-white flourescent lights (20 watt) on both sides of each chemoststst. This lighting arrangement allowed for an intensity of 400 ft-c. The chemoststs were housed in a walk-in environmental chamber maintained at 24 $\pm$ 1° C. Three peristaltic pumps were used to deliver the material to be bioassayed into the chemostats. Each pump had a 4-line capacity which allowed for a total of 12 lines (one for each chemostat). Usually, three replicate chemostats were run for each treatment. The positions of the

chemostats in the holding frames and the treatments assigned to the individual chemostats were determined for each bioassay run using a random numbers table. Samples to be bioassayed were maintained in 4-liter capacity wide-mouth jars and were pumped through small tygon tubing to the chemostats. The tops of the chemostats were fitted with No. 11 rubber stoppers with two openings. One opening contained a short length of glass tubing to act as a vent and sampling port. The other opening contained a shortened 15-ml volumetric pipette to receive the tygon tubing influent line. The end of the pipette reached to within about 5 cm of the overflow level. The sampling port was covered with a Pyrex test tube, and the undersides of the rubber stoppers were coated with silicone sealer.

Mixing in the chemostats was aided by magnetic stirrers and an air-CO_2 mixture which was bubbled in. The CO_2 content of the mixture was adjusted so that the pH would be maintained in the 7.0-8.5 range. The air-CO_2 mixture was filtered through a 0.45-micron Millipore filter in a Swinnex filter holder. The Swinnex holder was attached to tygon tubing leading to the base of the chemostat. The ends of the tubing were equipped with 20-gauge hypodermic needles which were inserted through a bead of silicone sealer between the watchglass and the chemostat base.

Sludge unit effluents to be bioassayed were usually autoclaved prior to testing. This procedure was instituted to control microbial contamination in the chemostats. However, one bioassay was run using non-sterile effluent that had been passed through a Foerst centrifuge (20,000 rpm) to remove solids. Effluents were spiked with phosphate-free NAAM nutrients to insure the presence of adequate levels for algal growth.

The selection of a hydraulic residence time (θ) is very important. The feed rate must not be so rapid as to wash the algal cells out before they can reproduce. If no toxicant is present, the limiting nutrient concentration determines how rapidly the algae reproduce. A feed solution rich in nutrients may be delivered at a relatively rapid rate because the algal reproductive rate will be equally rapid. At lower nutrient concentrations, however, the feed rate must be slower to prevent algal cell wash-out. Adjustments in the rates must be made if toxicants are present. Workable toxicant concentrations are those which reduce the algal growth rate without halting it altogether. Generally, a θ of three days was used in these studies, although one bioassay was run with a θ of four days. Fresh sample was provided every three days (enough for one θ).

Sufficient algal inoculum was added to each chemostat so that when filled to capacity, the initial cell concentration was 10^5 cells/ml. Data were recorded regularly (generally daily) in monitoring growth, flow rate, pH, and temperature. Continuous-flow bioassays should continue for at least one θ after steady state is reached. Steady state is defined as a period of at least one θ where daily growth levels do not vary more than 20% from the mean growth level over the period (Toerien et al. 1971). Continuous-flow bioassays were run for approximately three weeks. This length of time was required for the algae to become acclimated to the culture conditions and to achieve maximum growth. Including the time required for cleanup and inoculum preparation, the total time required for a continuous-flow bioassay was approximately one month.

All glassware used in both static and continuous-flow algal bioassays was prepared by scrubbing with a detergent, washing with 20% HCl, and rinsing seven times with deionized water.

Zinc Toxicity and Temperature Studies

Basically, the static algal bioassay procedures previously described were used to determine the toxicity of zinc to S. capricornutum. These studies, and those to determine temperature tolerance limits of the test organism, were conducted in a Percival environmental chamber equipped with lighting and temperature controls. The temperature tolerance studies were conducted by culturing S. capricornutum in NAAM for seven days at different temperatures. The algal standing crop levels after seven days (as measured by absorbance) were compared.

In the zinc toxicity studies, the algae were not continuously shaken in the environmental chamber, rather they were swirled manually once daily. Weiss and Helms (1971) found that manual swirling of algal cultures resulted in algal growth levels that were almost as high as continuously shaken cultures. The zinc concentrations were prepared from stock solutions of $ZnSO_4 \cdot 7H_2O$. The stock solutions were used to prepare test solutions in which NAAM acted as the dilution medium. The zinc concentrations in the test solutions were prepared so that 60 ml of the test solution plus 1 ml of the algal inoculum would result in the desired zinc concentration. The algal inoculum was prepared so that the addition of 1 ml to 60 ml of the test solution would result in a final cell concentration of 10^3 cells/ml. Algae to be used for inoculum preparation were acclimated to the temperature at which the bioassay was to be run for one week. Three replicate flasks were prepared for each zinc treatment. The cultures were incubated for approximately one week, and these procedures were followed for three different test temperatures. Growth was measured daily by absorbance at 600 nm. Lighting during these bioassays remained continuous at 400 ft-c.

The zinc standards used were checked for accuracy using a Norelco Unicam SP 90 A atomic absorption spectrophotometer.

The effects of temperature and zinc on algal photosynthetic rates as measured by oxygen production was tested also. Zinc standards were prepared and checked for accuracy in the manner previously described. The bioassays were carried out in 300-ml capacity BOD bottles with ground glass stoppers. The volumes of the stoppered bottles were measured prior to use. A much more concentrated algal inoculum was used for these studies. This was necessary because 10^3 cells/ml was not concentrated enough to yield significant levels of O_2 production over four to five hours of incubation. A series of screening tests resulted in the selection of 3×10^5 cells/ml as the desired cell concentration in the BOD bottles. The cells to be used for inoculum were acclimated to the test temperature for approximately one week prior to the test. Seven day old cells were not used for the O_2 production studies, rather four to five day old cells were used. This change was made since the maximum growth rate of the algae occurred after four to five days of incubation, thus their photosynthetic rates were greatest during this period. The inoculum was prepared so that 30 ml of inoculum would contain the proper number of cells to give a final cell concentration in the bottles of 3×10^5 cells/ml. The zinc test solutions were prepared so that filling the bottle to capacity after inoculum addition would result in the desired zinc concentrations. NAAM again acted as the dilution medium. Both light and dark bottles were prepared for each treatment group. Dark bottles were painted black and covered with aluminum foil. Two replicate BOD bottles were prepared for each set of light and dark treatments. This is in accordance with the

specified number of replicates for in situ productivity studies (American Public Health Association 1971). The bottles were incubated in the environmental chamber for variable periods (usually 4-5 hr). Dissolved oxygen (DO) levels were measured initially and at the end of the incubation period with a YSI Model 54 DO probe. The bottles were not allowed to incubate long enough to result in the production of O_2 bubbles around the stopper. The DO probe was calibrated using the azide modification of the Winkler test on deionized water that was allowed to equilibrate at the test temperature. The DO probe has an accuracy of 0.1 mg O_2/l and a precision of 0.05 mg O_2/l (American Public Health Association 1971). The pH of the solutions was checked before and after incubation. The test solutions which were added to the bottles were also allowed to equilibrate at the test temperature so that the algae would not be affected by thermal shocks.

Statistical Analyses

The statistical treatment of both the surfactant and the zinc toxicity growth data was carried out using computer programs that performed the following tests: the Kolmogorov-Smirnov test of goodness of fit, Bartlett's test of homogeneity of variances, single classification analysis of variance (ANOVA), and the Student-Newman-Keuls a posteriori multiple range test (Sokal and Rohlf 1969). The algal growth rates were calculated using computer programs of the Statistical Analysis System (SAS) (Barr and Goodnight 1972). In all statistical tests, the significance level was 0.05.

RESULTS

Surfactant Bioassays

Linear Tridecyl Benzene Sulfonate (LTBS)

Initial studies of LTBS involved tests of the intact (unbiodegraded) surfactant mixture for toxicity to S. capricornutum in NAAM. Static test procedures were used to evaluate a range of MBAS levels. The growth levels attained by the algae over the test period are presented in Table II. Figure 1 shows the corresponding growth curves. Statistical tests indicated that the maximum standing crop level of growth (after eight days) for each treatment was significantly different from all other growth levels. Thus, 5 mg MBAS/l increased the maximum standing crop over the control level, while 10, 15, and 20 mg MBAS/l successively decreased the growth levels.

The daily specific growth rates (Mu) were calculated for each replicate flask of each treatment according to the methods outlined by the Environmental Protection Agency (1971). Since absorbance readings were assumed linear only in the range of 0.05-1.0, specific growth rates were calculated only when the absorbance levels involved were in the 0.05-1.0 range. The maximum specific growth rates (Mu Max) were calculated for each treatment to determine if the various surfactant levels tested affected the rate of algal growth. The calculated Mu Max values are presented in Table III. Statistical tests indicated that the growth rates were not significantly different for the 5, 10, and 15 mg MBAS/l treatments. The control and 20 mg MBAS/l treatments were significantly higher and lower, respectively, than all other treatments.

Table II

Mean Daily Absorbance Levels (n=3) for Static Algal Bioassay of the Intact LTBS Mixture

Day	Control (NAAM)		5 mg MBAS/1		10 mg MBAS/1		15 mg MBAS/1		20 mg MBAS/1	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
2	0.003	21.7	0.003	17.3	0.003	21.7	0.002	24.7	0.002	24.7
3	0.013	24.1	0.022	11.3	0.013	7.7	0.008	7.5	0.005	20.0
4	0.060	7.6	0.106	6.8	0.055	13.3	0.028	10.8	0.012	16.9
5	0.225	9.6	0.316	4.4	0.178	3.4	0.083	8.0	0.038	13.1
6	0.559	2.5	0.643	2.5	0.506	2.1	0.332	4.2	0.147	17.4
7	0.718	0.5	0.769	1.4	0.658	0.3	0.549	1.0	0.409	7.6
8	0.793	0.3	0.831	1.1	0.735	1.2	0.704	1.4	0.595	4.2

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

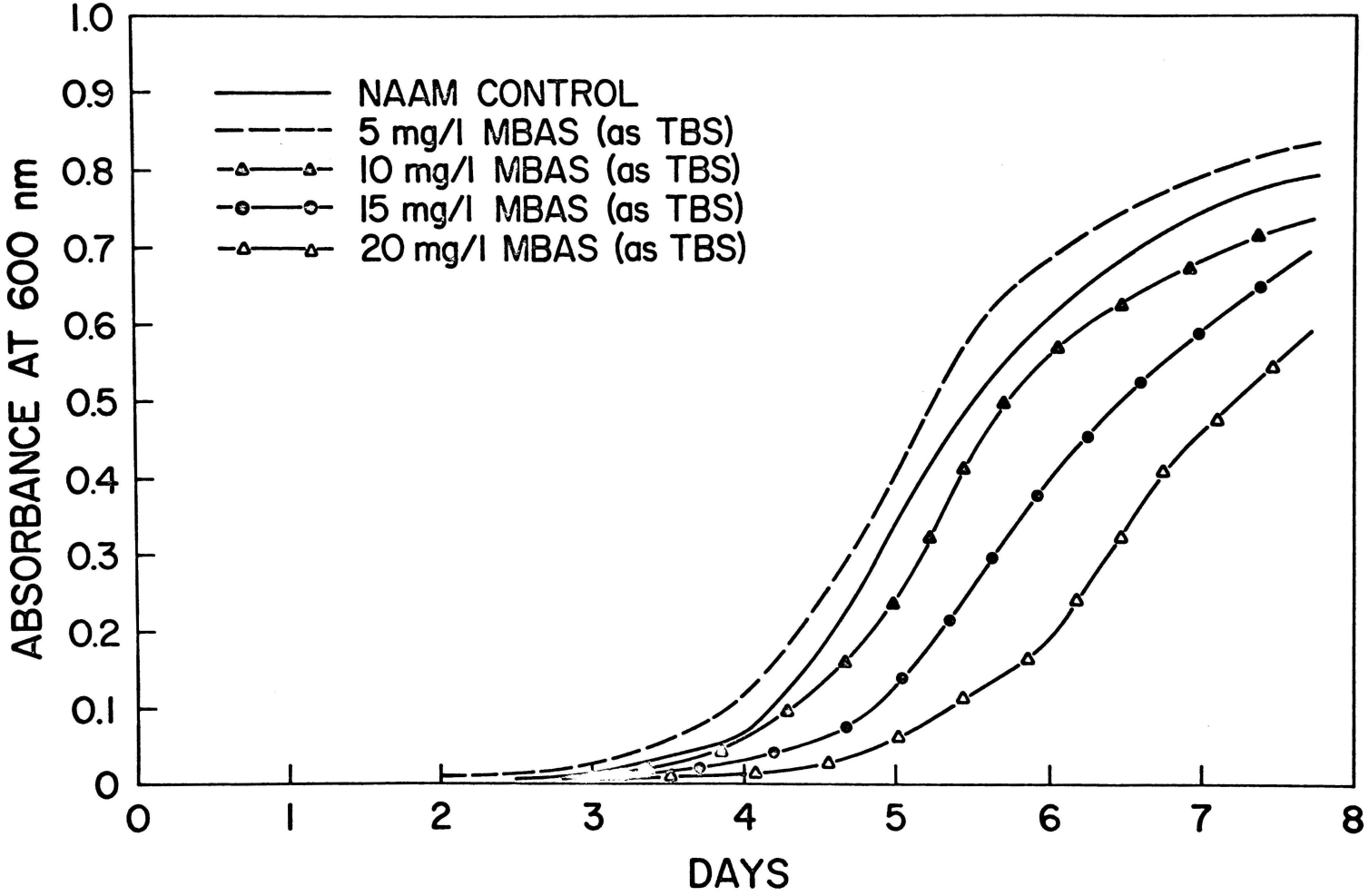

Figure 1. Algal growth curves for the static bioassay on the intact LTBS mixture.

Table III

Mean Maximum Specific Growth Rates
(n=3) for Static Algal Bioassay of
the Intact LTBS Mixture

Treatment	Mean Mu Max	C.V.
Control (NAAM)	1.67	1.7
5 mg MBAS/1	1.38	2.3
10 mg MBAS/1	1.29	15.4
15 mg MBAS/1	1.38	3.5
20 mg MBAS/1	1.04	10.2

Mu Max = Maximum specific growth rate (days $^{-1}$)

C.V. = Coefficient of variation (%)

Results of the initial continuous-flow bioassays on the intact LTBS indicated that foaming posed quite a problem. Agitation in the chemostats foamed the surfactant out; thus, fixed concentrations of MBAS could not be maintained during the test. Therefore, only static procedures were used for testing the intact surfactants.

The first continuous-flow bioassay of the sludge unit LTBS effluents was conducted for 15 days. A hydraulic residence time of approximately four days was used. Suspended solids were removed from the effluents with a Foerst centrifuge. Table IV shows the chemical parameters monitored on the sludge unit influents and effluents throughout the LTBS study. The growth levels attained by the algae in the chemostats over the test period are presented in Table V. Figure 2 shows the corresponding growth curves. Table VI presents the nitrogen and phosphorus levels in the sludge unit effluents during the 15-day bioassay period. Table VII shows the mean hydraulic residence times for the bioassay period.

A second continuous-flow bioassay on the sludge unit LTBS effluents was conducted for 19 days. The flow rate was increased so that the hydraulic residence time was reduced from four to three days. The use of CO_2 for pH control was instituted with this bioassay. All other experimental conditions were identical to those in the first bioassay. The algal growth levels attained in the chemostats are presented in Table VIII. Figure 3 shows the corresponding growth curves. Steady state levels of growth were approximated for each treatment by selecting the region of each curve where growth was most constant for at least one hydraulic residence time and calculating the mean growth level over this period. The approximated steady state levels of growth for the second continuous-flow

Table IV

Chemical Parameters Monitored on the Sludge Unit Influents and Effluents Throughout the Study of Linear Tridecyl Benzene Sulfonate (Dolan 1974)

	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
Parameter	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Influent pH	7.3	8.2	16	7.1	8.5	16	7.2	8.3	16	7.1	9.9	16
Effluent pH	6.6	7.6	16	6.6	7.6	16	6.8	7.4	16	6.8	8.8	16
Influent Total Alkalinity as $CaCO_3$ (mg/l)	45.9	34.6	16	44.8	17.6	16	42.0	7.1	16	41.6	4.8	16
Effluent Total Alkalinity as $CaCO_3$ (mg/l)	38.8	31.2	16	51.5	53.8	16	39.6	20.5	16	41.4	17.4	16
Influent Total Hardness as $CaCO_3$ (mg/l)	51.0	11.1	16	50.8	11.4	16	50.3	8.3	16	51.2	7.4	16
Effluent Total Hardness as $CaCO_3$ (mg/l)	48.0	12.1	16	50.6	15.8	16	51.2	6.2	16	52.0	10.2	16
Mixed Liquor Suspended Solids (mg/l)	2384	12.4	10	1242	15.2	9	2452	7.3	10	1717	9.3	10
Influent COD (mg/l)	132	56.8	8	141	85.1	7	178	90.4	8	113	109	9
Effluent COD (mg/l)	25.4	64.2	8	28.9	41.9	7	19.4	63.4	8	15.9	81.8	9
COD Reduction (%)	81.0	5.7	8	73.5	18.6	5	84.6	15.4	8	65.5	37.7	9

Table IV, Continued

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Influent MBAS (mg/l)	26.9	24.9	17	-	-	-	25.5	20.4	17	-	-	-
Effluent MBAS (mg/l)	0.6	50.0	18	-	-	-	0.3	33.3	18	-	-	-
MBAS Reduction (%)	97.6	1.5	17	-	-	-	98.7	0.3	17	-	-	-

C.V. = Coefficient of variation (%)

n = Number of replicates

Table V

Mean Daily Absorbance Levels (n=3) for the First Continuous-flow Algal Bioassay of the Sludge Unit LTBS Effluents

	4-hr residence				12-hr residence			
Day	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
1	0.047	10.1	0.031	3.7	0.035	7.1	0.035	5.7
2	0.056	9.9	0.045	23.2	0.075	2.0	0.074	7.9
3	0.149	13.2	0.153	18.9	0.166	11.4	0.161	7.8
4	0.247	7.4	0.287	14.3	0.383	10.1	0.319	15.6
5	0.366	3.3	0.429	10.6	0.566	7.6	0.443	17.8
6	0.469	4.1	0.553	8.9	0.637	9.5	0.540	22.0
7	0.522	6.4	0.657	7.4	0.654	11.0	0.600	23.6
8	0.543	9.0	0.756	10.0	0.615	15.6	0.624	21.1
9	0.564	11.8	0.829	13.7	0.543	18.8	0.602	17.7
10	0.557	9.9	0.831	12.9	0.504	19.5	0.564	21.8
11	0.562	5.4	0.830	12.9	0.471	21.3	0.525	24.3
12	0.509	5.5	0.750	10.9	0.416	17.5	0.451	24.4
13	0.471	7.7	0.672	9.1	0.368	18.1	0.391	23.9
14	0.447	13.5	0.609	10.2	0.321	22.2	0.336	23.4
15	0.411	18.1	0.527	5.9	0.280	25.8	0.282	21.0

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

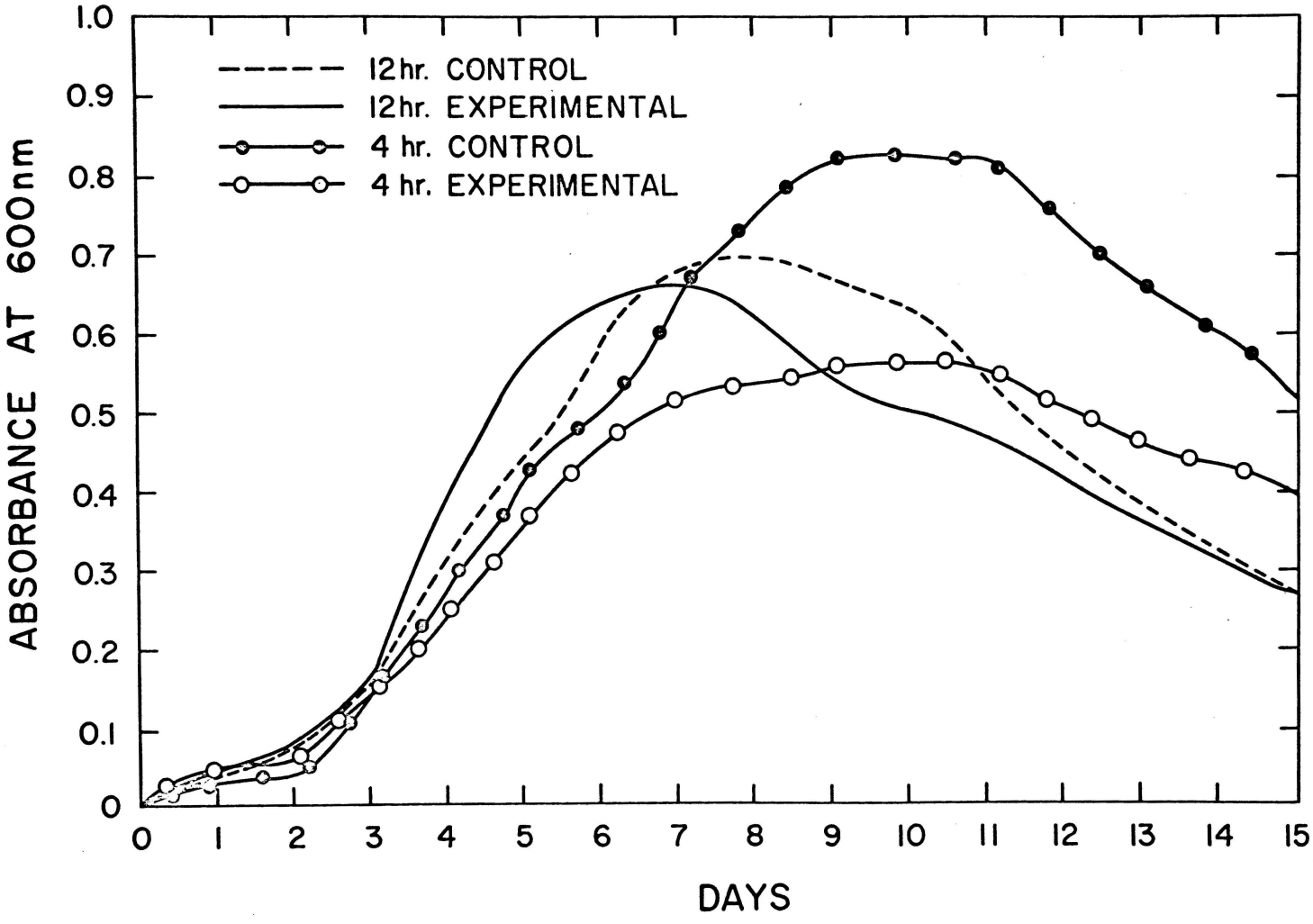

Figure 2. Algal growth curves for the first continuous-flow bioassay on the sludge unit LTBS effluents.

Table VI

Nutrient Chemical Parameters Monitored on the Sludge Unit Effluents during the First Continuous-flow Algal Bioassay of LTBS

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Ortho-PO_4 (mg P/1)	36.2	43.4	4	74.2	19.7	7	17.4	54.3	9	69.1	13.6	7
Total-PO_4 (mg P/1)	39.8	37.9	4	81.2	15.3	6	16.5	41.3	7	77.8	17.5	6
NH_3-N (mg N/1)	0.8	55.8	5	0.9	14.4	5	0.9	71.1	6	1.5	84.9	5
NO_3-N (mg N/1)	28.2	68.1	4	25.0	73.6	5	15.5	60.0	5	17.9	60.9	2

C.V. = Coefficient of variation (%)

n = Number of replicates

Table VII

Mean Hydraulic Residence Times for the Duration of the First Continuous-flow Algal Bioassay of LTBS Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean (days)	3.88	4.06	4.02	3.91
C.V.	14.4	20.0	13.7	19.7
n	42	42	41	42

C.V. = Coefficient of variation (%)

n = No. of replicates

Table VIII

Mean Daily Absorbance Levels (n=3) for the Second Continuous-flow Algal Bioassay of the Sludge Unit LTBS Effluents

	4-hr residence				12-hr residence			
Day	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
1	0.009	13.3	0.008	18.3	0.010	5.6	0.009	11.1
3	0.043	22.2	0.045	12.5	0.053	13.2	0.050	5.1
4	0.073	21.9	0.100	14.9	0.073	13.1	0.083	6.0
5	0.114	12.7	0.168	14.1	0.153	13.1	0.142	6.3
6	0.101	17.3	0.197	15.6	0.206	17.7	0.164	4.4
7	0.111*	19.1	0.186	20.1	0.245	20.5	0.180	5.0
8	0.101*	29.4	0.176	25.2	0.265	21.3	0.176	4.7
9	0.105*	29.6	0.176	31.2	0.236	27.1	0.164	7.9
11	0.132	23.9	0.232	30.6	0.192	39.8	0.154	8.4
13	0.163	10.9	0.314	24.7	0.209	54.1	0.094	15.2
16	0.167	38.7	0.410	6.3	0.218	40.3	0.157	27.9
17	0.119	57.5	0.352	7.4	0.153	40.9	0.185	9.9
18	0.091	63.6	0.304	16.1	0.123	45.4	0.151	11.9
19	0.104	49.0	0.231	13.7	0.133	34.5	0.110	14.6

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

* n=2

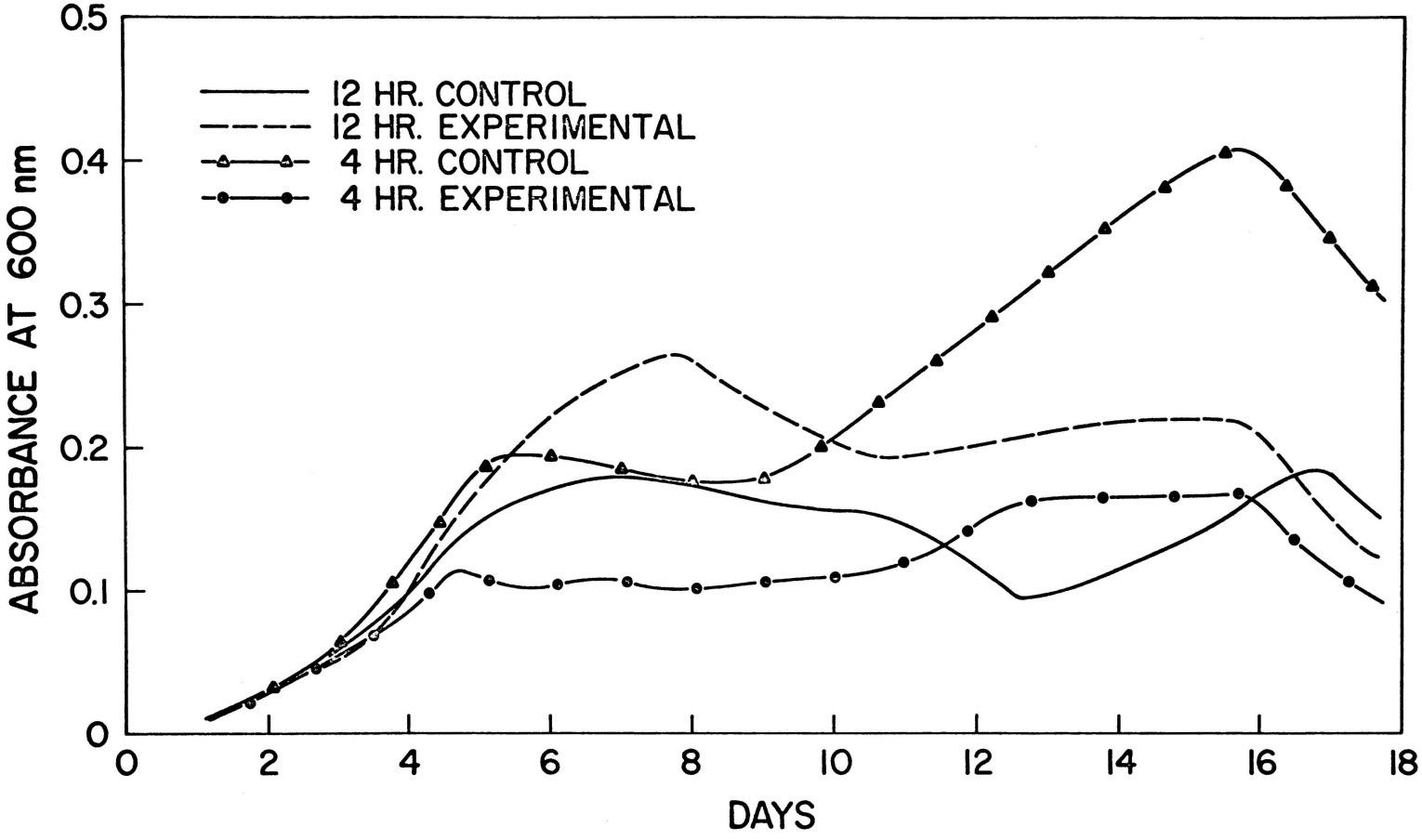

Figure 3. Algal growth curves for the second continuous-flow bioassay on the sludge unit LTBS effluents.

bioassay on the sludge unit LTBS effluents are presented in Table IX. The mean hydraulic residence times for only the approximated steady state regions of the curves are shown in Table X. The nitrogen and phosphorus levels in the sludge unit effluents during the second continuous-flow bioassay are shown in Table XI. The mean hydraulic residence times for the duration of the second continuous-flow bioassay are presented in Table XII.

The first static bioassay of the LTBS effluents was conducted for 11 days with growth readings taken only intermitantly. The daily algal growth levels attained in the flasks are presented in Table XIII. Statistical tests indicated that after 11 days, algal growth levels were significantly different among the 12-hr effluents but not among the 4-hr effluents.

A second static bioassay was conducted on LTBS effluents to verify the initial results. Growth levels were recorded daily and are shown in Table XIV. Figure 4 shows the corresponding growth curves. Statistical tests indicated that by the 11th and 12th days of the test, all algal growth levels were significantly different from each other. The daily specific growth rates were calculated for each flask over the test period. The Mu Max values corresponding to each treatment were calculated and are listed in Table XV. Statistical tests indicated that there was no significant difference in the maximum growth rates of the four treatments.

Linear Dodecyl Benzene Sulfonate (LDBS)

While the sludge units were being acclimated to the LDBS mixture, static algal bioassays were carried out on the intact LDBS to determine

Table IX

Approximations of Steady State Levels of Growth for the Second Continuous-flow Algal Bioassay of the Sludge Unit LTBS Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean Abs	0.107	0.184	0.206	0.171
C.V.	16.2	20.5	39.7	6.4
n	10	12	9	12
Time period (days)	3.0	3.0	5.1	3.0

Abs = Absorance at 600 nm

C.V. = Coefficient of variation

n = Number of replicates

Table X

Mean Hydraulic Residence Times for the Steady State Growth Periods of the Second Continuous-flow Algal Bioassay of the Sludge Unit LTBS Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean (days)	2.64	2.77	2.91	2.76
C.V.	2.6	6.7	5.3	6.6
n	5	9	9	9
Time period (days)	3.0	3.0	5.1	3.0

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XI

Nutrient Chemical Parameters Monitored on the Sludge Unit Effluents During the Second Continuous-flow Algal Bioassay of LTBS

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Ortho-PO_4 (mg P/l)	15.8	79.7	3	34.7	44.4	2	20.7	43.7	3	18.8	13.1	3
Total-PO_4 (mg P/l)	16.8	75.0	3	36.6	34.7	2	21.5	41.5	3	19.5	10.3	3
NH_3-N (mg N/l)	0.5	59.0	2	0.9	97.9	2	0.2	- -	1	0.8	68.8	2
NO_3-N (mg N/l)	6.2	- -	1	17.3	12.3	2	- -	- -	- -	15.6	31.7	2

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XII

Mean Hydraulic Residence Times for the Duration of the Second Continuous-flow Algal Bioassay of LTBS Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean (days)	2.90	2.86	2.78	2.84
C.V.	11.4	9.9	7.5	8.3
n	29	33	33	32

C.V. = Coefficient of variation (%)

n = No. of replicates

Table XIII

Mean Daily Absorbance Levels (n=3) for the
First Static Algal Bioassay of the
Sludge Unit LTBS Effluents

Day	4-hr residence				12-hr residence			
	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
1	0.002	91.7	0.003	21.7	0.002	24.7	0.004	43.3
2	0.004	35.3	0.006	27.0	0.005	28.6	0.008	33.1
4	0.050	14.1	0.056	9.1	0.066	9.7	0.054	8.7
5	0.109	8.4	0.126	9.7	0.143	10.9	0.130	18.9
11	0.650	5.4	0.758	11.6	0.812	12.2	0.591	13.7

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

Table XIV

Mean Daily Absorbance Levels (n=3) for the Second Static Algal Bioassay of the Sludge Unit LTBS Effluents

	4-hr residence				12-hr residence			
Day	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
1	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0
2	0.001	173	0.000	0.0	0.000	0.0	0.001	173
3	0.012	40.8	0.003	33.3	0.010	24.4	0.011	20.4
4	0.042	53.8	0.007	67.3	0.045	42.6	0.041	9.2
5	0.102	26.3	0.038	37.7	0.167	33.5	0.135	6.1
6	0.143	9.7	0.179	27.8	0.363	11.8	0.338	8.6
7	0.161	6.7	0.303	12.9	0.421	7.1	0.436	8.5
8	0.178	5.3	0.439	4.9	0.476	4.4	0.551	7.8
9	0.187	4.5	0.497	4.8	0.499	3.3	0.595	6.9
11	0.187	4.4	0.565	4.3	0.518	3.2	0.644	4.9
12	0.189	5.0	0.591	3.5	0.530	3.0	0.660	4.3

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

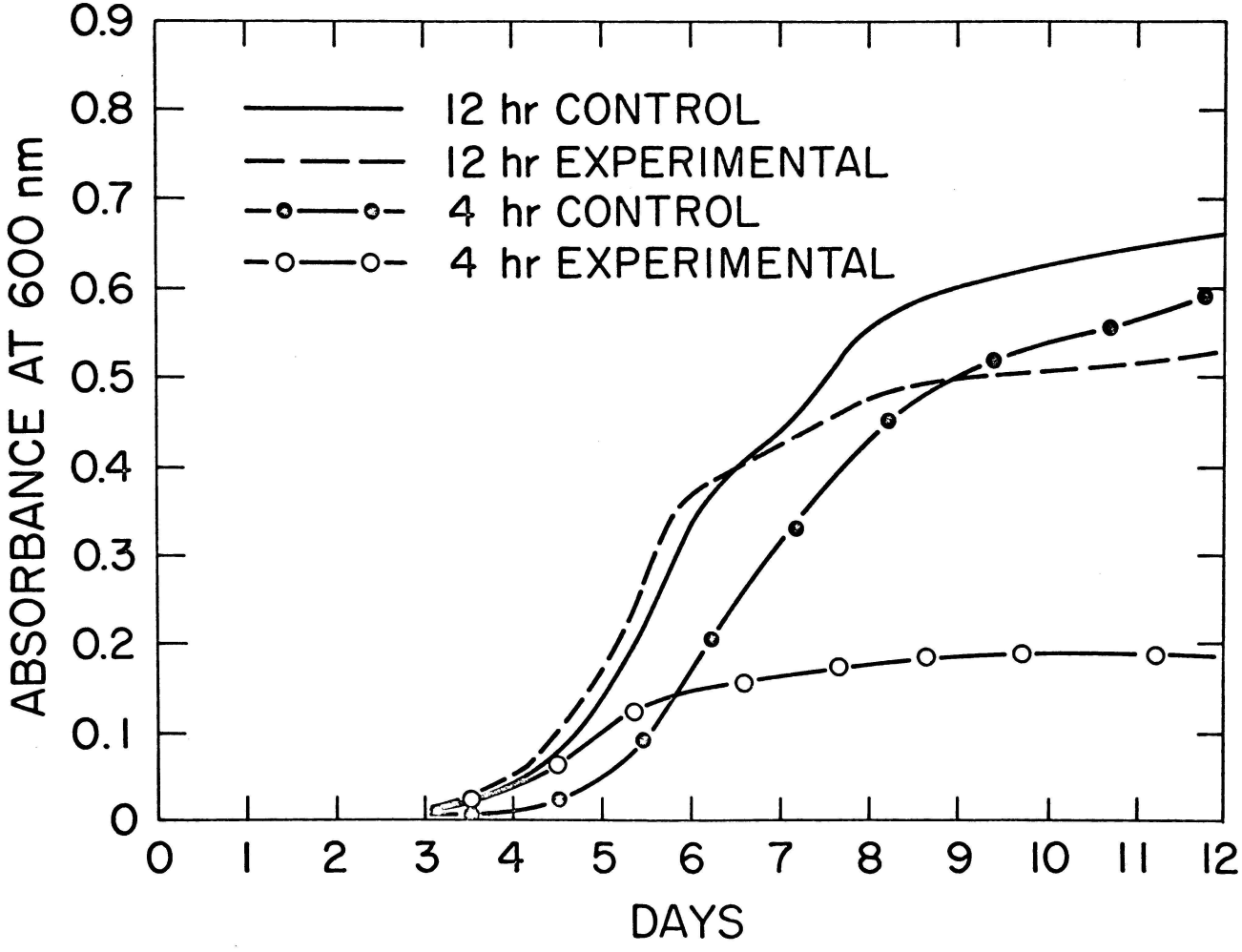

Figure 4. Algal growth curves for the second static bioassay on the sludge unit LTBS effluents.

Table XV

Mean Maximum Specific Growth Rates
(n=3) for the Second Static Algal Bioassay of
the Sludge Unit LTBS Effluents

Treatment	Mean Mu Max	C.V.
4-hr Experimental	0.70	15.4
4-hr Control	0.90	52.9
12-hr Experimental	1.03	21.3
12-hr Control	0.92	3.2

Mu Max = Maximum specific growth rate (day^{-1})

C.V. = Coefficient of variation (%)

its threshold of toxicity to S. capricornutum. The static test procedures were performed on LDBS concentrations of 0.1, 1.0, and 10.0 mg MBAS/1 with NAAM acting as the control and dilution medium. The growth levels attained by the algae over the test period are presented in Table XVI. Figure 5 shows the corresponding algal growth curves. Statistical tests on the standing crop levels during the last four days of the assay (days 6-9) indicated that the 10.0 mg MBAS/1 treatment supported significantly less growth than the other treatments. None of the other surfactant concentrations supported growth levels significantly different from control levels.

The maximum specific growth rates were calculated for each treatment of the intact LDBS and for the control. The calculated Mu Max values are presented in Table XVII. Statistical tests indicated that the growth rates were not significantly different for the 0.1 and 1.0 mg MBAS/1 treatments. The control and 10 mg/1 treatments were significantly higher and lower, respectively, than all other treatments.

Initial attempts to carry out continuous-flow algal bioassays on the sludge unit LDBS effluents were unsuccessful due to contamination of the chemostats by algae of other species. Tests of the sludge unit effluents indicated that algal contaminants were present. To correct this problem, effluents were autoclaved before feeding into the chemostats. This procedure was used previously by Scherfig and Dixon (1973) and Toerien et al. (1971). Nitrogen and phosphorus chemical tests as well as MBAS tests were run on effluents before and after autoclaving to check for changes in nutrient or surfactant levels. Since no differences in nutrient composition or MBAS levels were detected in the autoclaved effluents, this procedure was adopted for use.

Table XVI

Mean Daily Absorbance Levels (n=3)
for the Static Algal Bioassay of
the Intact LDBS Mixture

Day	Control (NAAM)		0.1 mg MBAS/1		1.0 mg MBAS/1		10 mg MBAS/1	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
1	0.010	14.8	0.010	29.9	0.011	23.6	0.009	11.1
2	0.070	23.2	0.069	27.5	0.065	11.7	0.040	12.2
3	0.256	8.0	0.227	14.6	0.215	14.0	0.149	18.2
4	0.471	3.0	0.423	7.3	0.411	11.9	0.346	11.3
5	0.553	3.5	0.519	6.0	0.511	9.0	0.444	8.5
6	0.647	2.3	0.620	4.2	0.605	8.1	0.524	6.3
7	0.675	2.3	0.658	3.0	0.642	6.6	0.573	4.5
8	0.695	2.1	0.680	2.2	0.666	6.1	0.598	3.9
9	0.703	1.8	0.693	1.8	0.676	5.9	0.616	3.0

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

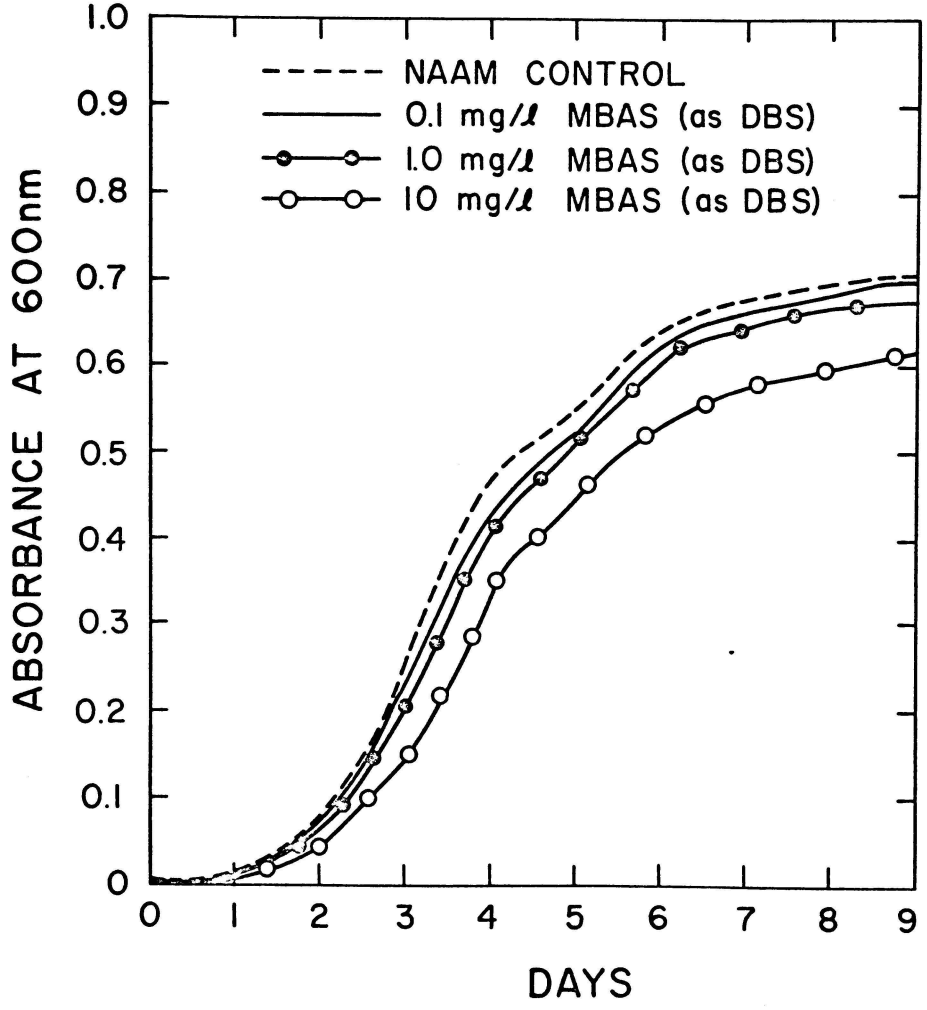

Figure 5. Algal growth curves for the static bioassay on the intact LDBS mixture.

Table XVII

Mean Maximum Specific Growth Rates
(n=3) for the Static Algal Bioassay of
the Intact LDBS Mixture

Treatment	Mean Mu Max	C.V.
Control (NAAM)	1.26	12.9
0.1 mg MBAS/l	0.94	26.3
1.0 mg MBAS/l	1.14	12.3
10.0 mg MBAS/l	0.78	8.2

Mu Max = Maximum specific growth rate (day^{-1})

C.V. = Coefficient of variation (%)

A subsequent continuous-flow bioassay on the sludge unit LDBS effluents was run for 19 days. All experimental conditions were identical to those used in the second LTBS continuous-flow assay with the exception of autoclaving the effluents before testing. Table XVIII shows the chemical parameters monitored on the sludge unit effluents throughout the LDBS study. The algal growth levels attained in the chemostats are presented in Table XIX. Figure 6 shows the corresponding algal growth curves. Steady state levels of growth were approximated for each treatment in the manner described previously. The approximated steady state levels of algal growth for the LDBS effluent continuous-flow assay are presented in Table XX. The mean hydraulic residence times for the approximated steady state regions of the curves are shown in Table XXI. The nitrogen and phosphorus levels in the sludge unit effluents during the 19-day bioassay are shown in Table XXII. The mean hydraulic residence times for the duration of the 19-day assay are given in Table XXIII.

A static bioassay on the LDBS effluents was run for nine days. The daily algal standing crop levels attained in the flasks are presented in Table XXIV. Figure 7 shows the corresponding algal growth curves. Statistical tests indicated that by the last two days of the test (days 8-9) the 12-hr control effluent supported significantly higher standing crop levels than all other effluents. There was no significant difference in growth levels among the other treatments. The daily specific growth rates were calculated for each flask over the test period. The Mu Max values corresponding to each treatment were calculated and are listed in Table XXV. Statistical tests indicated that there was no significant difference in maximum specific growth rates among the treatment groups.

Table XVIII

Chemical Parameters Monitored on the Sludge Unit Influents and Effluents Throughout the Study of Linear Dodecyl Benzene Sulfonate (Dolan 1974)

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Influent pH	7.3	4.1	21	7.3	4.1	21	7.2	4.2	21	7.1	7.0	21
Effluent pH	7.0	2.9	21	7.3	2.7	21	7.0	2.9	21	7.1	2.8	21
Influent Total Alkalinity as $CaCO_3$ (mg/l)	57.3	23.7	25	55.0	21.5	25	55.6	21.2	25	55.3	22.1	24
Effluent Total Alkalinity as $CaCO_3$ (mg/l)	48.3	17.8	24	55.0	26.2	24	47.3	24.7	24	50.0	17.2	24
Influent Total Hardness as $CaCO_3$ (mg/l)	55.7	11.7	25	52.3	18.2	25	55.0	9.5	25	54.9	10.2	25
Effluent Total Hardness as $CaCO_3$ (mg/l)	54.2	10.9	24	56.3	8.3	23	55.5	10.5	24	55.5	9.2	24
Mixed Liquor Suspended Solids (mg/l)	1769	24.0	13	1254	62.4	11	2341	18.6	13	1737	25.3	12
Influent COD (mg/l)	111	51.4	22	54	70.4	22	120	41.7	22	80	37.5	23
Effluent COD (mg/l)	24·7	61.1	22	28.4	55.6	24	23.1	44.6	25	23.4	43.6	23
COD reduction (%)	76.2	17.3	19	49.8	46.6	18	78.5	16.3	20	70.3	27.0	19

Table XVIII, Continued

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Influent MBAS (mg/l)	23.8	29.4	25	- -	- -	- -	22.8	29.4	25	- -	- -	- -
Effluent MBAS (mg/l)	0.5	40.0	25	- -	- -	- -	0.3	33.3	25	- -	- -	- -
MBAS reduction (%)	97.6	1.1	25	- -	- -	- -	98.8	0.3	25	- -	- -	- -

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XIX

Mean Daily Absorbance Levels (n=3)
for the Continuous-flow Algal Bioassay
of the Sludge Unit LDBS Effluents

Day	4-hr residence				12-hr residence			
	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs*	C.V.	Abs	C.V.
2	0.026	2.2	0.039	12.8	0.024	14.4	0.030	20.3
3	0.053	9.6	0.089	38.4	0.072	37.3	0.083	8.2
4	0.071	9.2	0.201	16.5	0.066	5.3	0.134	7.3
5	0.093	7.9	0.296	22.0	0.086	4.9	0.199	8.6
6	0.093	11.4	0.404	21.0	0.074	7.6	0.233	12.0
7	0.098	10.8	0.475	21.0	0.076	4.6	0.287	12.4
8	0.102	10.6	0.530	25.1	0.078	7.3	0.295	13.0
9	0.120	10.4	0.599	24.8	0.070	32.3	0.325	12.8
10	0.132	19.5	0.597	26.3	0.098	25.1	0.334	8.8
11	0.145	17.0	0.613	24.2	0.110	21.3	0.338	6.8
12	0.170	6.0	0.634	19.0	0.111	19.1	0.330	6.3
14	0.218	6.0	0.663	12.7	0.136	2.6	0.309	5.7
15	0.276	5.5	0.684	11.4	0.174	2.4	0.298	6.0
16	0.280	4.6	0.709	8.3	0.222	3.5	0.303	5.9

Table XIX, Continued

Day	4-hr residence				12-hr residence			
	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs*	C.V.	Abs	C.V.
17	0.254	3.8	0.725	7.0	0.257	2.2	0.327	6.3
18	0.232	2.1	0.752	8.6	0.299	0.5	0.381	9.2
19	0.224	8.7	0.718	7.9	0.348	0.6	0.429	8.5

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

* n=2

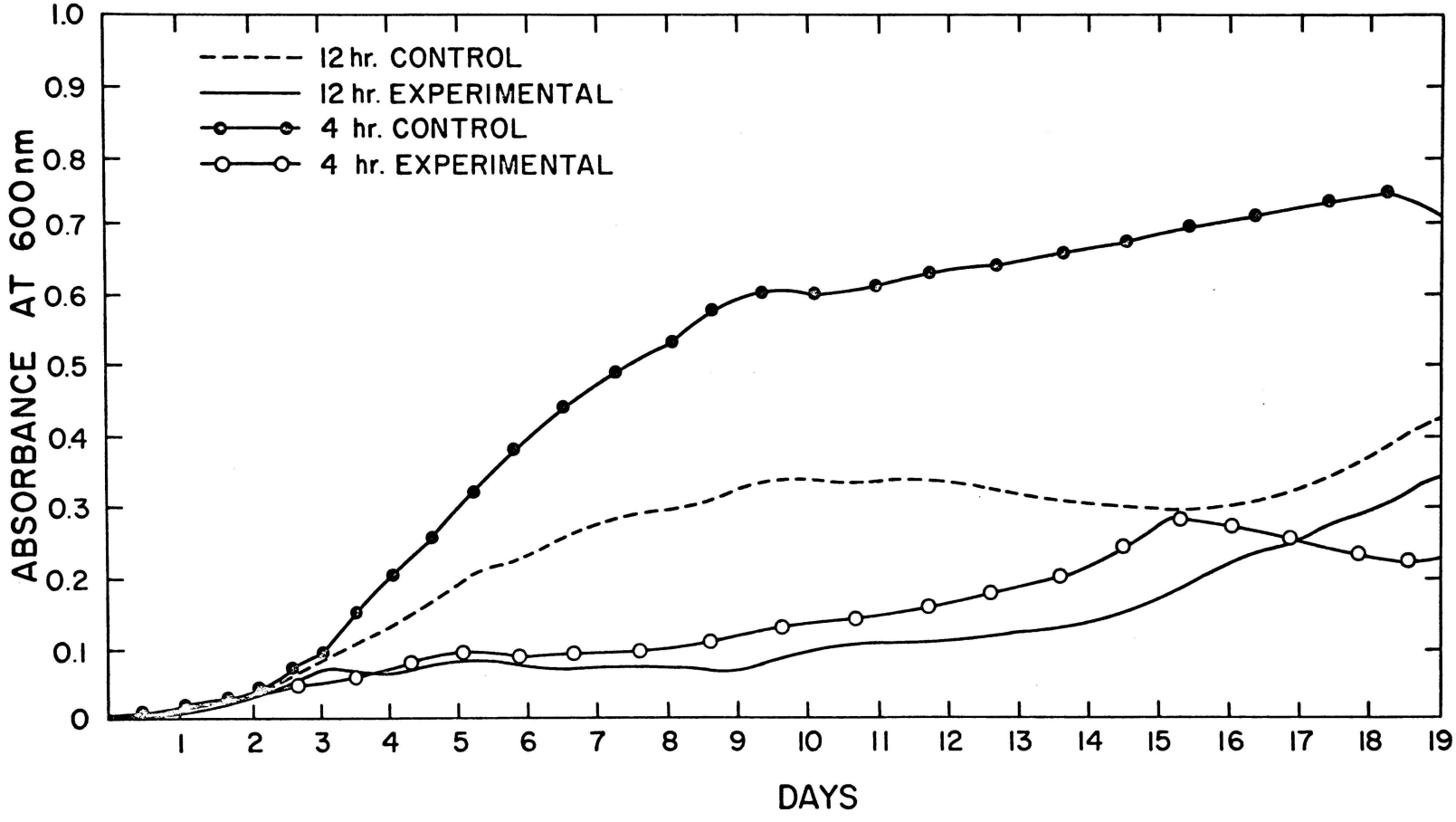

Figure 6. Algal growth curves for the continuous-flow bioassay on the sludge unit LDBS effluents.

Table XX

Approximations of Steady State
Levels of Growth for the
Continuous-flow Algal Bioassay
of the Sludge Unit LDBS Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean Abs	0.101	0.611	0.077	0.332
C.V.	13.4	20.3	12.9	7.8
n	15	12	10	12
Time period (days)	4.0	3.2	4.0	3.2

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XXI

Mean Hydraulic Residence Times for Steady State Growth Periods of the Continuous-flow Algal Bioassay of the Sludge Unit LDBS Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean (days)	3.09	3.12	3.45	3.07
C.V.	8.2	6.9	8.7	6.7
n	15	12	10	12
Time period (days)	4.0	3.2	4.0	3.2

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XXII

Nutrient Chemical Parameters Monitored on the Sludge Unit Effluents During the Continuous-flow Algal Bioassay of LDBS

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Ortho-PO_4 (mg P/l)	5.7	9.4	2	5.8	46.3	3	11.3	- -	1	8.8	10.0	2
Total-PO_4 (mg P/l)	6.9	25.4	2	6.4	50.6	3	8.5	- -	1	10.8	25.3	2
NH_3-N (mg N/l)	0.3	100	2	0.5	24.6	2	0.6	14.8	2	0.5	3.5	2
NO_3-N (mg N/l)	46.6	107	3	24.4	22.6	3	23.1	- -	1	38.5	37.1	3

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XXIII

Mean Hydraulic Residence Times for the
Duration of the Continuous-flow
Algal Bioassay of LDBS Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean (days)	3.12	3.14	3.41	3.09
C.V.	10.5	10.5	10.9	9.4
n	44	45	30	45

C.V. = Coefficient of variation (%)

n = No. of replicates

Table XXIV

Mean Daily Absorbance Levels (n=3) for the
Static Algal Bioassay of the Sludge Unit LDBS Effluents

Day	4-hr residence				12-hr residence			
	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
1	0.014	4.2	0.012	8.3	0.011	18.4	0.014	25.8
2	0.067	32.3	0.074	3.1	0.076	19.7	0.089	16.0
3	0.284	28.1	0.311	9.7	0.313	14.5	0.342	22.6
4	0.487	18.5	0.561	7.9	0.528	22.0	0.562	35.2
5	0.572	22.9	0.705	9.6	0.633	26.1	0.724	44.1
6	0.717	13.8	0.843	4.4	0.796	15.0	0.927	22.9
7	0.784	10.7	0.910	4.5	0.873	11.1	1.07	13.3
8	0.833	7.4	0.950	3.2	0.921	7.7	1.15	8.6
9	0.865	4.9	0.970	2.0	0.951	5.6	1.19	6.2

Abs = Absorbance at 600 nm

C.V.= Coefficient of variation (%)

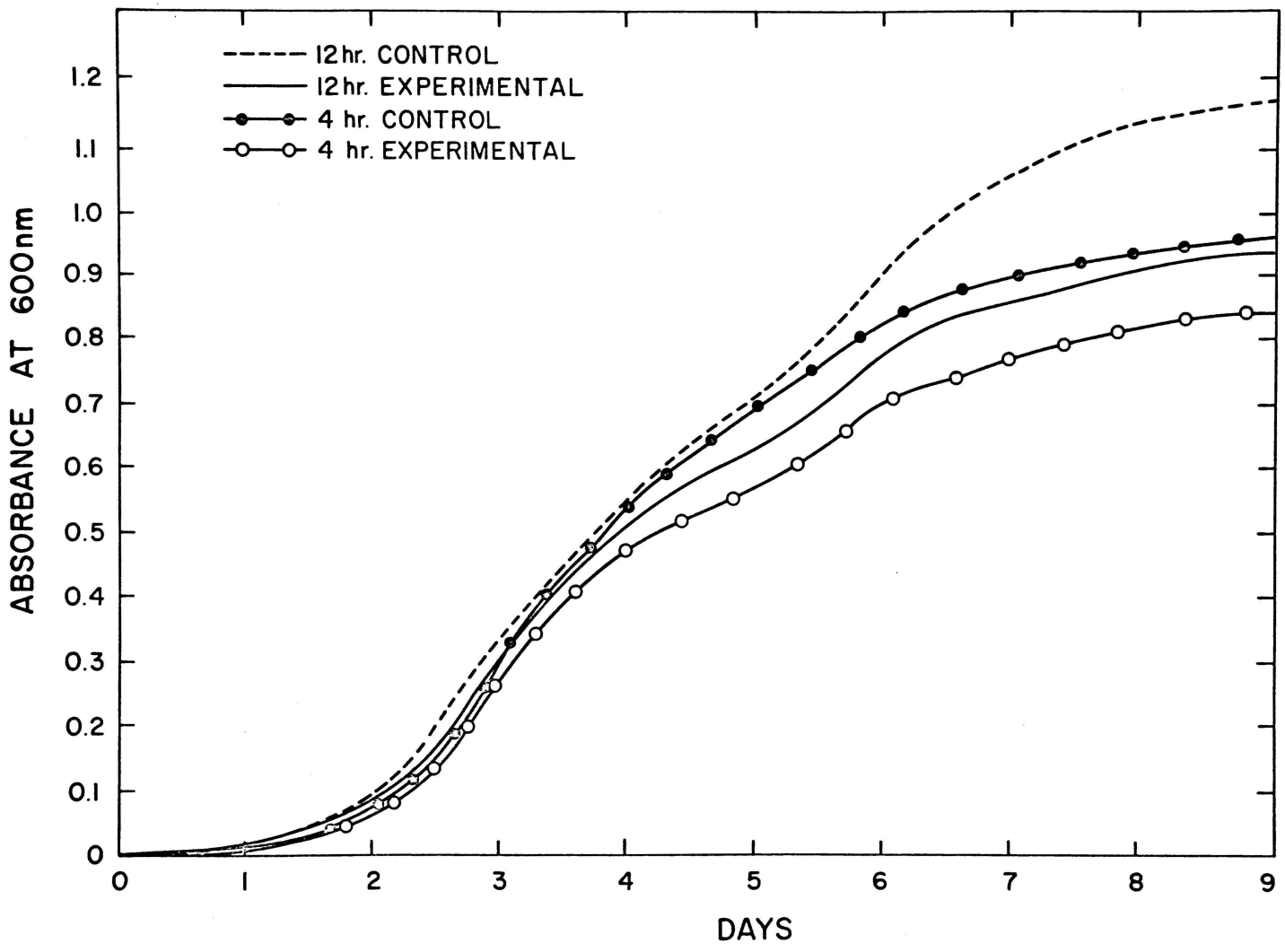

Figure 7. Algal growth curves for the static bioassay on the sludge unit LDBS effluents.

Table XXV

Mean Maximum Specific Growth Rates
(n=3) for the Static Algal Bioassay of
the Sludge Unit LDBS Effluents

Treatment	Mean Mu Max	C.V.
4-hr Experimental	1.09	38.5
4-hr Control	1.37	9.2
12-hr Experimental	1.36	5.9
12-hr Control	1.28	5.5

Mu Max = Maximum specific growth rate (day^{-1})

C.V. = Coefficient of variation (%)

Primary Alcohol Ethoxylate (Nonionic)

While the sludge units were being acclimated to the primary alcohol ethoxylate, static algal bioassays were carried out on the intact nonionic surfactant to determine its threshold of toxicity to S. capricornutum. The static test procedures were performed on primary alcohol ethoxylate concentrations of 0.1, 0.5, and 1.0 mg Nonionic/l with NAAM acting as the control and dilution medium. The growth levels attained by the algae over the test period are presented in Table XXVI. Figure 8 shows the corresponding algal growth curves. Statistical tests indicated that by the last day of the assay (day eight) the standing crop level supported by the 1.0 mg Nonionic/l treatment was significantly lower than all other treatments. None of the other surfactant concentrations supported growth levels significantly different from control levels.

The maximum specific growth rates were calculated for each treatment of the intact primary alcohol ethoxylate and for the control. The calculated Mu Max values are presented in Table XXVII. Analysis of variance and multiple range tests could not be used in evaluating the growth rates due to heterogeneous variances among the treatments.

The first continuous-flow algal bioassay of the primary alcohol ethoxylate effluents was run for 21 days. All experimental conditions were identical to those used in the LDBS continuous-flow assay. Table XXVIII shows the chemical parameters monitored on the sludge unit effluents throughout the primary alcohol ethoxylate study. The algal growth levels attained in the chemostats are presented in Table XXIX. Figure 9 shows the corresponding algal growth curves. Table XXX gives the nitrogen and phosphorus levels in the sludge unit effluents during the 21-day bioassay

Table XXVI

Mean Daily Absorbance Levels (n=3)
for the Static Algal Bioassay of
the Intact Primary Alcohol Ethoxylate

Day	Control (NAAM)		0.1 mg Nonionic/l		0.5 mg Nonionic/l		1.0 mg Nonionic/l	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
2	0.003	17.3	0.003	0.0	0.000	17.3	0.000	0.0
3	0.024	6.5	0.031	6.8	0.011	51.6	0.000	0.0
4	0.205	6.8	0.236	2.5	0.106	52.3	0.002	0.0
5	0.436	7.0	0.505	3.0	0.295	31.2	0.001	86.6
6	0.646	5.6	0.685	1.1	0.561	11.0	0.012	17.8
7	0.740	2.4	0.762	0.8	0.708	3.2	0.067	20.3
8	0.796	2.2	0.811	0.8	0.779	1.3	0.300	7.4

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

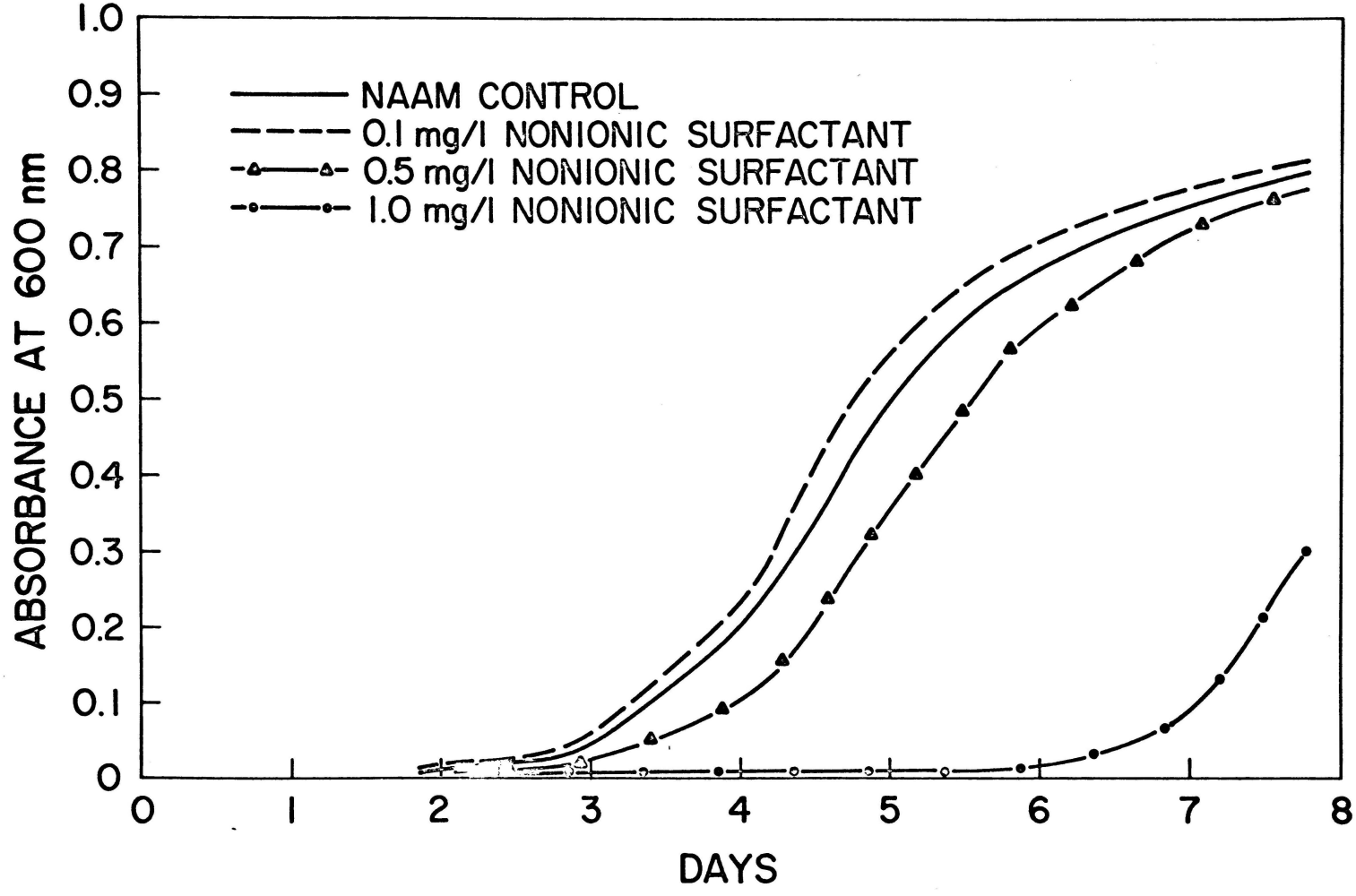

Figure 8. Algal growth curves for the static bioassay on primary alcohol ethoxylate.

Table XXVII

Mean Maximum Specific Growth Rates
(n=3) for the Static Algal Bioassay of
the Intact Primary Alcohol Ethoxylate

Treatment	Mean Mu Max	C.V.
Control (NAAM)	1.01	8.3
0.1 mg Nonionic/l	1.02	0.7
0.5 mg Nonionic/l	1.44	19.3
1.0 mg Nonionic/l	1.57	8.0

Mu Max = Maximum specific growth rate (day^{-1})

C.V. = Coefficient of variation (%)

Table XXVIII

Chemical Parameters Monitored on the Sludge Unit Influents and Effluents Throughout the Primary Alcohol Ethoxylate Study (Dolan 1974)

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Influent pH	7.7	3.9	22	7.7	2.6	22	7.7	3.9	21	7.6	3.9	22
Effluent pH	7.4	2.7	22	7.5	2.7	21	7.5	4.0	21	7.5	2.7	22
Influent Total Alkalinity as $CaCO_3$ (mg/l)	43.2	21.5	22	45.8	4.8	22	45.9	6.5	21	46.4	10.1	22
Effluent Total Alkalinity as $CaCO_3$ (mg/l)	49.0	16.9	22	47.8	17.2	22	46.8	20.7	21	46.5	9.5	22
Influent Total Hardness as $CaCO_3$ (mg/l)	65.1	16.1	22	67.8	9.3	22	67.6	12.0	22	62.5	23.5	21
Effluent Total Hardness as $CaCO_3$ (mg/l)	66.9	14.3	22	68.6	12.5	22	67.4	8.6	21	67.5	7.7	22
Mixed Liquor Suspended Solids (mg/l)	1759	31.8	6	1864	45.2	6	1735	40.4	6	1623	12.2	5
Influent COD (mg/l)	83.2	73.9	18	61.0	132	18	93.7	79.1	15	76.5	66.4	15
Effluent COD (mg/l)	35.0	62.9	19	42.2	34.1	17	33.0	64.2	17	45.2	53.1	19
COD reduction (%)	57.4	52.4	15	36.4	54.9	15	67.0	31.3	16	53.0	51.5	17

Table XXVIII, Continued

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Influent Nonionic (mg/1)	25.8	14.3	21	- -	- -	- -	27.3	17.6	21	- -	- -	- -
Effluent Nonionic (mg/1)	1.8	50.0	21	- -	- -	- -	1.4	57.1	21	- -	- -	- -
Nonionic reduction (%)	94.1	4.0	21	- -	- -	- -	94.0	3.5	21	- -	- -	- -

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XXIX

Mean Daily Absorbance Levels (n=3) for the First Continuous-flow Algal Bioassay of the Sludge Unit Primary Alcohol Ethoxylate Effluents

Day	4-hr residence				12-hr residence			
	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs*	C.V.	Abs	C.V.
2	0.063	26.1	0.050	12.3	0.044	9.6	0.044*	7.9
3	0.103	8.6	0.098	17.4	0.075	15.1	0.066*	11.7
4	0.181	12.3	0.165	20.3	0.132	15.6	0.112*	13.2
5	0.271	16.1	0.263	19.7	0.224	19.8	0.167	16.1
6	0.312	19.6	0.334	17.3	0.304	21.7	0.206	13.7
8	0.332	18.8	0.450	10.2	0.498	17.7	0.302	16.5
9	0.408	18.9	0.556	6.6	0.590	6.8	0.374	24.2
11	0.436	48.7	0.638	4.4	0.663	9.2	0.581	25.5
12	0.455	47.4	0.627	3.3	0.668	22.6	0.620	26.4
13	0.499	37.7	0.624	5.9	0.754	16.0	0.687	17.1
14	0.596	16.3	0.619	0.7	0.806	4.8	0.695	9.4
16	0.542	6.5	0.575	10.2	0.740	1.5	0.713	6.2
17	0.535	3.8	0.560	15.0	0.706	3.5	0.655	22.4
18	0.523	4.3	0.520	20.0	0.698	1.8	0.600	24.0

Table XXIX, Continued

Day	4-hr residence				12-hr residence			
	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs*	C.V.	Abs	C.V.
20	0.649	3.8	0.513	19.9	0.740	7.2	0.592	7.2
21	0.607	2.2	0.518	18.1	0.690	12.2	0.592	5.8

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

* n=2

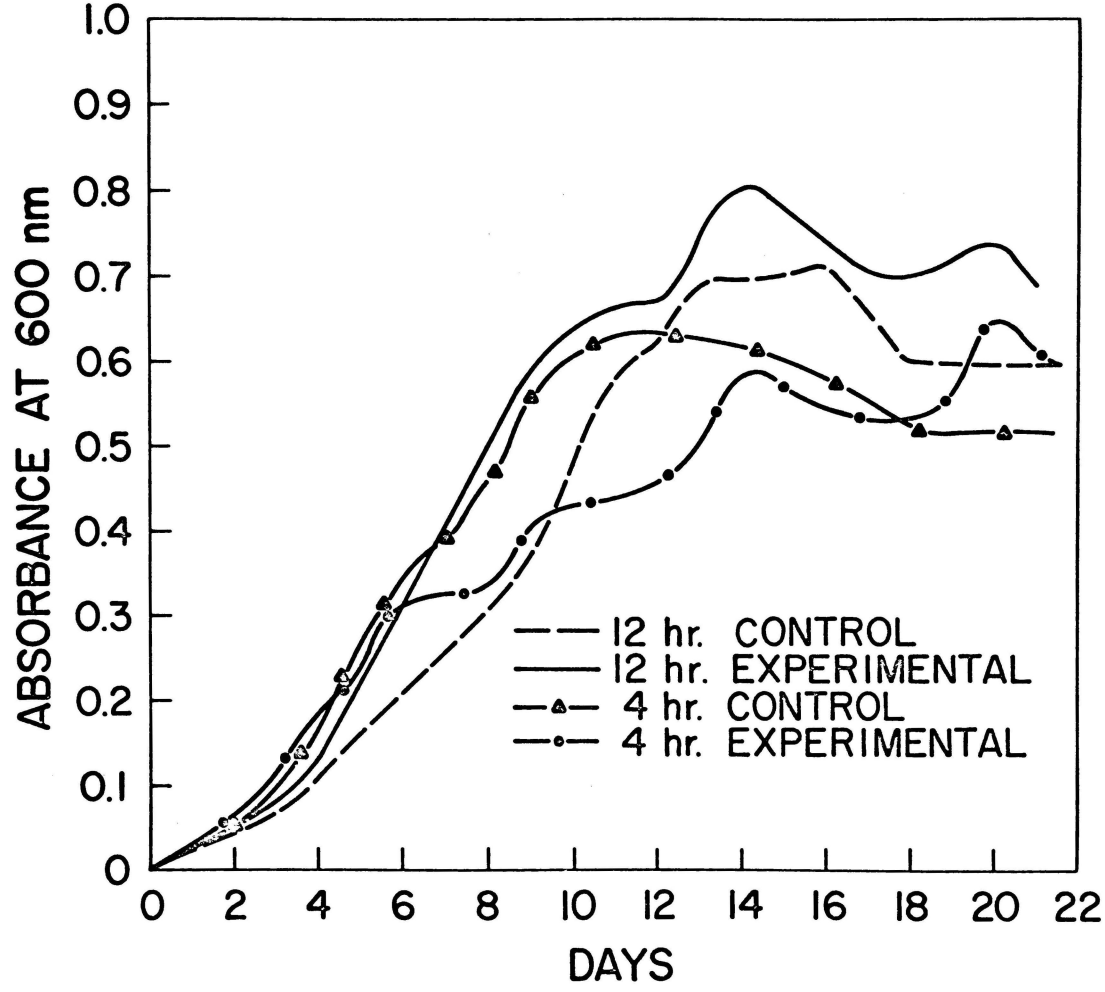

Figure 9. Algal growth curves for the first continuous-flow bioassay on the sludge unit primary alcohol ethoxylate effluents.

Table XXX

Nutrient Chemical Parameters Monitored on the Sludge Unit Effluents During the First Continuous-flow Algal Bioassay of Primary Alcohol Ethoxylate

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Ortho-PO_4 (mg P/l)	7.4	98.5	4	3.9	60.8	4	8.9	47.5	4	6.3	58.2	4
Total-PO_4 (mg P/l)	10.7	113	4	5.3	58.6	4	13.1	78.6	4	8.5	79.2	4
NH_3-N (mg N/l)	0.4	- -	1	1.5	- -	1	1.4	- -	1	0.6	- -	1
NO_3-N (mg N/l)	16.8	25.0	3	20.6	111	2	23.7	47.3	3	17.8	112	3

C.V. = Coefficient of variation (%)

n = Number of replicates

period. Table XXXI shows the mean hydraulic residence times for the bioassay period.

A second continuous-flow bioassay on the sludge unit primary alcohol ethoxylate effluents was run for 17 days. All experimental conditions were identical to those in the first bioassay. Table XXXII shows the algal growth levels attained in the chemostats. Figure 10 shows the corresponding algal growth curves. Steady state levels of growth were approximated for each treatment in the manner described previously. The approximated steady state levels of algal growth are given in Table XXXIII. The mean hydraulic residence times for the approximated steady state regions of the curves are shown in Table XXXIV. The nitrogen and phosphorus levels in the sludge unit effluents during the 17-day bioassay are shown in Table XXXV. The mean hydraulic residence times for the duration of the 17-day bioassay are presented in Table XXXVI.

Zinc Toxicity and Temperature Studies

Static Algal Bioassays

The initial investigations of the effects of temperature on the toxicity of zinc to S. capricornutum involved temperature tolerance studies. S. capricornutum was cultured in NAAM at a variety of temperatures above and below the recommended 24° C level. Results indicated that the algae would grow only slightly at 35° C and not at all a few degrees above this. Growth was slight at 11° C and a little greater at 15° C. Cultures grown at 18 and 30° C achieved roughly the same standing crop levels as the 24° C cultures except that the time required was greater at 18° C than at 30° C. Based on these findings, 19, 24, and 29° C were selected as the test temperatures for zinc toxicity studies.

Table XXXI

Mean Hydraulic Residence Times for the
Duration of the First Continuous-flow Algal Bioassay
of the Primary Alcohol Ethoxylate Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean (days)	2.95	3.14	3.05	2.96
C.V.	7.1	27.0	9.8	6.6
n	54	57	38	55

C.V. = Coefficient of variation (%)

n = No. of replicates

Table XXXII

Mean Daily Absorbance Levels (n=2) for the Second Continous-flow Algal Bioassay of the Sludge Unit Primary Alcohol Ethoxylate Effluents

	4-hr residence				12-hr residence			
Day	Experimental		Control		Experimental		Control	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
2	0.063*	7.9	0.060*	16.7	0.052*	3.0	0.057*	5.7
3	0.131*	12.1	0.137*	24.6	0.111*	7.2	0.110*	13.9
4	0.266*	10.8	0.285*	32.9	0.228*	4.4	0.224*	5.2
6	0.512	2.1	0.563	4.3	0.556	3.1	0.466	11.1
8	0.574	2.8	0.610	0.6	0.650	1.9	0.666	5.9
10	0.660	0.6	0.624	2.7	0.692	5.0	0.746	11.7
11	0.632	3.2	0.628	3.2	0.670	3.7	0.706	11.5
13	0.651	4.8	0.632	4.6	0.664	6.1	0.694	11.9
15	0.580	5.5	0.599	4.5	0.660	5.0	0.629	10.1
17	0.540	3.4	0.581	5.6	0.670	9.8	0.656	10.6

Abs = Absorbance at 600 nm

C.V.= Coefficient of variation (%)

* n=3

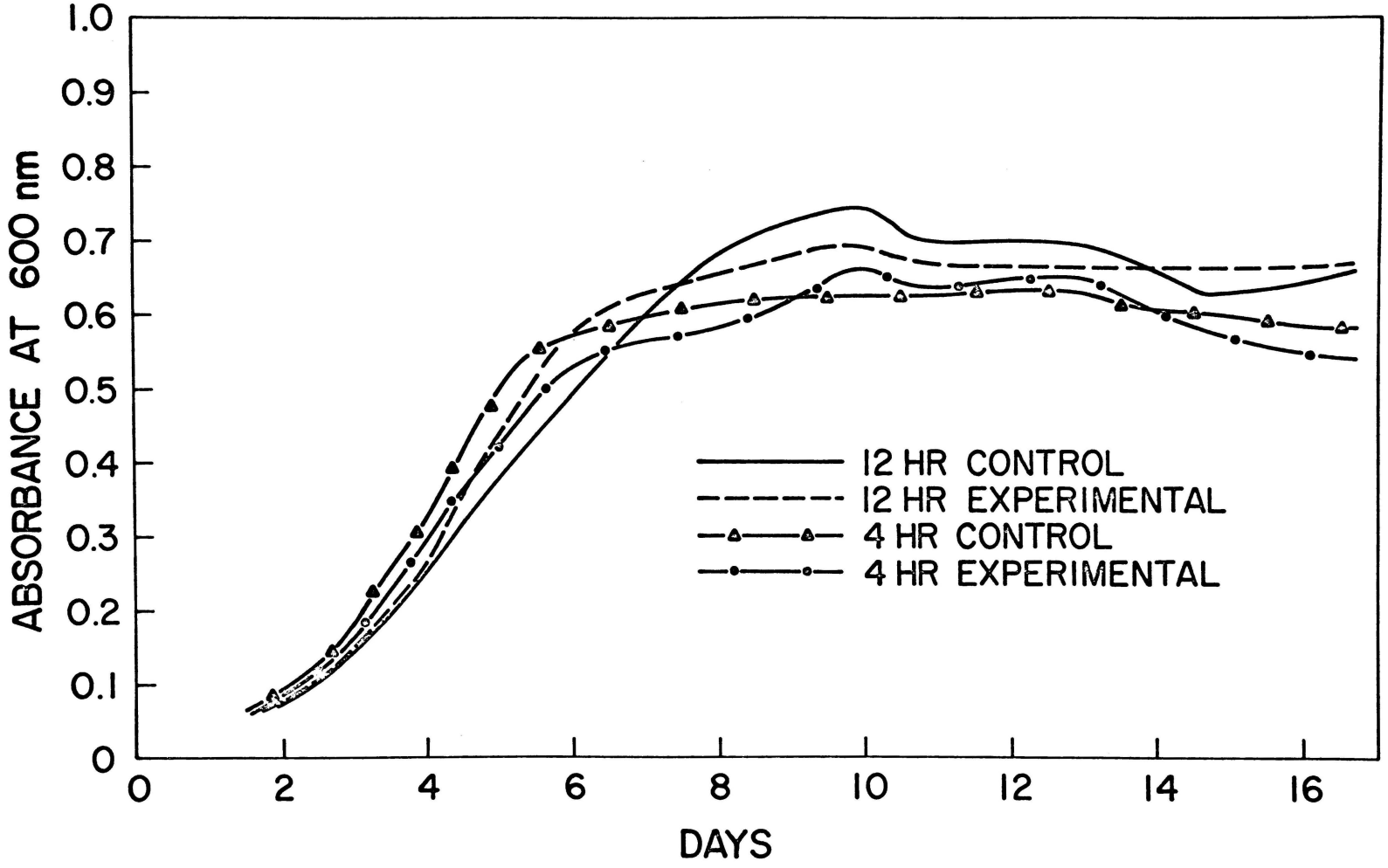

Figure 10. Algal growth curves for the second continuous-flow bioassay on the sludge unit primary alcohol ethoxylate effluents.

Table XXXIII

Approximations of Steady State Levels of Growth for the Second Continuous-flow Algal Bioassay of the Sludge Unit Primary Alcohol Ethoxylate Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean Abs	0.631	0.621	0.672	0.694
C.V.	6.0	3.6	4.3	10.8
n	8	8	8	8
Time period (days)	4.8	4.8	4.8	4.8

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XXXIV

Mean Hydraulic Residence Times for the Steady State Growth Periods of the Second Continuous-flow Algal Bioassay of the Sludge Unit Primary Alcohol Ethoxylate Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean (days)	3.14	2.93	3.03	3.05
C.V.	3.3	4.1	3.3	2.9
n	8	8	8	8
Time period (days)	4.8	4.8	4.8	4.8

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XXXV

Nutrient Chemical Parameters Monitored on the Sludge Unit Effluents During the Second Continuous-flow Algal Bioassay of Primary Alcohol Ethoxylate

Parameter	4-hr residence						12-hr residence					
	Experimental			Control			Experimental			Control		
	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n	Mean	C.V.	n
Ortho-PO_4 (mg P/l)	6.9	77.5	4	7.0	93.3	4	6.2	25.1	4	9.8	63.3	4
Total-PO_4 (mg P/l)	7.9	71.5	4	7.2	67.1	4	7.1	22.4	4	10.6	63.4	4
NH_3-N (mg N/l)	0.8	69.7	2	0.4	29.9	3	0.4	42.8	3	0.4	40.4	3
NO_3-N (mg N/l)	17.1	47.6	4	26.4	67.4	4	20.0	74.0	3	21.6	56.0	3

C.V. = Coefficient of variation (%)

n = Number of replicates

Table XXXVI

Mean Hydraulic Residence Times for the
Duration of the Second Continuous-flow Algal Bioassay
of Primary Alcohol Ethoxylate Effluents

	4-hr residence		12-hr residence	
	Experimental	Control	Experimental	Control
Mean (days)	3.22	2.98	3.05	3.09
C.V.	7.2	6.4	6.3	6.4
n	22	22	22	22

C.V. = Coefficient of variation (%)

n = No. of replicates

Preliminary static bioassays at 24° C indicated a threshold of zinc toxicity of approximately 0.06 mg Zn/1. Based on these findings, 0.02, 0.06, and 0.10 mg Zn/1 were selected as the test concentrations at each temperature. NAAM served as the control. A static algal bioassay was run on zinc at 24° C for eight days. The growth levels attained by the algae over the bioassay period are presented in Table XXXVII. Figure 11 shows the corresponding algal growth curves. Table XXXVIII gives the mean absorbance levels and % growth reduction by zinc after seven days of incubation. Statistical tests indicated that the 0.10 mg Zn/1 treatment supported standing crop levels significantly lower than all other treatments. None of the other zinc treatments supported growth levels significantly different from control levels.

The maximum specific growth rates were calculated for each zinc treatment and for the control. The calculated Mu Max values are presented in Table XXXIX. Statistical tests indicated that the 0.10 mg Zn/1 maximum growth rate was significantly lower than all other growth rates. None of the other zinc treatment maximum growth rates were significantly different from the control rate.

Another static algal bioassay was conducted on zinc at 29° C for 12 days. The growth levels attained by the algae over the bioassay period are presented in Table XL. Figure 12 shows the corresponding algal growth curves. Table XLI gives the mean absorbance levels and % growth reduction by zinc after seven days of incubation. The growth levels after seven days were selected because they closely approximated the growth levels attained after seven days at 24° C. Statistical tests indicated that both the 0.06 and 0.10 mg Zn/1 treatments supported standing crop levels

Table XXXVII

Mean Daily Absorbance Levels (n=3)
for the Static Algal Bioassay of
Zinc at 24°C

Day	Control (NAAM)		0.02 mg Zn/l		0.06 mg Zn/l		0.10 mg Zn/l	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
2	0.005	10.8	0.004	35.3	0.003	17.3	0.003	33.3
3	0.028	7.5	0.026	9.6	0.017	9.2	0.007	7.9
4	0.125	10.4	0.116	14.5	0.084	5.4	0.021	19.6
5	0.292	7.3	0.289	1.4	0.220	11.4	0.034	9.4
6	0.458	6.7	0.445	5.1	0.377	10.1	0.055	24.4
7	0.577	8.4	0.574	8.4	0.501	7.1	0.065	22.1
8	0.634	6.5	0.630	10.1	0.570	6.4	0.072	23.6

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

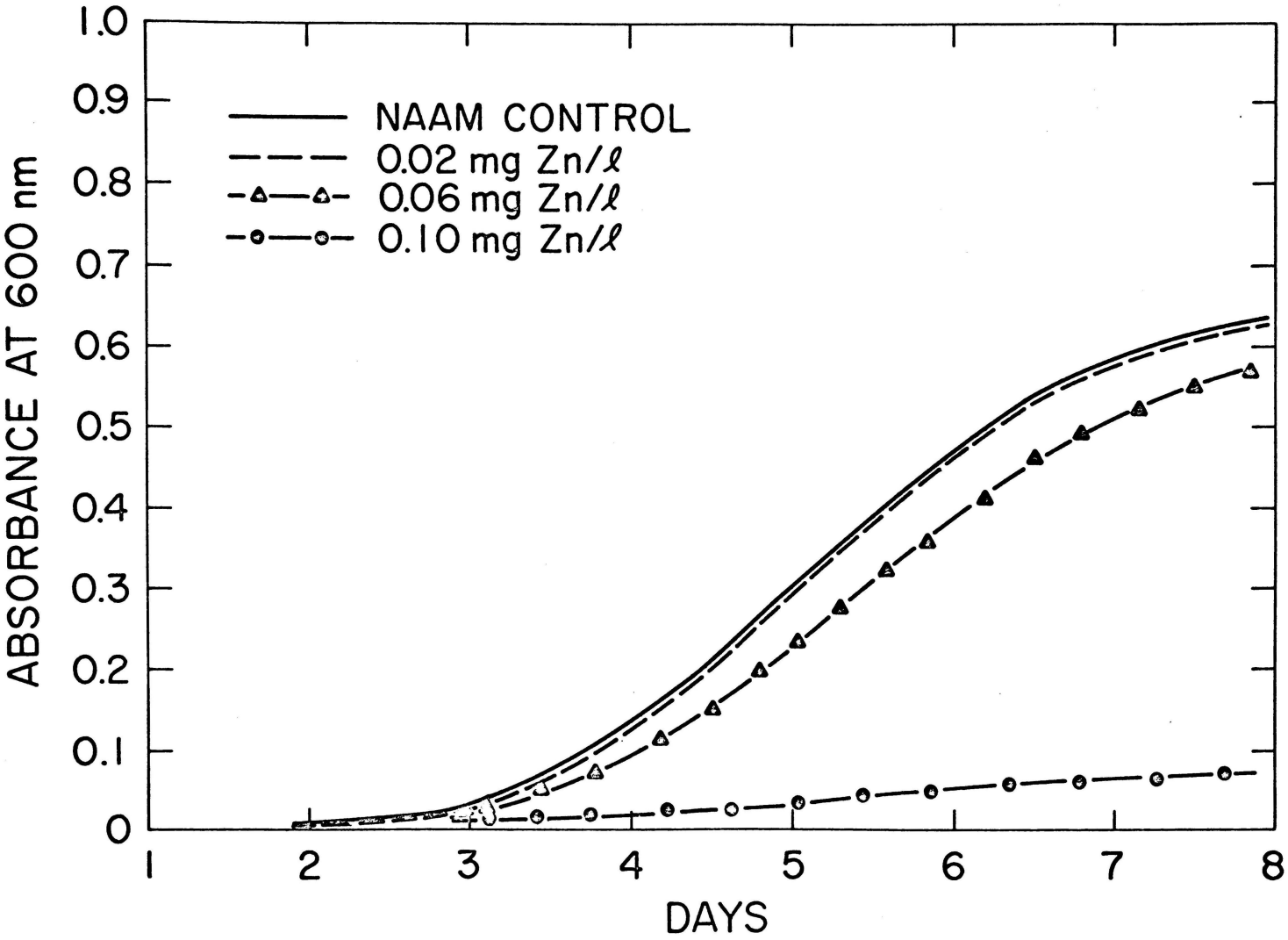

Figure 11. Algal growth curves for the static bioassay on zinc at 24° C.

Table XXXVIII

Mean Absorbance Levels (n=3) and Percent Growth Reduction by Zinc after Seven Days at 24° C

Treatment	Mean Abs	C.V.	% of Control
Control (NAAM)	0.577	8.4	100
0.02 mg Zn/l	0.574	8.4	99.5
0.06 mg Zn/l	0.501	7.1	86.8
0.10 mg Zn/l	0.065	22.1	11.3

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

Table XXXIX

Mean Maximum Specific Growth Rates (n=3) for the Static Algal Bioassay of Zinc at 24° C

Treatment	Mean Mu Max	C.V.
Control (NAAM)	0.85	12.3
0.02 mg Zn/l	0.92	15.9
0.06 mg Zn/l	0.96	6.3
0.10 mg Zn/l	0.18*	5.5

Mu Max = Maximum specific growth rate (day^{-1})

C.V. = Coefficient of variation (%)

* n=2

Table XL

Mean Daily Absorbance Levels (n=3) for the Static Algal Bioassay of Zinc at 29° C

Day	Control (NAAM)		0.02 mg Zn/l		0.06 mg Zn/l		0.10 mg Zn/l	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
2	0.002	24.7	0.003	33.3	0.000	0.0	0.001	173
3	0.026	7.7	0.029	12.3	0.007	37.8	0.001	173
5	0.251	14.1	0.282	8.6	0.085	10.5	0.004	74.2
6	0.423	0.7	0.427	3.1	0.131	8.6	0.006	63.8
7	0.493	0.8	0.499	2.8	0.153	10.2	0.008	94.4
12	0.672	2.3	0.676	2.3	0.312	26.8	0.022	75.1

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

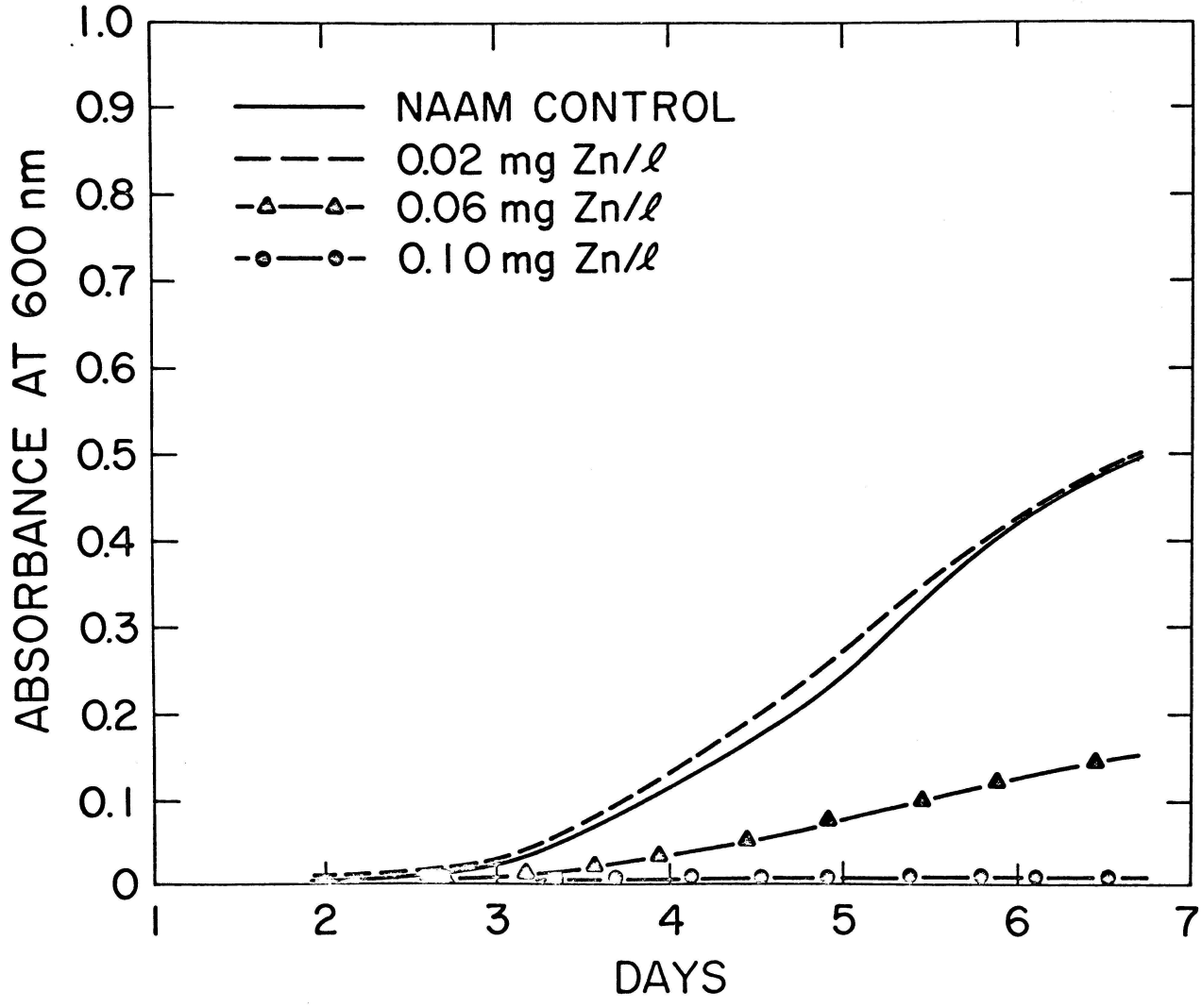

Figure 12. Algal growth curves for the static bioassay on zinc at 29° C.

Table XLI

Mean Absorbance Levels (n=3) and Percent Growth Reduction by Zinc after Seven Days at 29° C

Treatment	Mean Abs	C.V.	% of Control
Control (NAAM)	0.493	0.8	100
0.02 mg Zn/l	0.499	2.8	101
0.06 mg Zn/l	0.153	10.2	31.0
0.10 mg Zn/l	0.008	94.4	1.6

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

significantly different from all other treatments. There was no significant difference between the control and 0.02 mg Zn/l treatments after seven days.

The maximum specific growth rates were not calculated for this bioassay since absorbance readings were not taken on day four. This was the period during the bioassay at which the algal growth rate was greatest.

Another static algal bioassay was conducted on zinc at 19° C for 11 days. The growth levels attained by the algae over the bioassay period are presented in Table XLII. Figure 13 shows the corresponding algal growth curves. Table XLIII gives the mean abosrbance levels and % growth reduction by zinc after nine days of incubation. The growth levels after nine days were selected because they closely approximated the growth levels attained after seven days at both 24 and 29° C. Statistical tests indicated that both the 0.06 and 0.10 mg Zn/l treatments supported standing crop levels significantly different from all other treatments. There was no significant difference between the control and 0.02 mg Zn/l treatments after nine days.

The maximum specific growth rates were calculated for each zinc treatment and for the control. The calculated Mu Max values are presented in Table XLIV. Statistical tests indicated that the maximum growth rates for each treatment were significantly different from all other growth rates.

Photosynthesis and Respiration Rates

The effects of zinc and temperature on the net photosynthesis and respiration rates of S. capricornutum were evaluated using the same temperatures and zinc concentrations as previously described. The results

Table XLII

Mean Daily Absorbance Levels (n=3) for the Static Algal Bioassay of Zinc at 19° C

Day	Control (NAAM)		0.02 mg Zn/l		0.06 mg Zn/l		0.10 mg Zn/l	
	Abs	C.V.	Abs	C.V.	Abs	C.V.	Abs	C.V.
2	0.000	0.0	0.001	0.0	0.000	173	0.000	0.0
3	0.003	17.3	0.005	20.0	0.003	21.7	0.002	34.6
4	0.014	12.4	0.017	3.3	0.011	21.7	0.004	35.3
5	0.039	5.3	0.052	3.0	0.027	25.9	0.011	32.8
6	0.112	4.9	0.142	6.0	0.077	26.8	0.020	17.3
7	0.268	5.0	0.303	4.3	0.160	21.1	0.037	21.2
8	0.421	2.4	0.473	1.5	0.295	9.1	0.055	20.8
9	0.536	1.2	0.570	1.8	0.393	7.6	0.071	23.5
10	0.609	0.7	0.636	1.9	0.472	5.0	0.083	17.6
11	0.666	1.8	0.698	0.8	0.563	4.7	0.103	17.9

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

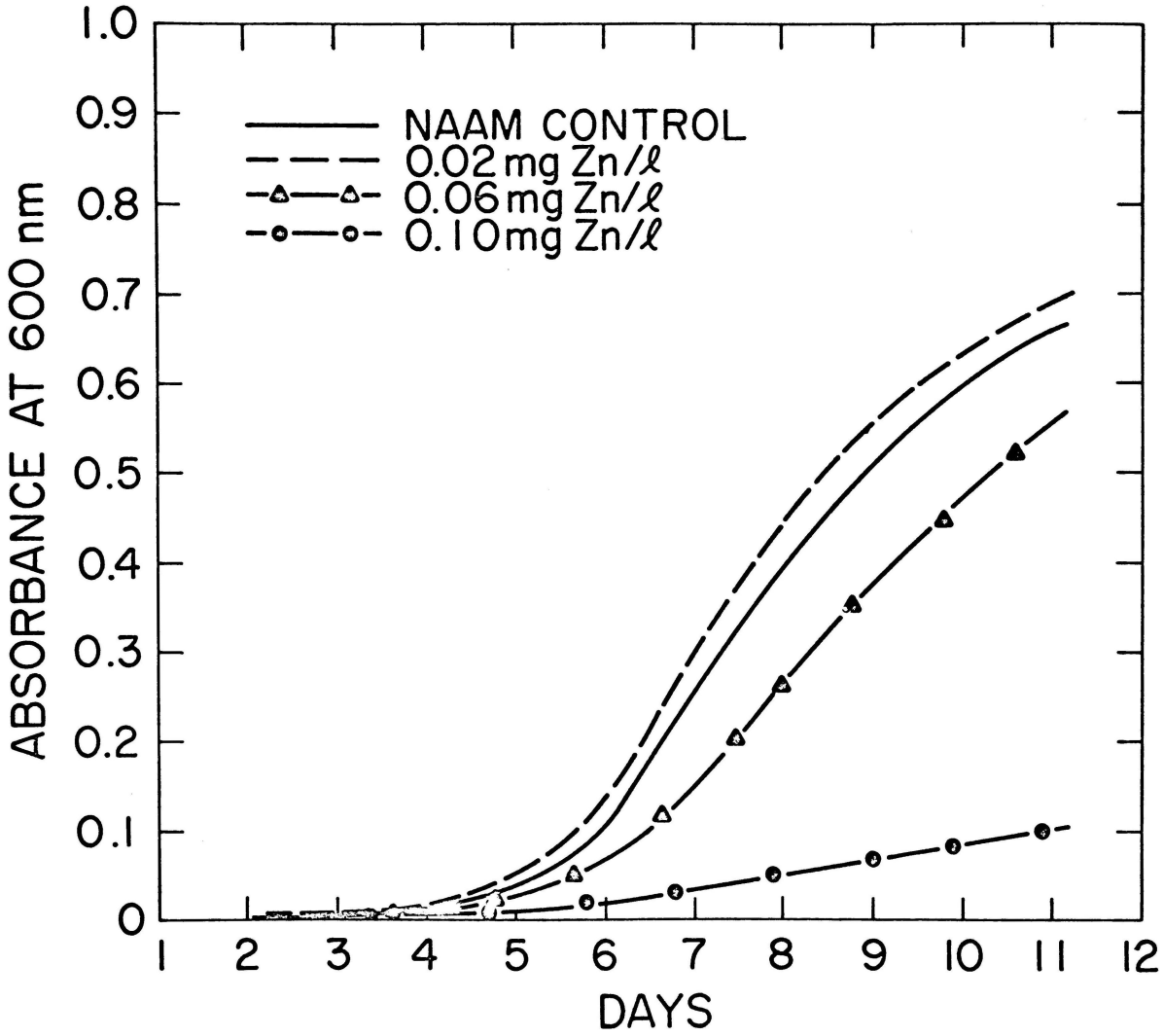

Figure 13. Algal growth curves for the static bioassay on zinc at 19° C.

Table XLIII

Mean Absorbance Levels (n=3) and Percent
Growth Reduction by Zinc after Nine Days at 19° C

Treatment	Mean Abs	C.V.	% of Control
Control (NAAM)	0.536	1.2	100
0.02 mg Zn/l	0.570	1.8	106
0.06 mg Zn/l	0.393	7.6	73.3
0.10 mg Zn/l	0.071	23.5	13.2

Abs = Absorbance at 600 nm

C.V. = Coefficient of variation (%)

Table XLIV

Mean Maximum Specific Growth Rates (n=3) for the Static Algal Bioassay of Zinc at 19° C

Treatment	Mean Mu Max	C.V.
Control (NAAM)	0.87	2.4
0.02 mg Zn/l	0.97	3.1
0.06 mg Zn/l	0.74	9.4
0.10 mg Zn/l	0.26	9.8

Mu Max = Maximum specific growth rate (day^{-1})

C.V. = Coefficient of variation (%)

of the first test at 24° C are presented in Table XLV. The % of control is listed for each zinc treatment for both net photosynthesis and respiration rates. Statistical tests indicated that the net photosynthesis rates for both the 0.06 and 0.10 mg Zn/l treatments were significantly different from those for all other treatments. There was no significant difference between the control and 0.02 mg Zn/l rates. Statistical tests also indicated that there was no significant difference between any of the respiration rates for any of the treatments.

The results of a second test at 24° C are presented in Table XLVI. The initial cell concentration for this test was slightly lower than that of the first 24° C test (2.63×10^5 as opposed to 3.0×10^5 cells/ml). Statistical tests indicated the same results as in the first test. The net photosynthetic rates for both the 0.06 and 0.10 mg Zn/l treatments were significantly different from those for all other treatments. There was no significant difference between the control and 0.02 mg Zn/l rates. Also, there was no significant difference between any of the respiration rates for any of the treatments.

The results of the first test at 29° C are presented in Table XLVII. Statistical tests indicated that the net photosynthesis rate for the 0.10 mg Zn/l treatment was significantly lower than those for all other treatments. There was no significant difference between the control, 0.02, or 0.06 mg Zn/l rates. Statistical tests indicated that the respiration rates for the 0.02 and 0.10 mg Zn/l treatments were significantly different from each other. No other combination of rates was significantly different.

The results of a second test at 29° C are presented in Table XLVIII. The initial cell concentration for this test was half that of the first

Table XLV

Mean Net Photosynthesis and Respiration Rates (n=2) for *S. capricornutum* During Exposure to Zinc at 24°C (first test)

Treatment	Net Photosynthesis (mg O_2/l/da)			Respiration (mg O_2/l/da)		
	Mean	C.V.	% of Control	Mean	C.V.	% of Control
Control (NAAM)	47.9	2.0	100	3.3	69.5	100
0.02 mg Zn/l	48.6	4.7	101	4.7	12.5	142
0.06 mg Zn/l	42.8	2.3	89.4	3.5	0.2	106
0.10 mg Zn/l	28.1	0.1	58.7	4.3	27.9	130

C.V. = Coefficient of variation (%)

Table XLVI

Mean Net Photosynthesis and Respiration Rates (n=2) for S. capricornutum During Exposure to Zinc at 24°C (second test)*

Treatment	Net Photosynthesis (mg O_2/l/da)			Respiration (mg O_2/l/da)		
	Mean	C.V.	% of Control	Mean	C.V.	% of Control
Control (NAAM)	53.1	5.3	100	6.3	9.0	100
0.02 mg Zn/l	56.6	6.1	107	6.0	0.4	95.2
0.06 mg Zn/l	44.8	0.9	84.4	6.9	14.8	110
0.10 mg Zn/l	23.3	0.1	43.9	6.9	16.1	110

C.V. = Coefficient of variation (%)

*Cell concentration = 2.63×10^5 cells/ml

Table XLVII

Mean Net Photosynthesis and Respiration Rates (n=2) for *S. capricornutum* During Exposure to Zinc at 29°C (first test)

Treatment	Net Photosynthesis (mg O_2/l/da)			Respiration (mg O_2/l/da)		
	Mean	C.V.	% of Control	Mean	C.V.	% of Control
Control (NAAM)	39.5	8.4	100	7.4	0.5	100
0.02 mg Zn/l	43.1	9.0	109	6.3	27.5	85.1
0.06 mg Zn/l	37.9	4.9	95.9	7.0	12.7	94.6
0.10 mg Zn/l	20.9	0.5	52.9	10.4	0.5	141

C.V. = Coefficient of variation (%)

Table XLVIII

Mean Net Photosynthesis and Respiration Rates (n=2) for *S. capricornutum* During Exposure to Zinc at 29° C (second test)*

Treatment	Net Photosynthesis (mg O_2/l/da)			Respiration (mg O_2/l/da)		
	Mean	C.V.	% of Control	Mean	C.V.	% of Control
Control (NAAM)	23.0	2.9	100	3.4	0.4	100
0.02 mg Zn/l	22.1	5.2	96.1	3.0	59.7	88.2
0.06 mg Zn/l	21.3	5.6	92.6	3.1	20.3	91.2
0.10 mg Zn/l	16.7	0.0	72.6	3.1	19.5	91.2

C.V. = Coefficient of variation (%)

*Cell concentration = 1.50×10^5 cells/ml

test (1.50×10^5 cells/ml as opposed to 3.0×10^5 cells/ml). Statistical tests indicated the same results as in the first test for the net photosynthesis rates but different results for the respiration rates. The net photosynthesis rate for the 0.10 mg Zn/l treatment was significantly lower than those for all other treatments. There was no significant difference between the control, 0.02, or 0.06 mg Zn/l rates. For the respiration rates, none were significantly different for any of the treatments.

The results of the first test at 19° C are presented in Table XLIX. Statistical tests indicated that the net photosynthesis rate for the 0.10 mg Zn/l treatment was significantly lower than those for all other treatments. There was no significant difference between the control, 0.02, or 0.06 mg Zn/l rates. Statistical tests also indicated that none of the respiration rates were significantly different for any of the treatments.

The results of a second test at 19° C are presented in Table L. Statistical tests indicated the same results as in the first test for the respiration rates but different results for the net photosynthesis rates. The net photosynthesis rates for both the 0.06 and 0.10 mg Zn/l treatments were significantly different from those for all other treatments. There was no significant difference between the control and 0.02 mg Zn/l rates. Also, there was no significant difference between any of the respiration rates for any of the treatments.

Table XLIX

Mean Net Photosynthesis and Respiration Rates (n=2) for *S. capricornutum* During Exposure to Zinc at 19° C (first test)

Treatment	Net Photosynthesis (mg O_2/l/da)			Respiration (mg O_2/l/da)		
	Mean	C.V.	% of Control	Mean	C.V.	% of Control
Control (NAAM)	37.8	5.6	100	5.7	21.9	100
0.02 mg Zn/l	39.1	3.1	103	4.9	0.3	86.0
0.06 mg Zn/l	35.2	0.0	93.1	5.0	0.4	87.7
0.10 mg Zn/l	24.6	4.7	65.1	5.9	19.4	104

C.V. = Coefficient of variation (%)

Table L

Mean Net Photosynthesis and Respiration Rates (n=2) for *S. capricornutum* During Exposure to Zinc at 19° C (second test)

Treatment	Net Photosynthesis (mg O_2/l/da)			Respiration (mg O_2/l/da)		
	Mean	C.V.	% of Control	Mean	C.V.	% of Control
Control (NAAM)	27.3	0.3	100	6.6	8.2	100
0.02 mg Zn/l	28.3	2.4	104	6.9	13.9	105
0.06 mg Zn/l	22.2	0.2	81.3	7.2	20.2	109
0.10 mg Zn/l	16.9	3.3	61.9	7.5	13.3	114

C.V. = Coefficient of variation (%)

DISCUSSION

Surfactant Bioassays

Linear Tridecyl Benzene Sulfonate (LTBS)

Static tests of the intact LTBS mixture for toxicity to S. capricornutum indicated that 5 mg MBAS/l increased standing crop levels, while 10, 15, and 20 mg MBAS/l successively decreased the levels (Figure 1). The growth stimulation by the lowest MBAS concentration was probably due to the surface-active properties of the compound. As was previously discussed, surfactants can alter the cell wall and membrane permeability which, in turn, alters the nutrient uptake and waste removal rates of the cell. MBAS concentrations of 10 mg/l and above were apparently great enough to override the stimulatory effects of lower concentrations and cause an inhibitory response. The threshold of toxicity (the maximum concentration that could be tolerated indefinitely without lethal effects) was approximately 10 mg MBAS/l. None of the inhibitory MBAS concentrations were algistatic. Since the cultures were not axenic, bacterial degradation of the surfactant during the bioassay period probably reduced the initial MBAS levels considerably. Analyses of the maximum specific growth rates for the treatments indicated that only the 20 mg MBAS/l treatment yielded a rate significantly different from the control (Table III). If the initial MBAS levels had been maintained for the duration of the assay, the maximum growth rates probably would have been more varied.

The results of the first continuous-flow bioassay on the sludge unit LTBS effluents indicate that all of the effluents supported substantial

levels of algal growth (Figure 2). The chemical parameter levels monitored on the sludge unit effluents throughout the LTBS study were similar for all effluents (Table IV). The nitrogen and phosphorus levels measured during the assay period were considerably more variable (Table VI). The numerous high coefficients of variation indicate that the nutrient levels fluctuated considerably during the assay period. The hydraulic residence times for all treatments were close to four days (Table VII). Table IV shows that the MBAS levels in the experimental effluents for both residence times were almost identical. Therefore, the difference in residence period failed to vary the degree of surfactant degradation. Because the MBAS levels in both experimental effluents were practically identical and in such small amounts, the differences in the algal growth curves probably reflect the fluctuation of algal nutrient levels within the sludge units. The elevated pH levels in the chemostats (which usually exceeded nine) necessitated a reduction of the hydraulic residence times and the use of CO_2 for pH control in further continuous-flow assays. These changes were made to prevent carbon limitation from affecting the algal growth levels.

The results of the second continuous-flow bioassay on the sludge unit LTBS effluents also indicate that all of the effluents supported substantial levels of algal growth (Figure 3). As was mentioned previously, the chemical parameter levels monitored on the sludge unit effluents throughout the LTBS study were very similar for all effluents (Table IV). However, as was true in the first continuous-flow bioassay, the nitrogen and phosphorus levels fluctuated considerably during the assay period (Table XI). The hydraulic residence times were all slightly less than three days (Table XII). Therefore, since the MBAS levels in both LTBS experimental

effluents were shown to be quite low and practically equal, the differences in the algal growth curves for the second continuous-flow assay probably reflect the fluctuations of algal nutrient levels within the sludge units. The approximations of steady state levels of growth for the treatments indicate that the 12-hr experimental effluent supported the highest level of stable algal growth for at least one hydraulic residence time (Table IX). These approximations were not made for the first continuous-flow assay because of the general lack of periods of stable algal growth among the treatments (Figure 2). The mean hydraulic residence times for the steady state periods of the second continuous-flow assay indicate that the flow rates were generally precise and accurate (Table X). Thus, the steady state level approximations represent the periods of the second continuous-flow assay during which the nutrient levels among the various effluents were most constant. The algal growth levels maintained during these periods indicate that nutrient levels rather than surfactant levels determined the algal growth potentials of the effluents.

The results of the first static bioassay on the LTBS effluents indicate that all of the effluents supported substantial levels of algal growth by day 11 (Table XIII). The differences in growth levels among the treatments were apparently due to nutrient level differences in the sludge unit effluents at the time the sample was taken. Maximum specific growth rates were not calculated since absorbance readings were not taken daily.

The results of the second static bioassay on the LTBS effluents indicate that the algal standing crops after 12 days were somewhat different from those in the first assay (Figure 4). The growth level of the 4-hr experimental effluent was considerably reduced below its level in the

first assay. The evaluation of the maximum specific growth rates for the treatments indicated that there were no significant differences in the maximum rates of growth among the treatments even though the corresponding standing crops attained were different (Table XV). Again, these results indicate that the differences in algal growth among the treatments were due to nutrient level variability within the sludge units.

The results of the LTBS studies indicate that the intact LTBS mixture was toxic to S. capricornutum at concentrations of 10 mg MBAS/l and above. However, the MBAS levels in both experimental effluents were far below the threshold toxicity level. Therefore, the effect of biodegradation on the toxicity of LTBS to S. capricornutum was to reduce the initial MBAS levels to sublethal concentrations. Also, the LTBS breakdown products were apparently not toxic since one test showed that the experimental effluent supported higher growth levels than the corresponding control. The two residence times used failed to vary the degree of surfactant degradation, however the algal bioassay procedures which were used successfully evaluated the capacities of the various solutions tested to support algal growth.

Linear Dodecyl Benzene Sulfonate (LDBS)

Static tests of the intact LDBS mixture for toxicity to S. capricornutum indicated that the 10 mg MBAS/l treatment was the only surfactant level tested that significantly decreased standing crop levels (Figure 5). Therefore, this concentration represents the approximate threshold of toxicity under the experimental conditions used. Analyses of the maximum specific growth rates for the treatments indicated that all surfactant concentrations decreased the growth rates, and the highest concentration resulted in the greatest degree of inhibition (Table XVII).

The procedure of autoclaving the sludge unit effluents before using them in bioassays worked well. The results of the continuous-flow bioassay on the sludge unit LDBS effluents indicate that all effluents supported algal growth but at widely varying levels (Figure 6). The chemical parameter levels monitored on the sludge unit effluents throughout the LDBS study were very similar for all effluents (Table XVIII). Also, the levels were similar in magnitude to those obtained in the LTBS study (Table IV). The nitrogen and phosphorus levels again were considerably variable (Table XXII). Also, the phosphorus levels were quite lower than those in the LTBS study. The hydraulic residence times were relatively close to three days, and the precision was good for each mean (Table XXIII). Table XVIII shows that the MBAS levels in the experimental effluents of both residence times were almost identical (as was true in the LTBS study). Therefore, the difference in residence period again failed to vary the degree of surfactant degradation. Because the MBAS levels in the experimental effluents were practically identical and in such small amounts, the differences in the algal growth curves probably reflect the fluctuations of algal nutrient levels within the sludge units. The approximations of steady state levels of growth for the treatments indicate that the growth levels for both experimental effluents were similar, but the control growth levels were both considerably greater (Table XX). The mean hydraulic residence times for the steady state periods indicate that the flow rates were generally precise and accurate (Table XXI). Thus, the steady state level approximations represent the periods of the bioassay during which the nutrient levels among the effluents were most constant. It is tempting to consider the reduced growth levels of the experimental

treatments and conclude that the breakdown products of LDBS biodegradation were toxic to the algae. However, there is no evidence to substantiate this hypothesis. It is more likely that the differences in the steady state levels of algal growth were determined by nutrient variability rather than by intact or degraded surfactant products.

The results of the static bioassay of the LDBS effluents indicate that all of the effluents supported substantial levels of algal growth by day nine (Figure 7). The 12-hr control effluent supported the greatest standing crop while there was no significant difference between the other three treatments. The evaluation of the maximum specific growth rates for the treatments indicated that there were no significant differences in the maximum rates of growth among the treatments even though the corresponding standing crop levels were different in certain instances (Table XXV). These results indicate again that the differences in algal growth levels among the treatments were due to nutrient level variability within the sludge units.

The results of the LDBS studies indicate that the LDBS mixture was toxic to S. capricornutum at concentrations of 10 mg MBAS/1. However, the MBAS levels in both experimental effluents were far below the threshold toxicity level. Therefore, the effect of biodegradation on the toxicity of LDBS to S. capricornutum was to reduce the initial MBAS levels to sublethal concentrations. The two residence times used failed to vary the degree of surfactant degradation, however the algal bioassay procedures which were used successfully evaluated the capacities of the various solutions tested to support algal growth.

Primary Alcohol Ethoxylate (Nonionic)

Static tests of the intact primary alcohol ethoxylate for toxicity to S. capricornutum indicated that the 1.0 mg Nonionic/l treatment was the only surfactant level that significantly decreased standing crop levels (Figure 8). Therefore, this concentration represents the approximate threshold of toxicity under the experimental conditions used. Analyses of the maximum specific growth rates for the treatments indicated that the highest surfactant concentration supported the highest growth rate (Table XXVII). However, this is deceiving as Figure 8 clearly shows. The abrupt growth stimulation after seven days in the 1.0 mg Nonionic/l treatment probably resulted from the bacterial breakdown of the initial surfactant level. Thus, when the toxic surfactant levels were reduced by the bacteria to sublethal levels, the algae then commenced to reproduce logarithmically. This also indicated that the 1.0 mg/l concentration was not algicidal but algistatic.

The results of the first continuous-flow bioassay on the sludge unit primary alcohol ethoxylate effluents indicate that all of the effluents supported substantial levels of algal growth (Figure 9). The chemical parameter levels monitored on the sludge unit effluents throughout the primary alcohol ethoxylate study were very similar for all effluents (Table XXVIII). Also, the levels were very close in magnitude to those obtained in the LTBS and LDBS studies (Table IV and Table XVIII, respectively). The nitrogen and phosphorus levels again were quite variable (Table XXX). The levels were very similar to those observed in the LDBS study (Table XXII). The numerous high coefficients of variation indicate that the nutrient levels fluctuated considerably during the assay period.

The hydraulic residence times for all treatments were very close to three days (Table XXXI). Table XXVIII shows that the surfactant levels in the experimental effluents of both residence times were almost identical. Therefore, the difference in residence period again failed to vary the degree of surfactant degradation. Because the surfactant levels in the experimental effluents were practically identical and in such small amounts, the differences in the algal growth curves probably reflect the fluctuations of algal nutrient levels within the sludge units.

The results of the second continuous-flow bioassay on the sludge unit primary alcohol ethoxylate effluents also indicate that all of the effluents supported substantial levels of algal growth (Figure 10). As was mentioned previously, the chemical parameter levels monitored on the sludge unit effluents throughout the primary alcohol ethoxylate study were very similar for all effluents (Table XXVII). However, as was true in the first continuous-flow bioassay, the nitrogen and phosphorus levels fluctuated considerably during the assay period (Table XXXV). The hydraulic residence times for all treatments were close to three days, and flow rate precision was good (Table XXXVI). Since the surfactant levels in the experimental effluents were both in excess of 1.0 mg/l, inhibition of algal growth might have been expected based on the threshold toxicity studies of the intact surfactant. However, the high coefficients of variation associated with the effluent surfactant levels indicate that the levels were quite variable during the assay period. Also, antagonistic effects by other substances present in the effluents may have reduced the toxicity of the surfactant. Therefore, since the surfactant levels in the primary alcohol ethoxylate experimental effluents were shown to be quite low and

practically equal, the differences in the algal growth curves for the second continuous-flow assay probably reflect the fluctuation of algal nutrient levels within the sludge units. The approximations of steady state levels of growth for the treatments indicate that all the treatments supported essentially the same levels of algal growth for at least one hydraulic residence time (Table XXXIII). These approximations were not made for the first continuous-flow assay because of the general lack of periods of stable algal growth among the treatments (Figure 9). The mean hydraulic residence times for the steady state periods of the second continuous-flow assay indicate that the flow rates were very precise and accurate (Table XXXIV). Thus, the steady state approximations represent the periods of the second continuous-flow assay during which the nutrient levels among the various effluents were most constant. The algal growth levels maintained during these periods indicate that nutrient levels rather than surfactant levels determined the algal growth potentials of the effluents.

The results of the primary alcohol ethoxylate studies indicate that the nonionic surfactant was toxic to S. capricornutum at 1.0 mg Nonionic/l. This an order of magnitude lower than the threshold toxicity levels for the LTBS and LDBS mixtures. The effect of biodegradation on the toxicity of the primary alcohol ethoxylate to S. capricornutum was to reduce the initial surfactant levels to sublethal concentrations. Also, the nonionic surfactant breakdown products were apparently not toxic since there were no substantial differences among the steady state levels of growth. The two residence times used failed to vary the degree of surfactant degradation, however the algal bioassay procedures successfully evaluated the capacities of the various solutions tested to support algal growth.

Zinc Toxicity and Temperature Studies

Static Algal Bioassays

The results of the static bioassay on zinc at 24° C indicate that only the 0.10 mg Zn/l treatment inhibited S. capricornutum growth (Figure 11). This zinc concentration allowed only 11% of the control level of growth after seven days (Table XXXVIII). Figure 8 also indicates that the 0.10 mg Zn/l treatment was essentially algistatic. The maximum specific growth rate data indicate that only the 0.10 mg Zn/l treatment resulted in a significantly lowered growth rate as compared to the control rate (Table XXXIX). It is also interesting to note the differences in maximum growth rates for S. capricornutum in NAAM during this study (Table XXXIX) and during the LTBS, LDBS, and nonionic studies (Tables III, XVII, and XXVII, respectively). The value in Table XXXIX is considerably less than 1.0 while all three values in the other tables are greater than 1.0. This indicates how agitation can affect the growth rates of algae. All of the static assays on the intact surfactants were carried out with continuous shaking. The zinc bioassays were carried out in an incubator, and the flasks were shaken manually once daily. The maximum growth rates for the continuously shaken cultures were, in each case, considerably greater than for the manually shaken cultures. However, the maximum standing crop attainable by the algae was not altered by the use of agitation.

The results of the static bioassay on zinc at 29° C indicate that both the 0.06 and 0.10 mg Zn/l treatments inhibited S. capricornutum growth (Figure 12). The 0.10 mg Zn/l treatment allowed only 2% of the control level of growth (as compared to 11% at 24° C), and the 0.06 mg Zn/l treatment allowed only 31% of the control level of growth (as compared to

87% at 24° C) (Table XLI). These findings indicate that the highest zinc concentration was more toxic at 29° C than at 24° C and that the intermediate zinc concentration was toxic at 29° C after minimal effects at 24° C.

The results of the static bioassay on zinc at 19° C indicate that both the 0.06 and 0.10 mg Zn/l treatments inhibited S. capricornutum growth (Figure 13). The 0.10 mg Zn/l treatment allowed 13% of the control level of growth (as compared to 11% at 24° C and 2% at 29° C), and the 0.06 mg Zn/l treatment allowed 73% of the control level of growth (as compared to 87% at 24° C and 31% at 29° C) (Table XLIII). The maximum specific growth rate data indicate that the 0.06 and 0.10 mg Zn/l rates were both significantly and successively lower than the control rate, while the 0.02 mg Zn/l rate was slightly higher than the control rate. The degree of algal growth inhibition for the various zinc concentrations tested were very similar for the 19 and 24° C studies. However, the degree of algal growth inhibition at 29° C was considerably greater for the two highest zinc concentrations than at the two lower temperatures. The temperature was apparently high enough at 29° C to significantly accelerate the rates of the physiological processes involved in the inhibition of algal growth by zinc. Various hypotheses of the mechanism(s) of the toxicity were described previously.

Photosynthesis and Respiration Rates

The effects of temperature on the toxicity of zinc to S. capricornutum were analyzed by measuring both algal standing crop levels and growth rates. Static algal bioassay procedures which were heretofore described were used in these tests. To supplement these results, the physiological responses of the test organism to zinc was measured by monitoring the

oxygen production and consumption rates using light and dark bottle techniques. The parameters measured were net photosynthesis and respiration. This study was extraneous to the evaluation of the use of algal growth potential techniques for toxicity studies, but it was conducted, nevertheless, to further characterize and define the responses of S. capricornutum to zinc within a range of temperatures.

The results of the first exposure to zinc at 24° C indicate that both the 0.06 and 0.10 mg Zn/l treatments inhibited oxygen production during algal photosynthesis (Table XLV). The lowest zinc concentration tested was not significantly different from the control. The 0.10 mg Zn/l treatment allowed an oxygen production rate of about 59% of the control level, while the 0.06 mg Zn/l treatment allowed a rate of about 89% of the control. Respiration was not affected by any of the zinc treatments, thus the toxic action of zinc must have inhibited a photosynthetic rather than a respiratory pathway.

The results of the second exposure to zinc at 24° C essentially confirmed the findings of the first test. The inoculum concentration for the second test was slightly reduced (to 2.63×10^5 cells/ml), but the resulting net photosynthetic rates were close to those measured in the first test (Table XLVI). Both the 0.06 and 0.10 mg Zn/l treatments inhibited oxygen production during algal photosynthesis. Again, the 0.02 mg Zn/l treatment response was not significantly different from the control response. The 0.10 mg Zn/l treatment allowed an oxygen production rate of about 44% of the control level (as compared to 59% in the first test), and the 0.06 mg Zn/l treatment allowed a rate of 84% of the control (as compared to 89% in the first test). Again, respiration was not

affected by any of the zinc treatments. Presumably, the reason that the 0.10 mg Zn/l percentages were higher in the light and dark bottle studies than in the static bioassays was that the initial cell concentration in the photosynthetic rate studies was generally 300 times greater than in the static assays. The initial cell concentration is important in characterizing the growth responses of algae in culture. If the initial cell concentration is great enough, the normal lag phase response may be eliminated entirely (Fogg 1965). This fact may be very important in cultures containing toxic substances. High initial cell concentrations may be able to tolerate a toxicant concentration that would be lethal to a smaller number of cells. In this respect the oxygen production and consumption studies are probably not strictly comparable (quantitatively) to the standing crop studies.

The results of the first exposure to zinc at 29° C indicate that only the 0.10 mg Zn/l treatment inhibited oxygen production during algal photosynthesis (Table XLVII). If zinc is more toxic at higher temperatures (as the static bioassays suggested), both of the two highest zinc concentrations in this test should have reduced the photosynthetic rate. Also, the degree of reduction should have been greater at 29° C. However, as was mentioned previously, this may not be the case when the initial cell concentration is substantially increased. The degree of inhibition of photosynthesis by 0.10 mg Zn/l at 29° C was similar to that at 24° C. Thus, at the cell concentrations used, temperature was not having a substantial effect on the degree of inhibition by zinc. In this test, the respiration rates were significantly different with the 0.02 mg Zn/l treatment consuming the least amount of oxygen and the 0.10 mg Zn/l

treatment consuming the greatest amount of oxygen. Of all the respiration rates measured throughout the oxygen studies, this was the only test in which the rates for the various treatments were significantly different, regardless of temperature. Therefore, the results of this particular test are probably erroneous.

The results of the second exposure to zinc at 29^{o} C yielded the same statistical findings as in the first test. The inoculum concentration for the second test was reduced by a factor of 2 (to 1.50×10^{5} cells/ml), and the resulting net photosynthetic rates were roughly half of those in the first test (Table XLVIII). Again, only the 0.10 mg Zn/l treatment inhibited oxygen production during algal photosynthesis. The respiration rates also were roughly half of those in the first test, but in this test there was no significant difference among any of the treatments. Neither of the two exposures at 29^{o} C indicated that the increase in temperature from 24^{o} C substantially affected the degree of photosynthetic rate inhibition by zinc.

The results of the first exposure to zinc at 19^{o} C indicated that only the 0.10 mg Zn/l treatment inhibited oxygen production during algal photosynthesis (Table XLIX). The 0.10 mg Zn/l treatment allowed an oxygen production rate of about 65% of the control level. Again, this degree of inhibition is comparable to those previously discussed for the 0.10 mg Zn/l treatment at other temperatures. There was no significant difference in any of the respiration rates for any of the treatments.

The results of the second exposure to zinc at 19^{o} C indicated findings similar to those of the first test. The main difference was that in the second test, the 0.06 mg Zn/l treatment (along with the 0.10 mg Zn/l

treatment) inhibited oxygen production during algal photosynthesis (Table L). The 0.10 mg Zn/l treatment allowed an oxygen production rate of about 62% of the control level (as compared to 65% in the first test), and the 0.06 mg Zn/l treatment allowed a rate of about 81% of the control. The degree of inhibition by the 0.10 mg Zn/l treatment in the second test was very close to that in the first test. As was found in the first test, none of the respiration rates were significantly different for any of the treatments.

The static bioassays on zinc at different temperatures indicated that with an initial cell concentration of 10^3 cells/ml, an inhibitory zinc concentration at 24° C (0.10 mg Zn/l) was more inhibitory at 29° C. Also, a zinc concentration that was not inhibitory at 24° C (0.06 mg Zn/l) became very inhibitory at 29° C. The algal growth levels produced at 19° C were very close to those at 24° C, thus a temperature increase from 19 to 24° C did not enhance the toxicity of zinc.

The light and dark bottle studies indicated that a concentration of 0.10 mg Zn/l inhibited oxygen production by S. capricornutum at all temperatures tested. The differences in the degree of inhibition at the various temperatures were not great enough to show a synergistic effect between temperature and zinc. The results also show that zinc inhibited (at least initially) a photosynthetic pathway rather than a respiratory pathway. The fact that 0.06 mg Zn/l was inhibitory in some tests but not in others indicates that this concentration was very close to the threshold of toxicity for the conditions used. Thus, any normal variations in procedures due to inherent experimental error would cause this treatment to elicit responses that might be statistically significant in one case

but not in another. These studies also confirm the toxic levels of zinc to S. capricornutum reported by Bartlett et al. (1974) and the general site of toxic action of zinc in eucaryotic algae reported by Greenfield (1942).

SUMMARY

This investigation was undertaken to evaluate the use of algal growth potential techniques for toxicity studies of surfactants and zinc. The results show that these procedures can accurately and precisely evaluate the capacity of a given medium to support algal growth under the conditions specified. The capacity for detecting the presence and the physiological effects of toxic substances was also demonstrated. Results such as these are valuable supplements to physical and chemical data.

The studies of the three surfactants indicated that sufficient concentrations of all three compounds were toxic to *S. capricornutum*. These concentrations were never attained in the sludge unit effluents since varying the residence period failed to vary the degree of surfactant degradation. Thus, the algae never were inhibited by excessive surfactant levels in the effluents. However, the algal bioassay procedures used were successful in evaluating the algal growth potentials of the various effluents tested. These assay procedures also were successful in determining the approximate thresholds of toxicity for all three intact surfactants in algal nutrient medium. The results of these tests showed that the LTBS and LDBS mixtures were toxic to *S. capricornutum* at approximately the same levels (10 mg MBAS/l). Also, the nonionic surfactant was much more toxic to the algae than the other two mixtures (with a threshold of 1.0 mg Non-ionic/l). The results of both static and continuous-flow tests reflected the suitability for algal growth of the physical and chemical conditions of the samples at given times during an assay. Nutrient fluctuations

were indirectly monitored by observance of the changes in algal growth levels when toxic substances were present in sublethal amounts. Through the use of algal bioassay techniques, the effects of biodegradation on the toxicity of surfactants to S. capricornutum were successfully evaluated. For all surfactants, these effects were to reduce the surfactant concentrations to sublethal levels.

Algal bioassay procedures were also successful in evaluating the toxicity of zinc to S. capricornutum. The threshold of toxicity was determined and confirmed several times (0.06 mg Zn/l), and the effects of temperature on zinc toxicity to the algae were demonstrated under controlled conditions. These results indicate that a temperature increase from 19 to 24° C did not substantially change the degree of toxicity of a given zinc concentration. However, the same concentrations were significantly more toxic at 29° C than at the two lower temperatures. Assuming that the metabolic rate is a primary factor in zinc toxicity (which was suggested by several authors), these findings indicate that the change in metabolic rate (and consequently, the degree of zinc toxicity) in increasing from 19 to 24° C is not linearly proportional to the change in increasing from 24 to 29° C.

The oxygen studies showed that zinc toxicity affects the photosynthetic rate but not the respiratory rate. However, significant differences in the degree of toxicity due to changes in temperature could not be demonstrated. The reason for this was probably the increase in the initial cell concentration which was necessary to elicit measurable changes in oxygen production over relatively short periods of time. The results confirm earlier reports of the role of zinc in the inhibition of the photosynthetic process. The discovery of the exact site of toxic action

of zinc will involve the isolation and evaluation of the individual steps of various photosynthetic metabolic pathways.

The results of both the surfactant and zinc studies have demonstrated the applicability of algal bioassay techniques for both algal growth potential and toxicity studies. Use of these procedures in future studies with similar objectives is certainly recommended.

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THE APPLICATION OF ALGAL GROWTH POTENTIAL TECHNIQUES TO SURFACTANT AND ZINC TOXICITY STUDIES

by

Frank A. Camp, III

(ABSTRACT)

Studies were undertaken to evaluate the suitability of algal growth potential techniques for testing the toxic effects of surfactants and zinc to an algal test organism. The algal growth potential techniques used included both static and continuous-flow algal bioassay procedures. The algal test organism selected was Selenastrum capricornutum Printz. The surfactants tested included two anionic mixtures (linear tridecyl benzene sulfonate and linear dodecyl benzene sulfonate) and a nonionic surfactant (primary alcohol ethoxylate).

The surfactant toxicity studies were conducted by feeding a surfactant-synthetic sewage mixture into a series of bench-scale activated sludge units. Both control and experimental sludge units were operated for each of two residence times. The effluents from the sludge units were bioassayed to evaluate the effects of biodegradation on the toxicity of the surfactants to S. capricornutum. The effluents were bioassayed using static and continuous-flow algal bioassay procedures. Toxic effects to the alga were determined by measuring variations in standing crop levels and maximum specific growth rates. Also, algal bioassays were conducted on selected concentrations of the intact (unbiodegraded) surfactants in algal nutrient medium.

Results of these studies indicated that the effects of biodegradation on the toxicity of surfactants to S. capricornutum were to reduce the surfactant concentrations in the effluents to sublethal levels. For all three surfactants tested, the levels in the effluents were less than or equal to 1 mg/l. Bioassays of the intact surfactants indicated thresholds of toxicity of 10 mg MBAS/l for both anionic mixtures and 1 mg/l for the nonionic surfactant. Although the effluents were not toxic to the alga, the bioassay procedures used successfully evaluated the algal growth potentials of the solutions tested. Also, the algal bioassays of the intact surfactants successfully determined the toxic levels of the respective compounds.

Additional studies were undertaken to determine if the toxicity of the heavy metal zinc to S. capricornutum was affected by temperature. Static algal bioassay procedures were used. The zinc concentrations tested included 0.02, 0.06, and 0.10 mg Zn/l. Incubation temperatures selected included 19, 24, and 29^{o} C. Toxic effects again were determined by measuring standing crop and maximum specific growth rate levels.

Results indicated that the threshold of zinc toxicity to S. capricornutum was approximately 0.06 mg Zn/l. Results also indicated that the degree of algal growth inhibition by 0.06 and 0.10 mg Zn/l was significantly greater at 29^{o} C than at the two lower test temperatures. Thus, increased incubation temperatures apparently increased the degree of algal growth inhibition by a given zinc concentration.

Light and dark bottle studies were used to test the effects of the previously described zinc concentrations and temperatures on the photosynthesis and respiration rates of S. capricornutum. Results showed that

zinc toxicity affected photosynthetic rates but not respiration rates. No differences in photosynthetic rate inhibition by zinc at the three temperatures tested could be demonstrated. This possibly was due to the need for a highly concentrated algal inoculum in the bottles so that measurable oxygen levels would be produced in a short time period. Inoculum concentration is an important factor in toxic responses of algae.