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Technical Report

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ABSTRACT

Effects of Support Media and Media Precoating on Anaerobic Biofilm Development

Fixed film (attached growth) anaerobic reactors often require long periods of time to obtain optimum levels of treatment. This is primarily due to the slow accumulation of methanogenic and anaerobic bacteria on the support surfaces within the reactor. Two experiments were performed in order to investigate if the rate of anaerobic biofilm development could be enhanced. The first experiment explored the use of four different materials on anaerobic and methanogenic biofilm development. The second experiment explored the possibility of enhancing anaerobic and methanogenic biofilm development through the use of denitrifying bacterial precoating. Two support materials were used in the second experiment.

All experiments were carried out in a 15 liter anaerobic slurry reactor. The reactor's solids retention time (SRT) was maintained at five days with a nutrient broth/sucrose feed of 5,600 mg COD/l strength. A relative increase of methanogenic bacteria in the attached biofilm was monitored by fluorimetric techniques (nanomoles F-420/cm²). The accumulation of anaerobic bacteria on the support media was monitored by COD analysis of the biofilm materials ($\mu\text{g COD/cm}^2$). Initial rates of anaerobic development were determined from the linear slope of μg

COD/cm² data versus time. Fluorimetric analyses, for the most part, proved inconclusive for this study.

Results from the first experiment, support media variation, indicated that materials with very high or very low critical surface tensions provide faster rates of anaerobic attachment. The results of the first experiment also indicated that materials which corrode in the anaerobic environment, or exhibit toxic effects on anaerobic bacteria produce lower rates of anaerobic biofilm attachment. Roughly speaking, a 30 to 50 percent increase in anaerobic biofilm accumulation rates was observed by using Teflon and stainless steel as support media rather than PVC and aluminum.

Results from the second experiment, support media precoating, indicated that support media precoated by five days of denitrifying growth exhibited slightly better rates of anaerobic accumulation than for media precoated for only one day and dipped and air-dried (into denitrifying culture). Denitrifying precoating caused anaerobic biofilm accumulation rates to lag uncoated rates up to ten days. However, final rates of attachment were generally the same for uncoated and precoated materials averaged over a 15 day experimental period.

Based on the findings of the two experiments, the start-up times of anaerobic fixed reactors may be reduced by constructing reactors which utilize support materials with very high and very low critical surface tensions, and which exhibit no toxic effects or surface degradation in the anaerobic environment. Support media precoating with

denitrifying bacterial biofilms does not appear to enhance initial anaerobic biofilm accumulation. Other forms of support media precoating should be investigated to determine if this technique may in fact reduce fixed film reactor start-up.

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CHAPTER I

INTRODUCTION

Since the early 60's, when McCarty (32) and others (40) first began to investigate the fundamentals of anaerobic waste treatment, rapid advances have taken place in both the basic science and applied aspects of anaerobic methane fermentation. With the onslaught of the environmental movement and the energy crisis of the 70's, further emphasis was placed on anaerobic waste treatment technology. In the hope that conventional, energy intensive, aerobic waste treatment systems would yield way to low-energy requiring systems, researchers have made enormous advances in the understanding of the anaerobic process over the past ten years.

There are advantages and disadvantages to the anaerobic treatment of wastes. When compared with aerobic treatment, anaerobic treatment has the advantages of 1) having a higher degree of stabilization; 2) producing lower volumes of sludge; 3) requiring fewer nutrients; 4) lacking oxygen or the need for aeration; and 5) producing methane as a useful by-product. However, the disadvantages include: 1) the slow growth rate of methanogenic bacteria; 2) the sensitivity of methanogens to environmental stress; 3) high temperature requirements for optimum growth (35°C); 4) the long solids retention times required; and 5) an unwarranted belief that anaerobic treatment is unreliable (32,43).

Several research engineers and scientists have examined a number of anaerobic reactor configurations and designs. Many of these systems display rather novel techniques for enhancing the anaerobic process, and methods for increasing reliability and efficiency. One trend among the newer anaerobic treatment schemes is the utilization of the bacteria's ability to attach to solid surfaces. Fixed film processes, as they are called have proven to be capable of maintaining high solids retention times (SRT) while being run at hydraulic retention times which are lower than slurry systems (27,41,52). These systems also obtain a higher degree of waste stabilization by concentrating the biomass within the reactor volume.

Although some of the newer anaerobic reactor configurations hold promise as being versatile treatment systems, most share a common problem of having prolonged start-up times. Once a reactor has been started, development of a stable biofilm may take several months. Periods of six to twelve months to reach optimum performance have been reported for fluidized bed anaerobic reactors (42,48). Since, for the most part, the growth rate for methanogenic bacteria cannot be altered significantly through physical and biochemical means, methods for decreasing the start-up period by increasing the attachment rate of anaerobic bacteria to a support surface may be one particular method of improving anaerobic process start-up times.

One such means of reducing start-up in anaerobic fixed film reactors may be by using materials for which methanogens have a higher

affinity for adsorption. If a particular group or type of material were to prove favorable for methanogenic attachment, it might be wise to build future anaerobic fixed film reactors utilizing these materials as a support surface. Another means of accelerating start-up may be the use of biologically precoated support media.

The major objective of this study was to investigate the possibility of reducing anaerobic reactor start-up times through the use of different support surfaces, and through the use of biological precoating as a method of enhancing anaerobic biofilm development. In regards to determining a preferred support material, four materials, polyvinylchloride (PVC), tetrafluoroethylene (teflon), stainless steel and aluminum were examined. These materials represent a wide range of common materials, and differ by their own unique surface chemistries. Biological precoating of support surfaces was accomplished by subjecting surfaces to denitrifying bacteria prior to anaerobic treatment. Two support media were examined in this second phase: polyvinylchloride and aluminum. These investigations and the methods involved with them will be discussed in greater detail in the following sections.

A previous study conducted at the University of Massachusetts investigated methods of monitoring methanogenic activity in anaerobic reactors (35), and another investigated the effects of SRT, organic loading, and fluid shear velocity variations on anaerobic biofilm development (38). The research performed through this study is a

follow-up to these investigations, and utilizes many of the theories and methods which they have set forth.

C H A P T E R I I

BACKGROUND

2.1. Anaerobic Microbiology. Anaerobic treatment of wastes, put in simplest terms, is the conversion of complex organic matter to CH_4 and CO_2 by an interdependent group of bacteria which metabolize in the absence of molecular oxygen. This is quite different from aerobic operations which contain several diverse microbiological communities and complex food chains (18). The anaerobic process is a multi-step process in which each bacterial group carries out a particular function. This process is depicted step-wise in Figure 1.

Organic matter in waste streams may take several forms. Large organic molecules must be reduced in size in order for cellular uptake and metabolism to occur. This action takes place during the first step of the anaerobic process, and is referred to as hydrolysis. Reactions during hydrolysis are catalyzed by enzymes released to the medium by the fermentative bacteria and are usually hydrolytic in nature (18).

By-products of the hydrolysis step, smaller organic molecules, are utilized by a rather diverse group of facultative and obligatory anaerobic bacteria as carbon and energy sources. The metabolic pathways of these bacteria ultimately result in the fermentative end-products of several short-chain volatile organic acids such as acetic, propionic, butyric, isobutyric, valeric and iso-valeric, as well as hydrogen and

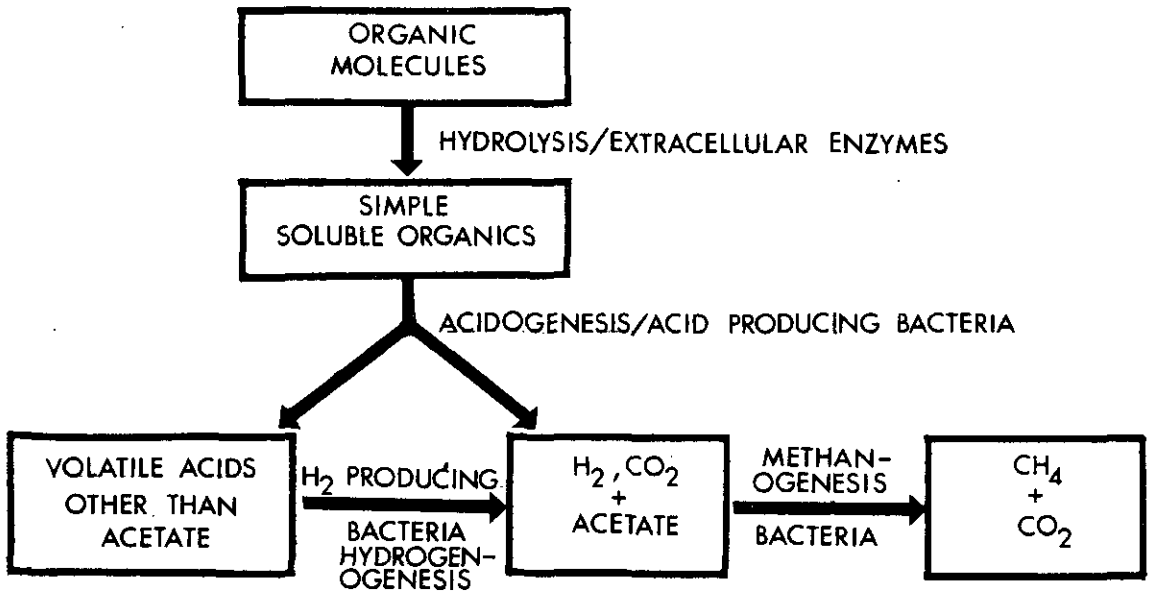


Figure 1. Anaerobic degradation of organic matter.

carbon dioxide. The production of the organic acids, acidogenesis, is mediated by acid-producing bacteria. Some of the acid-producing bacteria possess a specialized enzyme system which allows them to oxidize reduced coenzymes without passing the electrons to an organic acceptor, thereby releasing hydrogen gas to the medium (18,19)). Organic acids larger than acetic acid can be utilized by bacteria to produce acetic acid, CO_2 and H_2 . This step is vitally important to the complete process as nearly 72 percent of the methane produced originates from acetate during the reduction of a complex waste (32). The bacteria carrying out this conversion of large organic acids are called hydrogen-producing bacteria, and their process step is referred to as hydrogenogenesis (18).

The production rate of acids is high compared to the methane production rate, which means a sudden increase in easily degradable (soluble) organics will result in increased acid production with a subsequent drop in the system pH (if the system has a low alkalinity) due to the accumulations of acids.

The final step in the anaerobic process is the production of methane from acetic acid, hydrogen and carbon dioxide. Methane production is a slow process, and is the rate-limiting step of anaerobic degradation of soluble compounds. It is carried out by two groups of methanogens which are different from one another. One group of bacteria obtains its energy from the oxidation of molecular hydrogen whereas the other group oxidizes acetate. With the exception of losses due to

microbial inefficiency and growth yield, almost all of the energy removed from the electron donor is recovered in the methane. One mole of methane requires two moles of oxygen to be oxidized to CO_2 and H_2O . Consequently each 16 grams of methane produced corresponds to a removal of 64 grams of COD. At standard temperature and pressure this corresponds to 5.62 ft^3 of methane for each pound of COD stabilized (18,32).

2.2. Anaerobic Kinetics. An approach to the mathematical modelling of microbial growth was developed by Monod. Since Monod's time, the question of which mathematical formula best expresses microbial growth has been the subject of much debate, and several amended versions of Monod's equation have resulted. Consequently, Monod's work has obtained the greatest historical precedence and acceptance as a mathematical tool for kinetic study (18,25). The use of bacterial growth kinetics is two fold: 1) to translate interesting scientific hypotheses into predictions of process performance which can be tested by experiment, and 2) to translate the qualitative observations of the microbiologists into process design parameters that can be used for the design and operation of the process (34).

A completely mixed anaerobic digester operated with continuous feed and withdrawal will closely resemble a chemostat reactor. If it is operated at a steady flow rate using a medium of constant strength and composition, it will eventually achieve a steady state condition. The

relationship between biological growth and substrate uptake for such a system can then be mathematically described through a combination of two equations: one which describes the net growth rate of microorganisms in relation to substrate utilization, and one which relates the rate of substrate utilization to: 1) the concentration of substrate surrounding the microorganisms, and 2) to the concentration of microorganisms in the reactor (25,34).

Several important design parameters result from the kinetic study of microbial processes. Henze and Harremoës (19) analyzed data from several sources in regards to the growth coefficients, maximum specific growth rate, yield, and maximum substrate removal rate for anaerobic slurry bacterial cultures (acid-producers, methane-producers, and mixed) to obtain the growth constants of Table 1.

In fixed film reactors, kinetics of microbial reactions take on another form. The requirement for movement of the organic matter from the bulk phase through the boundary layer (see Figure 2) and into the biofilm causes the substrate concentrations surrounding the microorganisms to be less than the concentration the liquid phase (i.e. a substrate concentration gradient is present). Since the rates of microbial reactions are determined by the concentration of substrate surrounding the microbes, it is necessary to combine physical mass transport theory with microbial reaction theory when modelling fixed film reactors (18).

Table 1. Growth Constants of Anaerobic Cultures (19)

Parameter	μ_{\max} Maximum specific growth rate at 35°C, days ⁻¹	y_{\max} Maximum yield coefficient, kg VSS/kg COD	$r_{\max} = \mu_{\max}/Y_{\max}$ Maximum substrate removal rate at 35°C, kgCOD/kg VSS · day 50% 100% active 50% active	
Culture				
↓				
Acetic acid producing bacteria	1.5	0.15	10	5
Methane- producing bacteria	0.30	0.03	1.7	0.85
Combined culture	0.30	0.18	1.7	0.85

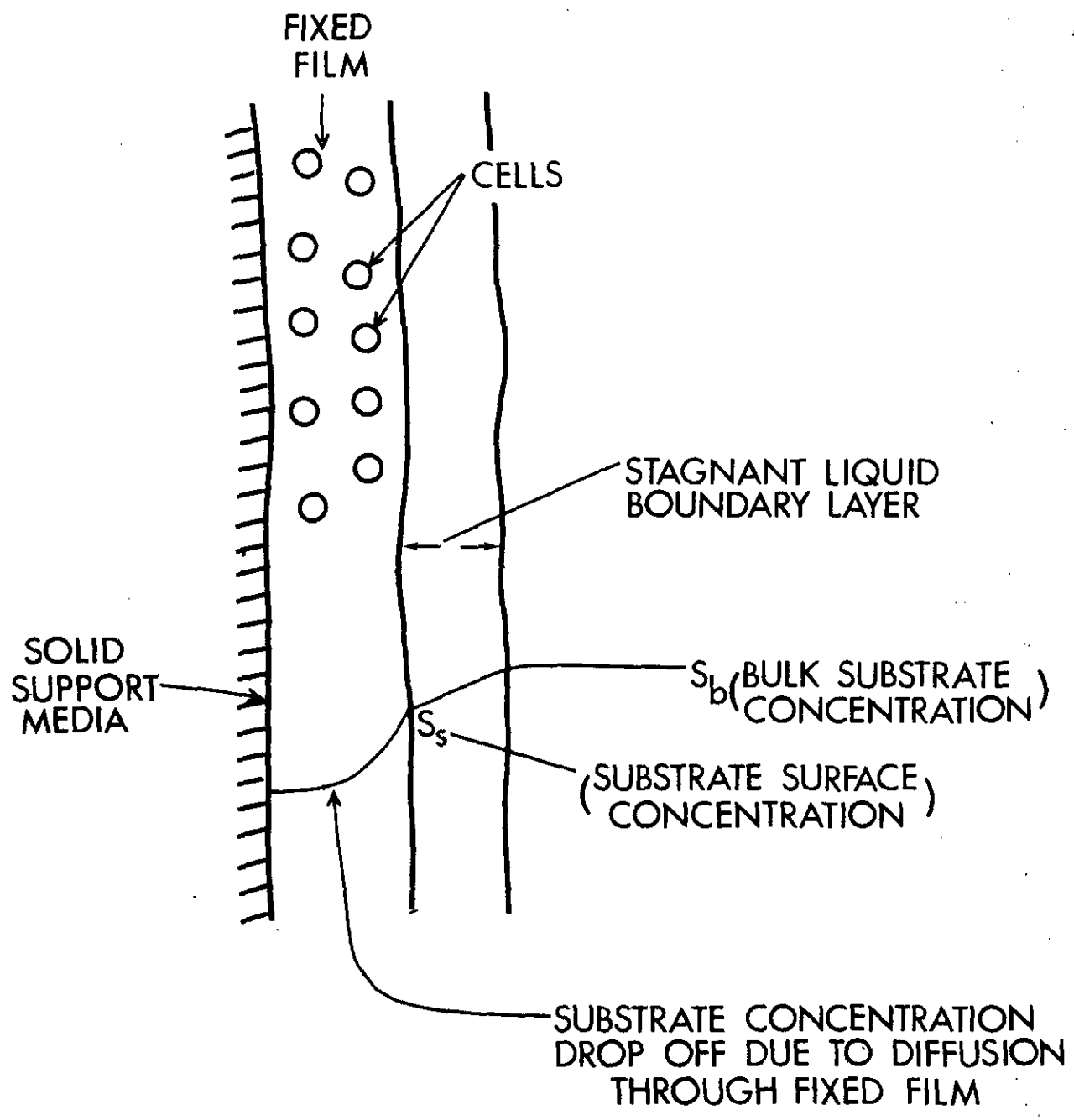


Figure 2. Substrate concentration profiles for reactions within a thick biofilm.

To account for this change in substrate concentrations, internal and external effectiveness factors are applied to Monod kinetics. Effectiveness factors may be regarded as correction factors, which when multiplied by rate equations without mass transfer resistance, give the actual rates in the presence of mass transfer resistance.

Although more thorough presentations of anaerobic and biofilm kinetics could be presented at this point, such material is beyond the scope of this report. At the present moment current science and experimental techniques do not allow one to accurately study and measure the actual kinetic parameters involved in anaerobic biofilm kinetics. More research is needed to verify the microbial-substrate interactions which take place in biofilms. This condensed material is presented to the reader as insight. For further understanding of this subject the readers is referred to other references (2,18,21,26,34).

2.3. Bacterial Attachment. Bacteria in the natural environment are often attached firmly to some surface. Sometimes bacteria will attach to certain surfaces more readily than others. Work done by marine biologists and engineers has shown that colonization of a solid surface by bacteria is a rather selective process which is affected by many environmental conditions. Ionic strength, pH, substrate and nutrient concentration, water temperature, and the surface properties of the solid, are all thought to influence biofilm attachment and development.

Although there is some knowledge about the dynamics of biofilm development, and a considerable body of information about the kinetics of substrate removal by biofilms, the mechanisms and factors governing microbial attachment and biofilm development are still unclear.

There exists little scientific literature on the mechanisms of microbial attachment onto surfaces in anaerobic reactors. Most of the research which has taken place in the area of bacterial attachment has involved biofilm fouling and its prevention; places where biofilms cause more harm than good (i.e., heat exchangers, functional resistance in pipes, boat hulls, etc.). Therefore the status of our understanding of the start-up of anaerobic fixed-film reactors is experimental rather than scientific (24,37).

2.3.1 The Process of Attachment and Influencing Factors. Characklis and co-workers summarized the steps which take place during biofilm formation: 1) the initial transport of organics and bacteria to the support media, 2) the exponential growth of biofilm, and 3) the steady-state or constant thickness phase in which the detachment rate of biomass is equal to its accumulation rate (3,5).

The first stage of attachment, where bacteria and organics are being absorbed to the support surface, is characterized by the formation of a conditioning film from which biofilm growth and development takes place. Bacterial cells are thought to contact submerged surfaces by a variety of physio-chemical and biological attraction mechanisms. These

include Brownian motion, electrostatic attraction, convective currents, hydrophobicity and possibly chemotaxis (24).

Daniels (9) and Fletcher (16) have done extensive research into the factors affecting adsorption to the support surface. A list of these factors is presented in Table 2. Almost all of these factors have a direct effect, or deal exclusively with the electrostatic surface charges exhibited by the support media and bacteria. These surface charge interactions between the bacteria and media contribute the most of any force affecting the adsorption, or the desorption of cells which are adsorbed (24). Marshall observed that the initial adsorption of bacteria to submerged surfaces occurred almost instantaneously (31). Daniels, in his review of the subject, found that, most of the time, adsorption had for all practical purposes halted after 15 minutes (9). Fletcher studied the effects of culture age, time, temperature and bacterial growth phase on adsorption. Cells were found to adsorb best in log growth and worst in the death phase (16).

The second state of attachment, in which biofilm thickness undergoes exponential growth, is characterized with the production by the adsorbed bacteria of extracellular polysaccharides. These extracellular polymers extend from the cell surface to the media to form a matrix of tangled fibers. This phenomenon was closely observed by Costerton et al. who termed this matrix of polymer a "glycocalyx" (8). Corpe examined the chemical composition of these polysaccharides while examining various species of marine bacteria (7). After recovering some

Table 2. Variables Affecting the Sorption of Microorganisms to Solid Surfaces [from Daniels (30)]

1. Character of the microorganisms

- a. Species
- b. Culture medium
- c. Culture age
- d. Concentration of biomass in the medium

2. Character of the support media

- a. Material surface charge
- b. Concentration
- c. Media size
- d. Surface to volume ratio

3. Character of the environment

- a. pH
- b. Ionic strength of the solution
- c. Fluid shear forces
- d. Presence of toxins
- e. Temperature

of these polysaccharides, Corpe found them to be acidic mucopolysaccharides chiefly composed of polyanionic carbohydrates.

The final stage of the attachment process, or steady-state thickness phase, where the detachment rate of biomass is equal to its accumulation rate, has little importance in the study of attachment. It is merely an indicator for the final stage of the attachment process, where the effects of mass transfer, diffusion of substrate and nutrients into the biofilm, and cell lysis appear to weigh equally with cell growth.

2.3.2. Bacterial Attachment in Various Anaerobic Reactor

Configurations. Several research engineers and scientists have examined a number of anaerobic fixed-film reactors. Unlike conventional systems which are incapable of separating solids retention time from hydraulic retention time without solids recycle, fixed-film reactors utilize bacterial attachment to achieve this. Four types of anaerobic fixed film reactors will be presented in this discussion. These include the anaerobic filter (AF) the upflow anaerobic sludge blanket (UASB), the fluidized or expanded bed reactor (AEB), and the downflow stationary fixed film reactor (DSFF).

The AF reactor was developed by Young and McCarty (52), and resembles an upflow trickling filter. Waste enters in the bottom and flows upwards through packing composed of rocks or plastic media, with biomass collecting in the void spaces on the surface. Much of the

biomass is present in suspended form in the void spaces with a relatively small portion attached to the surface. Since the suspended growth tends to collect in the bottom of the reactor, most of the activity is in the reactor bottom. The growth on the surfaces provides a polishing action, while the packing serves to separate the gas and to provide quiescent areas for settling of suspended growth (50).

The UASB reactor was developed by Lettinga and co-workers to avoid clogging problems associated with the AF-reactor (28). The support packing is removed and the associated biomass flocs are kept in suspension primarily by the effect of gas bubbles. The flocs that are suspended have a biofilm structure and are formed as granules of 1-5 mm diameter. Although the UASB reactor resembles a contact process, it is a fixed film process in that the bacteria are attaching to one another. For this reason many (19,28,44) consider UASB granules to be fixed films.

A problem with the UASB reactors is the loss of granules due to the entrainment of process gases. It is important to equip these reactors with a gas-solids separator in their upper portions. This separator breaks up entrained gas from the granules, allowing them to settle back to the blanket portion of the reactor, and thereby removing them from the process flow.

The AEB reactor is similar to UASB reactors in that the active biomass is present in the form of readily settleable aggregates. These aggregates are obtained by having the biomass grow on small inert

particles such as fine sand or aluminum. The rate of liquid flow through the reactor and the resulting degree of expansion determines whether the reactor is called a fluidized (more than doubling of the static reactor volume) or an expanded (less than doubling) bed reactor. Waste and recycle are pumped up through the bed and process gases are collected at the top. Jewell originally developed the technology to apply fluidized bed reactors to anaerobic treatment (20). Switzenbaum has performed extensive research toward the use of AEB reactors for dilute wastewater and municipal wastewater treatment (41,42,43). Others have investigated the use of these reactors for the treatment of higher strength industrial wastes (19).

The DSFF reactor was also developed from the anaerobic filter to avoid plugging problems. In this case the packing was left in and the suspended growth in the reactor was removed by operating the reactor in the downflow mode. The need for an elaborate distribution system is eliminated because water entering at the top of the reactor is readily dispersed by the gas escaping from the packing, which is then collected at the top of the reactor. An important consideration in this reactor is the formation and stability of an active biomass film on the surfaces provided. The DSFF reactor is capable of handling a wide variety of wastes because of its configuration and the addition of waste at the top of the reactor. Wastes high in suspended solids are easily degradable, although their degradation depends on the time they spend in the reactor, in contact with active biomass (49,50). This type of system is

used by Bicardi Rum Corporation in one of the most successful applications of anaerobic wastewater treatment technology.

2.4. Methods for the Reduction of Anaerobic Fixed Film Reactor Start-up and the Enhancement of Anaerobic Microbial Attachment. It has

previously been discussed that the start-up of an anaerobic fixed film reactor may require a long and unpredictable period of time. This long start up is due to the slow accumulation and subsequent growth of methanogenic bacteria and anaerobic biofilm on the solid support surfaces in the reactor. In the past five to ten years, much research has been carried out to investigate the means of reducing anaerobic fixed film reactor start-up. These means may be categorized into two basic areas: 1) those means which optimize anaerobic growth and biofilm development through environmental controls (i.e., organic loading and SRT variations, substrate and nutritional enrichment, temperature, and pH controls) and 2) those means which enhance anaerobic attachment and growth through process controls (i.e., support media variation and support media precoating). Some of these means will be addressed and discussed below.

2.4.1. Anaerobic Environmental Controls. From the point of view of energy metabolism, optimum environmental conditions should be maintained in order to achieve rapid attachment and development of a stable biofilm in anaerobic fixed film reactors. The temperature during the start-up

period should be kept at 35°C and not allowed to differ significantly with time, while the pH of the reactor medium should be maintained as close to neutrality as possible. The latter may require the addition of alkalinity to prevent significant pH depression due to the over production of volatile organic acids by the acid-forming bacteria. To control pH depression, the use of calcium rather than soda lime for neutralization has proven beneficial during start-up (24,38). Should a particular waste be deficient in the nutrients required for anaerobic growth, it may be necessary to supplement the waste stream with adequate salts of those which are deficient (11).

Several methods to reduce start-up times for anaerobic fixed film reactors through environmental means have been reported. Initial use of low organic reactor loading rates with gradual increases to the operating reactor loading was cited as beneficial for start-up (4,14,23). Others have done the same with synthetic feeds and gradually replaced them with the waste to be treated (11,29). This maintained volatile acid production at a minimum and prevented pH depression. Kennedy reported that methanogenic activity decreased with higher organic loading rates during the start-up period (21).

Seeding of the reactor volume with a rich inoculum of active solids from a working reactor treating a similar waste is a necessity. Experience has shown that during the start-up, the ratio of feed to seed should be kept low (keep biomass density over 20 kg VSS/m³), and organic loading low (food to microorganism ratio less than 0.1 kg COD/kg VSS .

d) (19,37) where VSS = volatile suspended solids. Salkinoja-Salonen and co-workers suggest a seed inoculum of 30-50 percent of the reactor volume to speed the start-up period (37). Henze and Harremoes confer that organic loading rates should be increased by 50 percent per week to operating levels on each observation of increased gas production (19).

The use of methanol as an initial substrate has been studied by Tait and Friedman, who found that this procedure favored a high carbon to nitrogen ratio (45). This may be important in extracellular polymer production and the growth of methanol utilizing methanogenic bacteria, one of the more rapid growth methanogens (45). Bull and co-workers reduced start-up time by using methanol as initial substrate, then gradually changing to the influent to be treated (4).

More recent experimentation by Shapiro who investigated the effects effects of organic loading per unit reactor volume and reactor SRT on anaerobic biofilm development. Contrary to earlier research, Shapiro's results indicated that the initial rates of methanogenic and anaerobic biofilm accumulation increased with increased organic space loading (in a well buffered system). In addition, initial rates were greater at a bulk liquid SRT of 15 days rather than 5 days. When the organic space loading was increased from 0.070 to 1.5 kg COD/m · d at 5 day SRT, the difference in initial rates for the five and 15 day SRT became much less significant when plotted against the corresponding VSS concentration of the bulk liquid. Thus, the VSS concentration of the bulk liquid was found to be a very important parameter affecting initial

biofilm development. This suggests that a reduction of start-up time in anaerobic fixed film reactors might be achieved by maintaining a high organic space loading. High organic space loading provides a large VSS concentration in the bulk liquid during the start-up period (38,39).

2.4.2. Support Media Variation. The propensity of microorganisms to attach to solid surfaces may vary significantly depending on the type of material used. For this reason, the use of a material to which methanogenic and anaerobic bacteria have a high affinity for adsorption may prove favorable for the construction of fixed film reactors. The utilization of a preferred material, rather than one which is not preferred, could significantly reduce the start-up period.

Since the late 1940's, researchers have attempted to determine the effects of various support materials on biological attachment. Weiss and Blumenson were the first to introduce the concepts of wettability, contact angle, and critical surface tension to the field of bioadhesion (51). Critical surface tension is defined as the intercept of the extrapolated straight line plot (Figure 3 and 4) of cosine θ versus the vapor/liquid surface tension. Critical surface tension provides information on the nature of the solid itself, since it is a characteristic of the solid only. It is used as a means of empirically ranking solids by their relative surface energies.

The critical surface tension of a material is believed to play an important role on the adsorption of molecular organic films during the

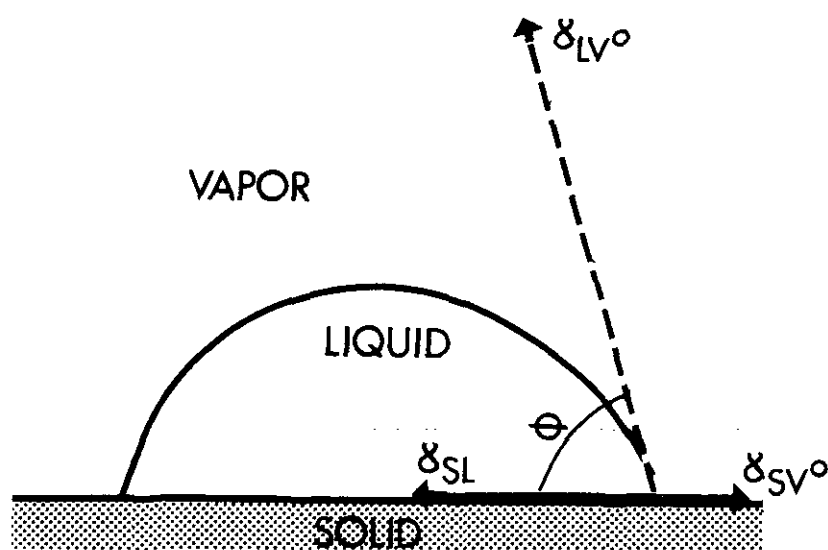


Figure 3. Schematic diagram of a finite contact angle formed by a sessile drop resting on a solid surface (from Weiss and Blumenson (51)).

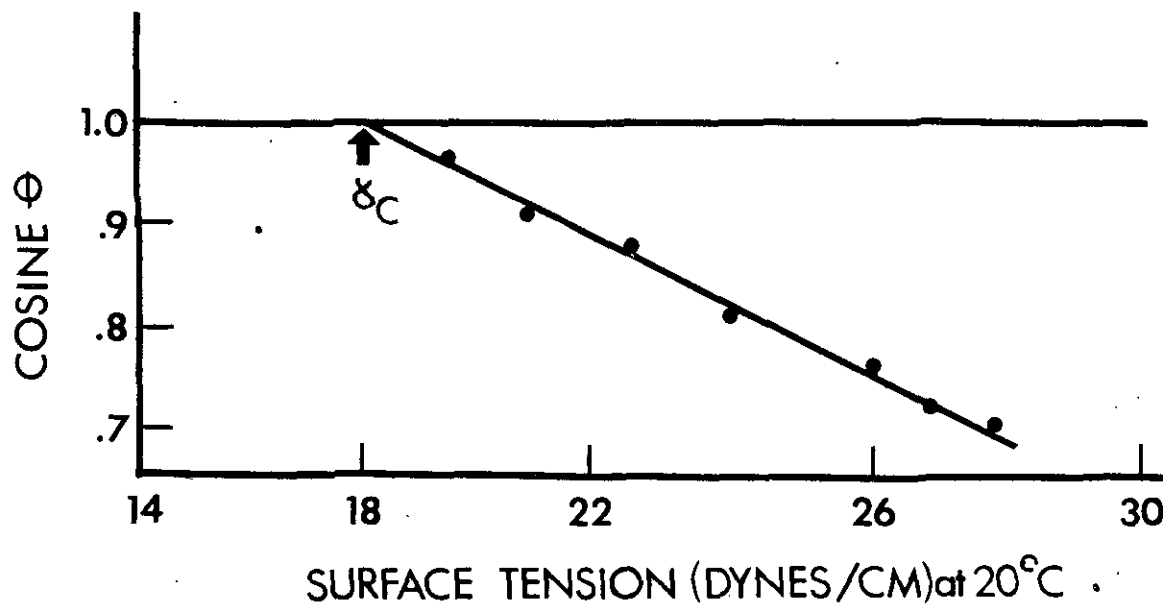


Figure 4. Wettability of polytetrafluoroethylene by the n-alkanes (from Weiss and Bluemenson (51)).

first stage of the attachment process (1,13,36). The adsorption of a molecular organic film happens nearly instantaneously and the layer which is created has been termed the "conditioning film" (13). It has been suggested that the role of the conditioning film is to make the surface more suitable for biological attachment, thus encouraging primary biofilm formation (7,13,31). A dependence of primary biofilm formation rate on the original surface properties of a material has been demonstrated by others (1,12).

Values of critical surface tension are commonly expressed in terms of dynes per centimeter. Typical values range from 18 dyne/cm for a hydrophobic material such as polytetrafluoroethylene (teflon), to 46 dyne/cm for glass which is considered hydrophilic (13). Goupil et al. found that relatively strong adhesion and biofilm growth was exhibited by both very low and high critical surface tension support media, and that minimal bioadhesion was noted in a zone of intermediate critical surface tensions, from 20-30 dyn/cm (17). This work was later confirmed by Dexter, who examined the effects of material critical surface tension on marine bacterial attachment in lower Delaware Bay. Figure 5 is a display of his findings (12).

In regards to anaerobic biofilm attachment, very little work has been done to investigate the effects of support media variation on biofilm development. Marchand and Le Duy investigated the effect of ceramic and stainless steel packings on the start-up and steady-state performance of anaerobic downflow packed column reactors (30). They

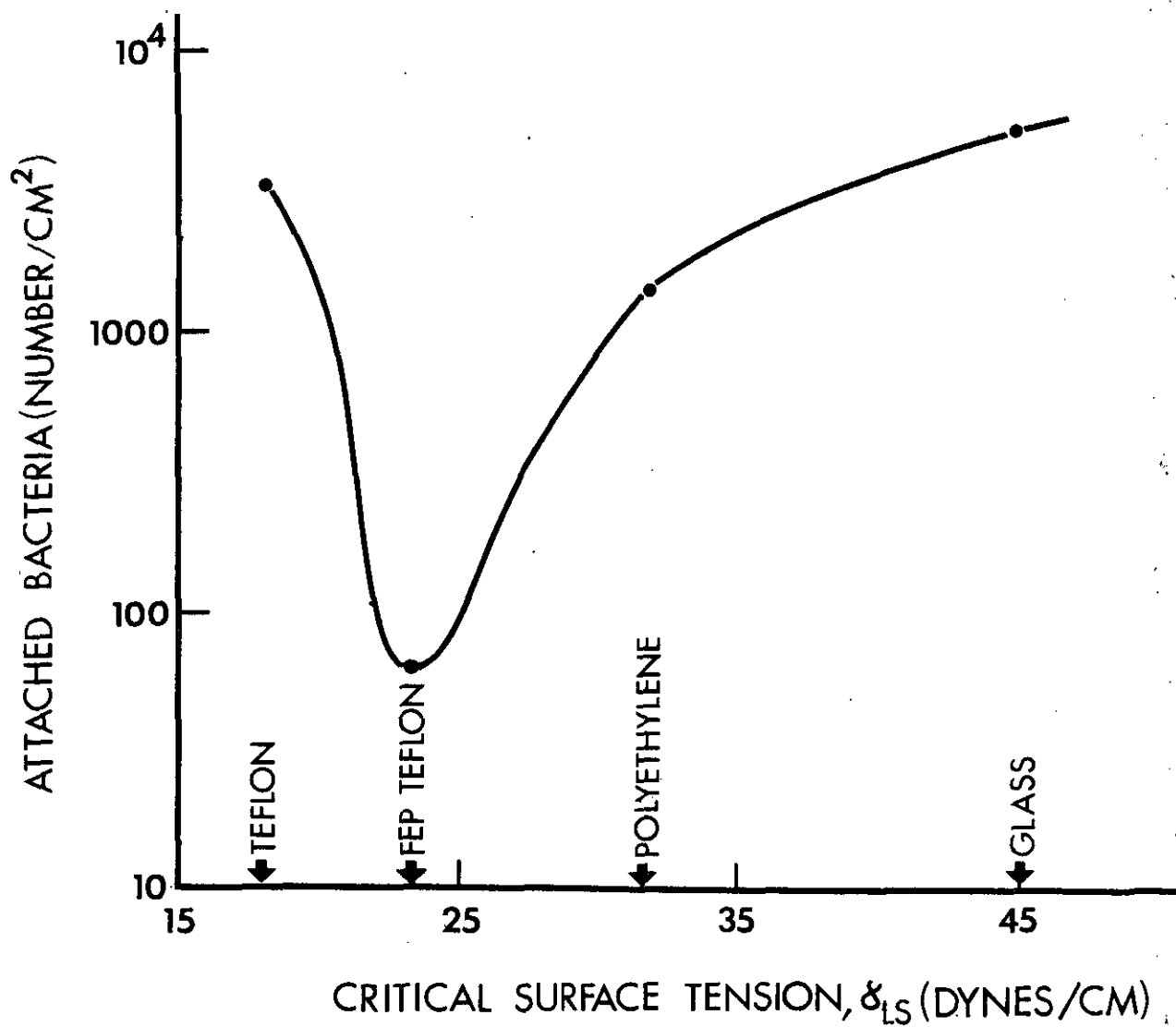


Figure 5. Number of attached bacteria vs. media critical surface tension for exposure of 24 hours in lower Delaware Bay (from Dexter (13)).

concluded that the type of material used had no effect on the steady-state performance of the reactor, but that a reduction of the start-up period could be expected by using stainless steel support media. Since this study only addressed the relationship of support media to reactor performance, one may only assume that faster bacterial attachment rates can be expected for anaerobic fixed film reactors which utilize stainless steel as a support surface. More research is definitely required to further the understanding of the effects of support media variation on anaerobic biofilm development.

2.4.3. Support Media Precoating. Support media precoating is a novel, although not necessarily new, process control for enhancing microbial attachment in fixed film reactors. There has been no literature to date dealing with the subject of support media precoating in anaerobic processes, however several researchers have investigated this technique in aerobic systems (8,24). It has been suggested by many that support media precoating may prove favorable in reducing anaerobic fixed film reactor start-up (8,19,24,38,39).

As stated previously, the second phase of the attachment process is characterized by the production of extracellular polysaccharides by the adsorbed bacteria. Corpe, in his work with marine bacteria, was able to isolate and purify these polysaccharides. He smeared some of the recovered polymer on glass slides and allowed them to air dry. After three days he submerged them along with clear slides into both

natural and artificial seawater. In both cases the presence of polymer significantly enhanced attachment (7).

Torteson and Corpe repeated the same experiment for algal cells (47). The polymer of the marine pseudomonad caused algal attachment. If the polymer was added to a suspended culture of algae, it caused the cells to clump or aggregate.

Since it is apparent that attachment is controlled to a fairly large extent by surface properties of the support media, the surface properties of the substratum surface may be altered via the application of a surface modifier such as chemical polymer. Because of the many adsorptive sites, polymers are virtually insensitive to variations on localized surface conditions. Polymers may extend adsorption sites out various distances from the substratum, thereby increasing the chance for bacterial attachment.

La Motta and Hickey investigated the effect of support surface precoating on the attachment of aerobic mixed and nitrifying bacterial cultures treating domestic wastewater (24). Their experiments were done using a twin-chamber rotating disk reactor. The two chambers of the bench-scale unit worked in parallel, one as the experimental unit, and the others as a control. A number of various water and wastewater treatment polymers were used to precoat the experimental chamber. After the optimum concentration of each polymer was determined, the solution was allowed to sit in the experimental chamber for 24 hours. After this application the chamber was drained and air dried. The experiment was

started by running primary sewage or secondary effluent continuously, at the same flow rate, through both chambers. The reactor was operated under aerobic, completely mixed conditions. The growth of biological film was monitored through the aid of removable slides over a ten day period. Precoating the support surfaces with synthetic cationic polymers was found to significantly enhance the initial film attachment. In the case of nitrifying bacteria, no nitrification was observed during six days in the control chamber, while a low level of nitrification could be observed as early as one day in the precoated chamber.

Besides precoating support media with natural and synthetic polymers, precoating support media with aerobic or denitrifying biofilms may promote faster anaerobic biofilm attachment. This technique may in fact be favorable as the toxic effect of synthetic polymers on anaerobic growth has yet to be determined. It may also be difficult to keep water soluble polymers from dissolving into the bulk liquid after the support surface has been subjected to the anaerobic slurry. Since aerobic and denitrifying bacteria metabolize much faster than anaerobes, they can quickly produce a matrix of polymers which anaerobes may adhere to during the start-up process.

More anaerobic fixed film processes are amenable to precoating with aerobic or denitrifying biofilms. Once treatment begins it would be relatively easy to add nitrate salt, along with similar inoculum, to the reactor. However, the construction of aeration facilities solely for the purpose of start-up is likely to be cost prohibitive. After a

few days of operation in this mode, the procedure can be halted and the reactor seeded with anaerobic culture.

Whether precoating support media with aerobic or denitrifying biofilms has a positive effect on increasing anaerobic biofilm formation and decreasing reactor start-up time has yet to be determined. Certainly prior research would lead one to believe that it should. This study investigated this question in regards to the use of denitrifying precoat biofilms.

2.5. Factor F-420. Methanogenic bacteria possess a cellular coenzyme, F-420, which at the present time is believed to exist only in this anaerobic bacterial group (15). It is a low molecular weight compound, about 770 gm/mole (46) and is involved as an electron carrier in both metabolic and catabolic reactions (6).

Much research has been focused on F-420 because it can be useful as a relative measure of methanogen concentration in anaerobic processes (120). Further research has shown that F-420 fluorescence varies with SRT and substrate, and continues after heat treatment has killed methanogens (35). The amount of F-420 fluorescence also varies among the different species of methanogenic bacteria (15). These findings suggest that it may only be used as an indicator of active mass under certain conditions. One condition is when SRT and feed is constant and F-420 concentration is found to increase in the mixed liquor or

anaerobic biofilm. An increase could only occur when methanogenic cells are growing and hence in an active state (38).

C H A P T E R I I I

MATERIALS AND METHODS

3.1. Reactor Design

3.1.1. Anaerobic Biofilm Experimentation Reactor. Two experimental 15 l volume reactors consisting of six inch inside diameter, extruded acrylic were in service at any one time (Figure 6). The reactors were housed at 35°C in a constant temperature incubator at the UMASS Environmental Engineering Laboratory. The contents of each reactor were intermittently mixed by a pump/timer combination which drew slurry from the bottom of the reactor and recycled it back through the top of the reactor. Mixing took place for 15 minutes every two hours. This mixing system maintained quasi-quiescent conditions in the reactor and minimized the effect of shear along the support media surfaces. Removable flanges at the bottom and top of each reactor sealed the reactors to the atmosphere while allowing periodic access to the reactor contents. The top flange was fitted with ports for feeding/recycle, support media sections, and process gas venting. The gas vent and feeding/recycle ports were contained appropriate fittings in order to accommodate plastic tubing.

Rod-shaped support media were suspended in the reactor through the media ports and held in place with hollowed-out rubber stoppers. These

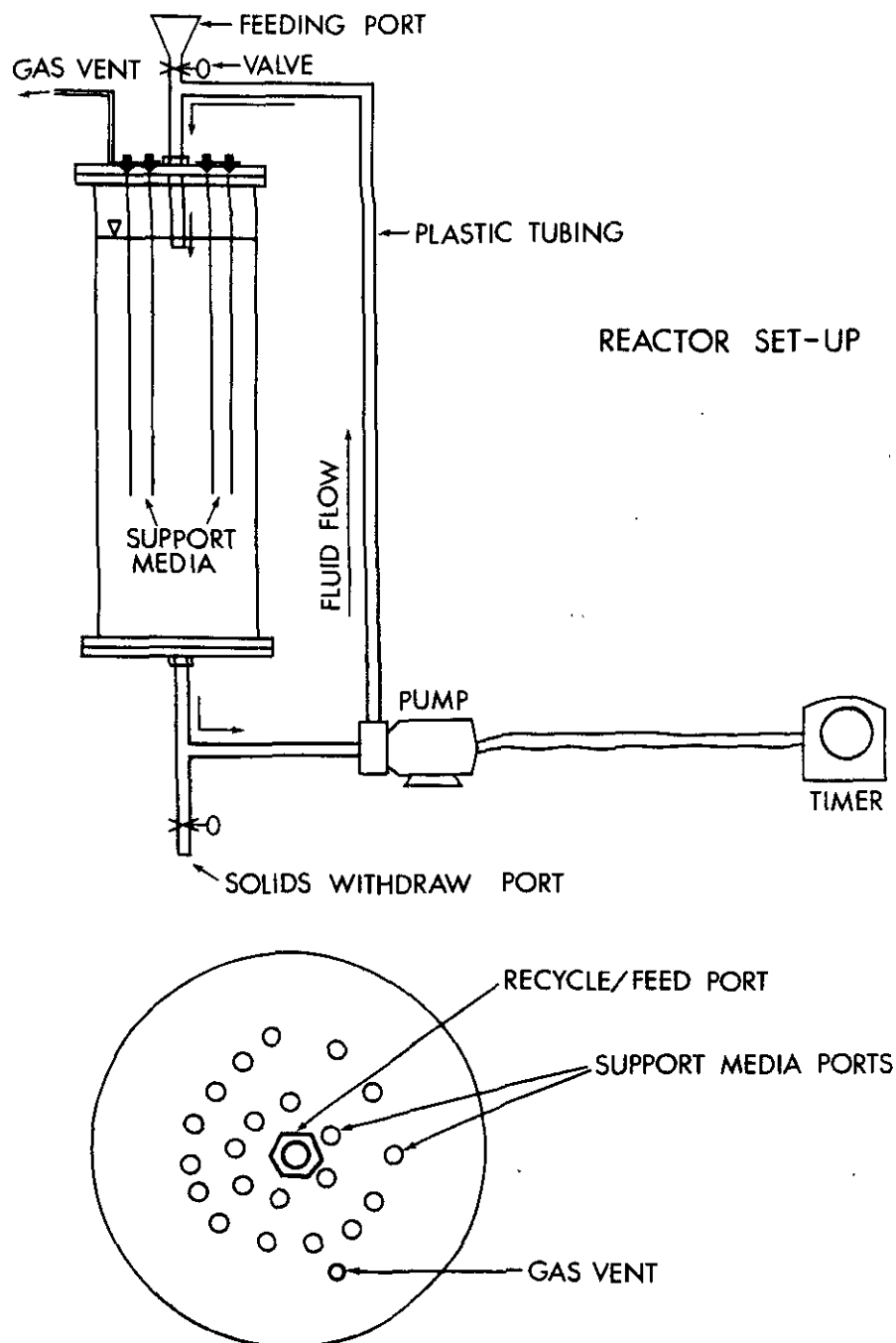


Figure 6. Reactor set-up and downward view of the top flange.

stoppers fit tightly around the rods and sealed the port to the atmosphere. There were a total of 21 media ports per reactor which allowed each reactor to hold 21 support media "test sections" (a support media "test section" will be described later).

A port for the recycle of the reactor contents was located on the bottom flange of the reactor. From a "T"-fitting off of this port a valve was located for the evacuation of waste solids during the feed process. Anaerobic inoculum for the reactors was obtained from active cultures maintained at the UMASS Wastewater Treatment Pilot Facility.

3.1.2. Denitrifying Biofilm Precoating Reactor. The precoating of support media with denitrifying biofilm was accomplished in a reactor of similar construction to that of the anaerobic reactors. The bottom flange of this reactor was sealed entirely, whereas the top flange was identical to that of the anaerobic reactors. This allowed easy transfer of precoated support media to the anaerobic reactor as the top flanges were interchangeable.

The denitrifying slurry was mixed by means of a pump/timer combination. Mixing took place for ten minutes every three hours. Slurry was drawn from the bottom of the reactor via plastic tubing and recycled back to the top of the liquid volume. Recycling in a vortex-like manner maintained well-mixed conditions and eliminated the problem of stratification.

3.2. Biofilm Attachment Support Media.

3.2.1. Rods. Thin rods were chosen to serve as the biofilm support media for this experiment. These rods were easily manipulated for laboratory analysis and numerous materials are manufactured in this format. Rods were 0.1875 inches (0.476 cm) in diameter, and 2.0 inches (5.08 cm) in length. Sections of rod were connected together by 0.5 inch (1.27 cm) lengths of tight fitting silicone tubing at each end, so that 1.5 inches of rod surface length was exposed to the reactor slurry during experimentation. This corresponds to a section surface area of 0.884 in^2 (5.700 cm^2).

A "test section" of a specific support media consisted of 12-2.0 inch rod sections (see Figure 7). Six of these sections were used for COD analysis and six were used for F-420 analysis at each test interval. Each test section was submerged below the reactor gas/liquid interface by means of a 12-inch length of PVC rod of equal diameter. This PVC rod was fitted into a hollowed-out rubber stopper which was seated in the top flange of the reactor. The total length of a test section was 36 inches (91.4 cm).

3.2.2. Support Materials. Four materials, polytetrafluoroethylene (teflon), polyvinylchloride (PVC), aluminum and stainless steel were examined in the first phase of this study, support media variations. Two materials, PVC and aluminum were examined in the second phase of

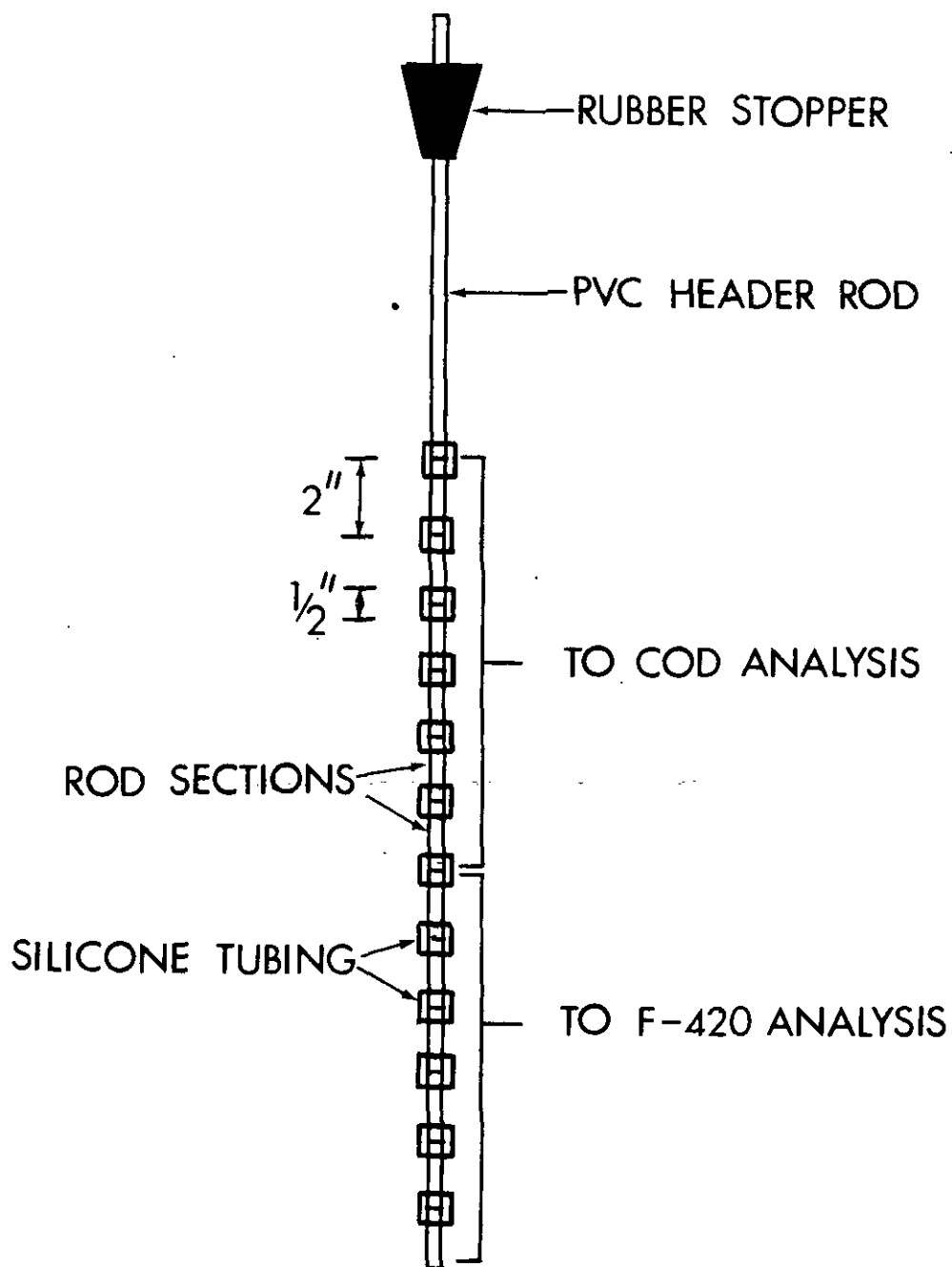


Figure 7. Support media "test section".

this study, support media precoating. All of these materials are uniquely different from one another. Aluminum and stainless steel are considered hydrophilic materials, whereas teflon and PVC are hydrophobic. As previously described, hydrophobicity and hydrophobicity refer to the ability of a material to be wetted and are properties related to the critical surface tension of the material. Water does not spread readily on hydrophobic surfaces, whereas it does on hydrophilic surfaces. The critical surface tensions of aluminum and stainless steel are about 45 dynes/cm, while for PVC and teflon they are 35 dynes/cm and 18 dynes/cm respectively.

Stainless steel and teflon are rather inert materials. That is to say, both are resistant to corrosion and biodegradation. PVC and aluminum, although somewhat resistant to corrosion, are not as resistant as stainless steel and teflon. Stainless steel has been previously shown to be beneficial for anaerobic biofilm development (30), and although certain plastics may exhibit toxic effects when used in culturing bacteria, teflon and PVC are not expected to be toxic in their pure forms (13).

Support materials used in the denitrifier precoat study were exposed to the denitrifier slurry for three different time spans. A first set of PVC and aluminum test sections was left in the denitrifier slurry for five days, then transferred to the anaerobic reactor. A second set was exposed for 24 hours prior to anaerobic treatment. The final set was dipped into the denitrifier slurry, removed and air dried

before it was placed in the anaerobic reactor. This provided information regarding the effect of precoating duration on anaerobic biofilm development.

3.3. Reactor Feeding. Two important considerations in regards to reactor feeding are the feed composition and SRT. The feed composition must provide the anaerobic bacteria with an organic substrate and carbon source, while at the same time providing all the nutritional requirements for growth in a buffered solution. The SRT in a draw and fill reactor is analogous with the hydraulic retention time, and together with feed strength governs the amount of mixed liquor present in the reactor.

The feed for the anaerobic reactor consisted of five basic ingredients which are listed in Table 3. Nutrient salts #1 and #2 contained the essential trace nutrients for anaerobic growth as determined by Speece and McCarty (40) and later modified by Pause and Switzenbaum (35). Mixing nutrient salts in this fashion avoided co-precipitation of the nutrient salt solutions. Tap water (town of Amherst, Massachusetts) was used in order to supplement the feed with trace quantities of micronutrients. Tap water was allowed to sit in a 25 l carboy overnight in order to remove residual chlorine. This avoided problems from chlorination.

The anaerobic reactor was maintained at a five day SRT; 3.0 l of sucrose/nutrient broth feed per day per reactor and a 3.0 l volume of

Table 3. Components of Stock Feed Solution

Sucrose/Nutrient Broth Feed (3 liters)

7.95 g sucrose
 7.95 g nutrient broth
 160 ml nutrient salt #1
 160 ml nutrient salt #2
 320 ml buffer solution
 2,360 ml tap water

<u>Salt #1*</u>	<u>Salt #2*</u>	<u>Buffer*</u>
11.4 g $(\text{NH}_4)_2\text{HPO}_4$	2.0 g KCl	50 g NaHCO_3
3.9 g CaCl_2	8.3 g FeCl_3	
	0.3 g CoCl_2	
	7.0 g MgCl_2	
	0.6 g NiCl_2	

*dissolved in 1.0 l tap water

mixed liquor removed. This five day SRT was chosen as research has shown that F-420 is at a maximum concentration per unit of VSS the closer the SRT is to the critical washout rate for methanogenic bacteria. This is approximately four days (35). A feed strength of 5600 mg/l COD was chosen on the basis that this is a typical industrial waste strength, and therefore a candidate for anaerobic treatment. Feeding the reactor in this manner has been observed to produce adequate levels of COD and F-420 detectability, while not presenting a problem with mechanical reactor fouling.

Feed for the denitrifying reactor was identical to that of the anaerobic reactor with the exception that Sodium Nitrate was added to provide a terminal electron acceptor for the denitrifying bacteria. Sodium Nitrate was added at 5.29 g/l to the sucrose/nutrient broth feed, based on the stoichiometry of the denitrification process with an additional 20 percent by weight of NaNO_3 as a reaction driving force (33). An inoculum of denitrifying bacteria from activated sludge (Amherst, MA Wastewater Treatment Facility) was placed in the reactor with 13l of NaNO_3 spiked feed. On each consecutive day of operation 3l of slurry was extracted from this reactor, and 3l of denitrifier feed was added.

3.4. Biofilm Attachment Analysis. Two chemical analyses were used to determine the amounts, or relative amounts, of anaerobic and

methanogenic bacteria present on support media at any given time. These techniques were chemical oxygen demand analysis and F-420 analysis.

A time study was performed for both uncoated and precoated support materials by testing anaerobic exposure at five, ten, and 15 days. A total of six replicates of each material and precoat duration were obtained at each test interval for the statistical integrity of the experiment. By testing in this fashion the rates of biofilm accumulation for each clean and precoated support material could be compared.

3.4.1. COD Analysis. The anaerobic biofilm accumulation was determined through chemical oxygen demand analysis run on the support media sections. This analysis was conducted according to the procedure outlined by Knetchel (22) and later modified by Shapiro (38). A 2.0 inch section of support media was removed from the test section and placed in 20 ml of 1 N H_2SO_4 . The test tube was placed in a boiling bath for 14 minutes. Following this, the test tube was cooled to room temperature and a 10 ml aliquot of the boiled extract was taken for use in the COD analysis. The digestion was carried out in Kimax brand culture tubes while being incubated at $150^{\circ}C$ for two hours. The COD was determined by measuring the increase of Cr(III) concentrations using a Spectronic 20 set at 620 nm. A COD standard curve was prepared regularly using phthalate acid. Controls for uncoated and precoated

materials were run through this analysis in order to determine the extent, if any, of their COD background.

3.4.2. Factor F-420 Analysis. The fact that methanogenic bacteria possess a fluorescent coenzyme, F-420, is useful for determining the relative accumulation of methanogens in the anaerobic biofilm. The procedure used to detect the quantity of F-420 was developed by De Zeeuw and co-workers (14) who modified the procedure of Delafontaine and co-workers (10). Shapiro utilized the De Zeeuw method to extract F-420 from biofilms (38). The procedures used in this experiment involved placing a 2.0 inch section of support media from a test section in a test tube and adding 20 ml of glycine-EDTA buffer. Test tubes were placed in a boiling water bath for 14 minutes. After this time, the support sections were removed and the solution was cooled and centrifuged at 10,000 rpm for ten minutes at 4°C. After centrifugation, a 5 ml aliquot of the supernatant was mixed with 10 ml of isopropanol in a second test tube. The pH was adjusted to 8.8 with a few drops of 0.1 N KOH and then another centrifugation was carried out under the same conditions.

The resultant mixture was analyzed for the presence of F-420 coenzyme. This was done by pumping the mixture through an F.S. 970 Fluorometer (Kratos Instruments) at approximately 50 ml/hr. The excitation energy of the fluorometer was set at 420 nm and the emission filter selected for 470 nm. The readings on the fluorometer were

recorded by a Fisher "Recordall" chart recorder. An F-420 standard curve was prepared by running known concentrations of crystalline F-420 through the fluorometer. The crystalline coenzyme was kindly provided by Dr. R. S. Wolfe.

CHAPTER IV

RESULTS

4.1. Effect of Support Media Variation on Methanogens and Anaerobic Biofilm Development. Figures 8 and 9 represent the effect of support media variation on anaerobic and methanogenic biofilm development over a 15 day period. Values for these figures are listed in Table 4, and are based on six determinations for each support material at each test interval.

Anaerobic biofilm accumulation data for all materials increase from zero to 15 days. Accumulations were more rapid for stainless steel and teflon, than for PVC and aluminum.

Methanogen accumulations in the anaerobic biofilm displayed identical patterns for all materials. From zero to five days there was a rapid increase in F-420 levels followed by a less drastic increase from five to ten days. From ten to 15 days there were drastic decreases in F-420 measurements. Intermaterial differences for methanogen accumulations were, for the most part, consistent over the 15 day period. Stainless steel displayed the largest F-420 levels at each test interval, followed by teflon, aluminum, and PVC.

4.2. Effect of Denitrifying Biofilm Support Media Precoating on Methanogen and Anaerobic Biofilm Development. The effects of

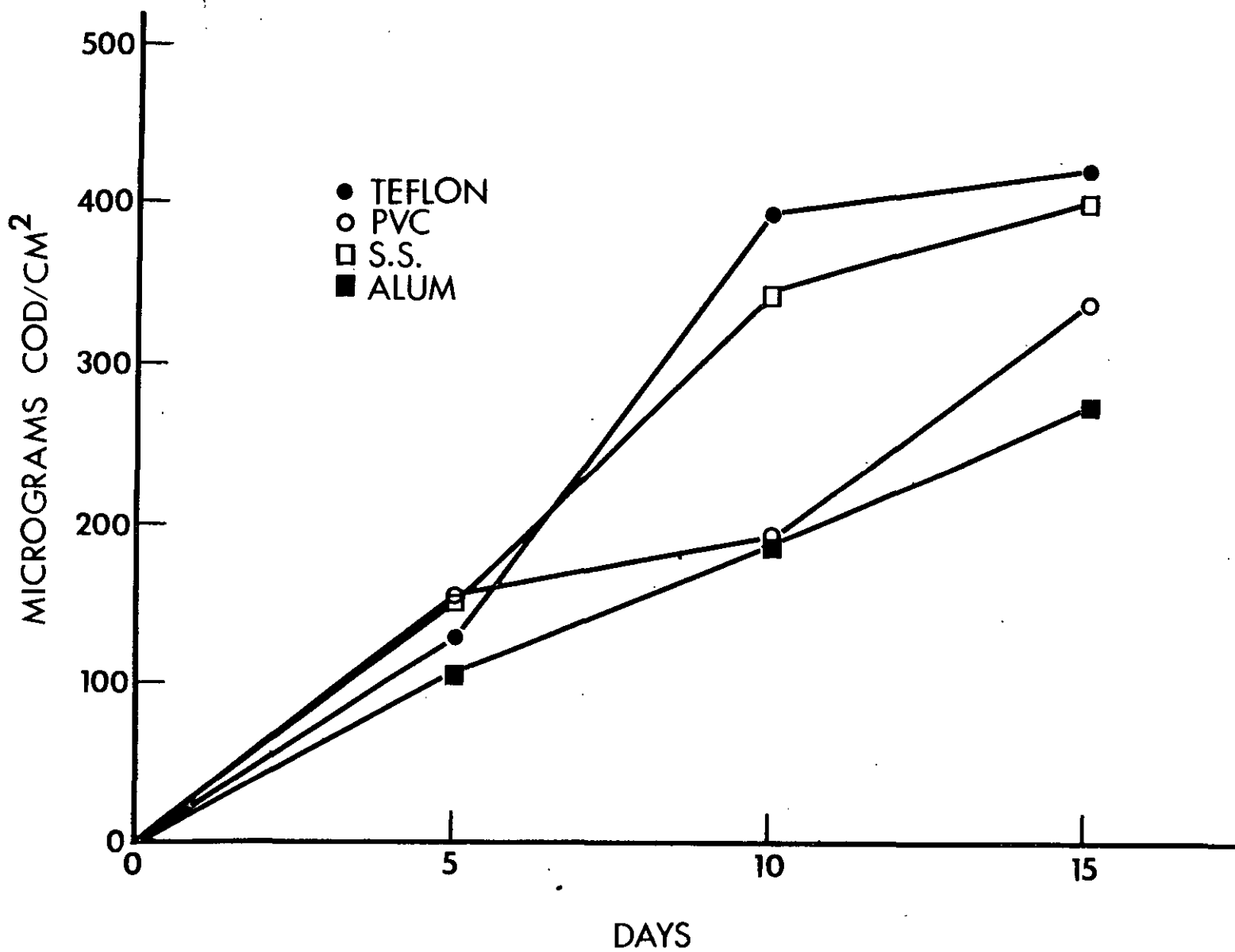


Figure 8. Uncoated materials: anaerobic biofilm accumulations ($\mu\text{g COD/cm}^2$) vs. time (days).

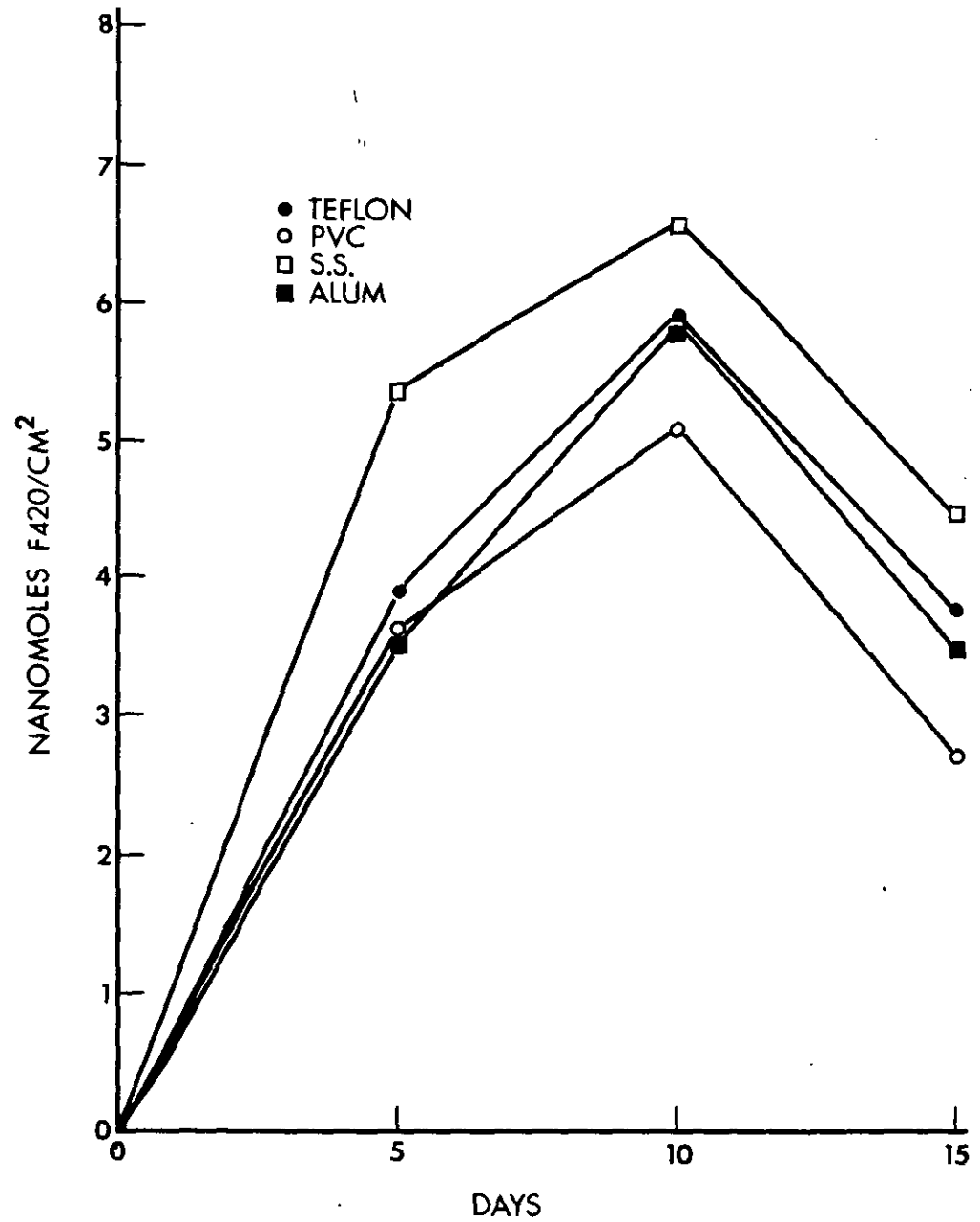


Figure 9. Uncoated materials: Methanogen biofilm accumulation (nanomoles F_{420}) vs. time (days).

Table 4. Anaerobic and Methanogenic Biofilm Accumulation Data
(Clean Materials)

Material	ANAEROBIC ACCUMULATION $\mu\text{g COD}/\text{cm}^2$			METHANOGENIC ACCUMULATION nanomoles F-420/ cm^2		
	5d	10d	15d	5d	10d	15d
Teflon	128	395	422	3.9	5.91	3.75
PVC	155	193	337	3.62	5.09	2.68
S.S.	152	345	402	5.35	6.58	4.44
Aluminum	107	189	274	3.52	5.83	3.46

denitrifying biofilm support media precoating on anaerobic and methanogen biofilm accumulation are portrayed in Figures 10 through 13. For each material tested, three precoating durations; five day, one day, and dipped/air dried, are displayed. Values for these figures are listed in Table 5, and are the result of six determinations for each support material and precoating duration of each test interval. For Table 5 and Figures 10-13, background levels of COD due to the denitrification film have been subtracted from the gross COD accumulation. Thus the data are net values.

Anaerobic biofilm accumulation data for aluminum and PVC, under all three precoating durations, display the same patterns with time. From zero to five days there was very little accumulation of biofilm, followed by rapid linear increases in biofilm accumulations from five to 15 days. There were only slight differences between the levels associated with the various precoating durations, and intermaterial differences were negligible.

Methanogen accumulations in the anaerobic biofilm displayed identical patterns for all materials and precoating durations with time. After five days exposure in the anaerobic reactors, support media exhibited low levels of F-420. These low F-420 levels were consistent through the duration of the experiment, and only increased or decreased by small percentages.

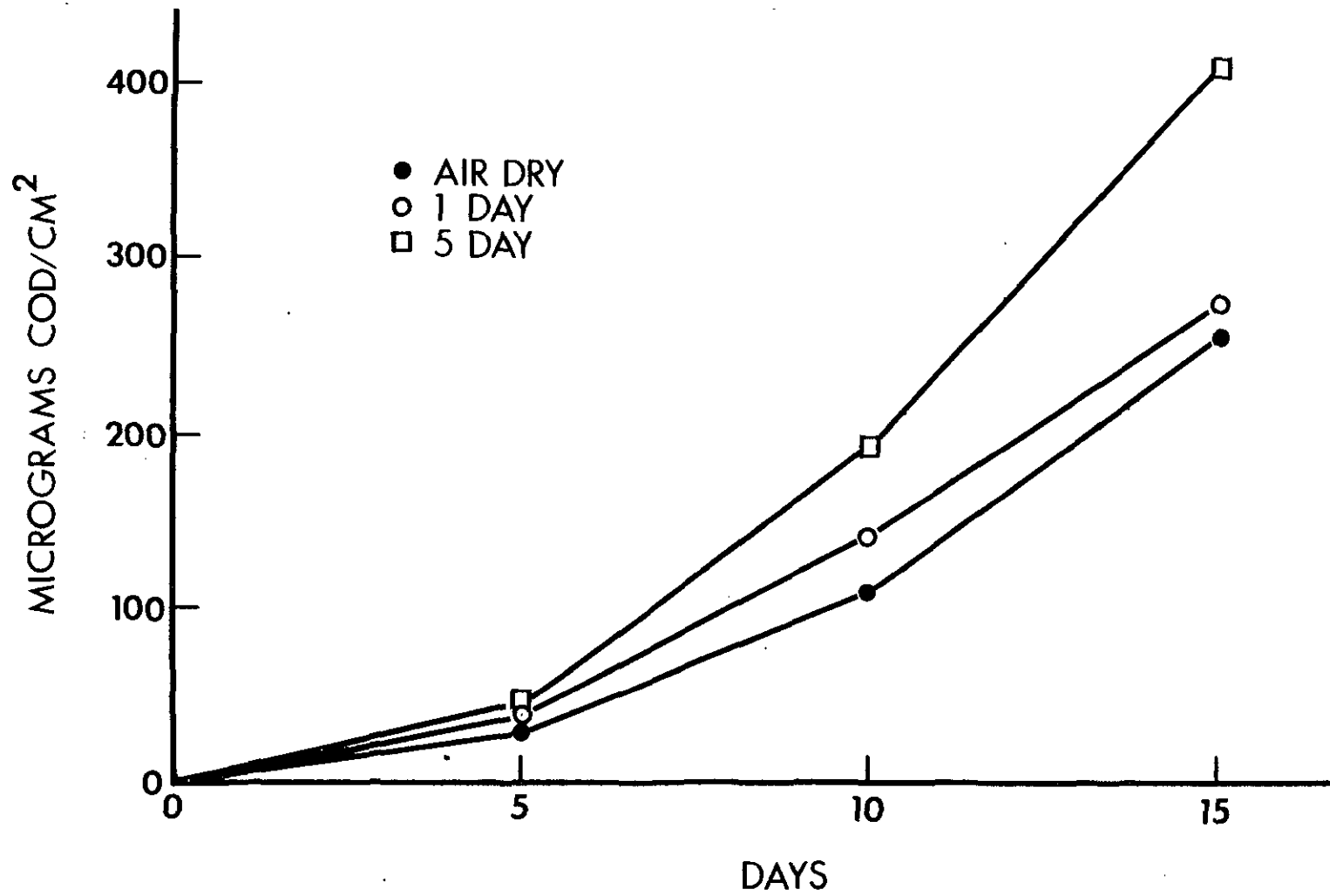


Figure 10. Precoated PVC ($\mu\text{g COD/cm}^2$) vs. time (days).

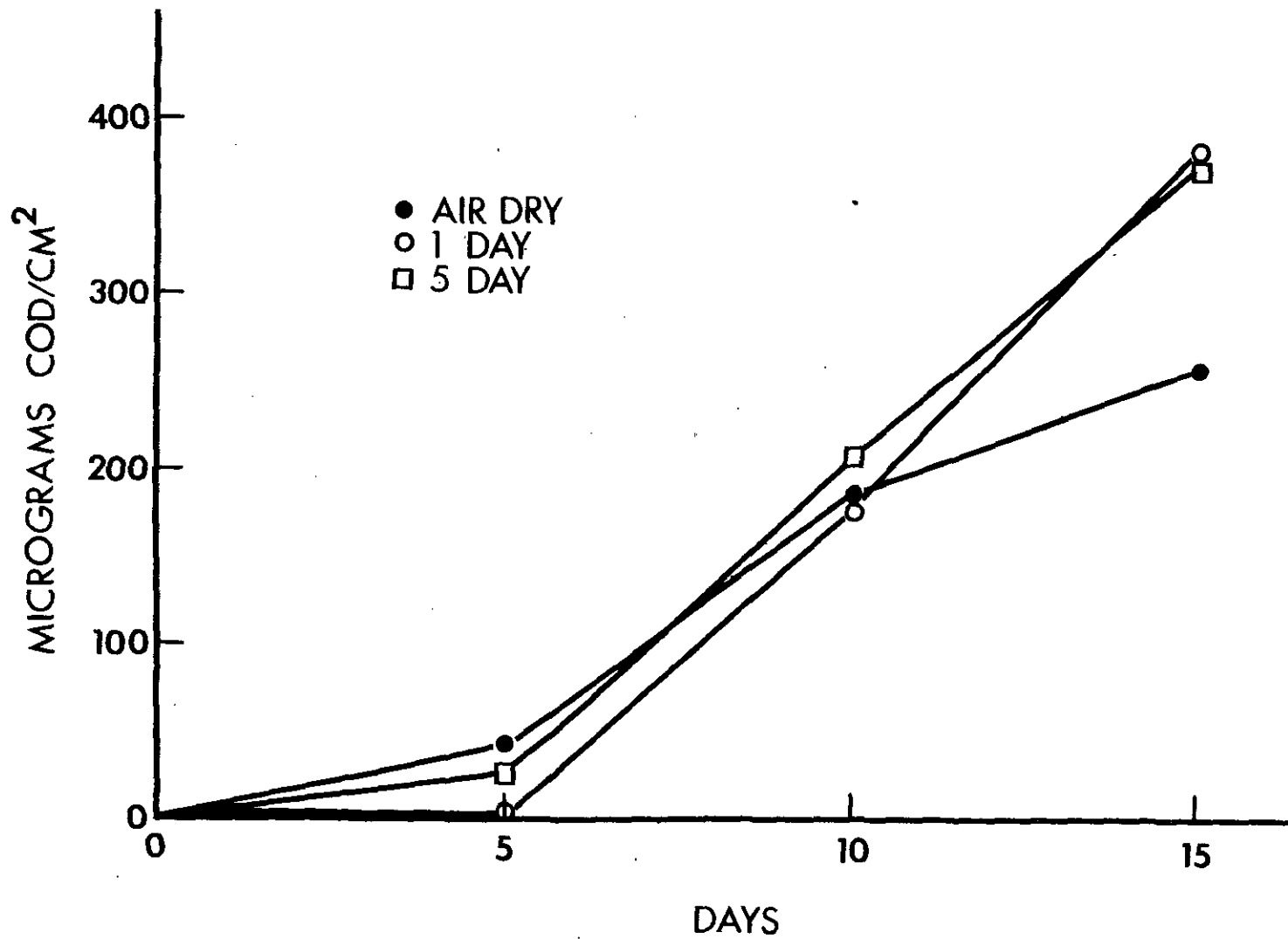


Figure 11. Precoated aluminum ($\mu\text{g COD/cm}^2$) vs. time (days).

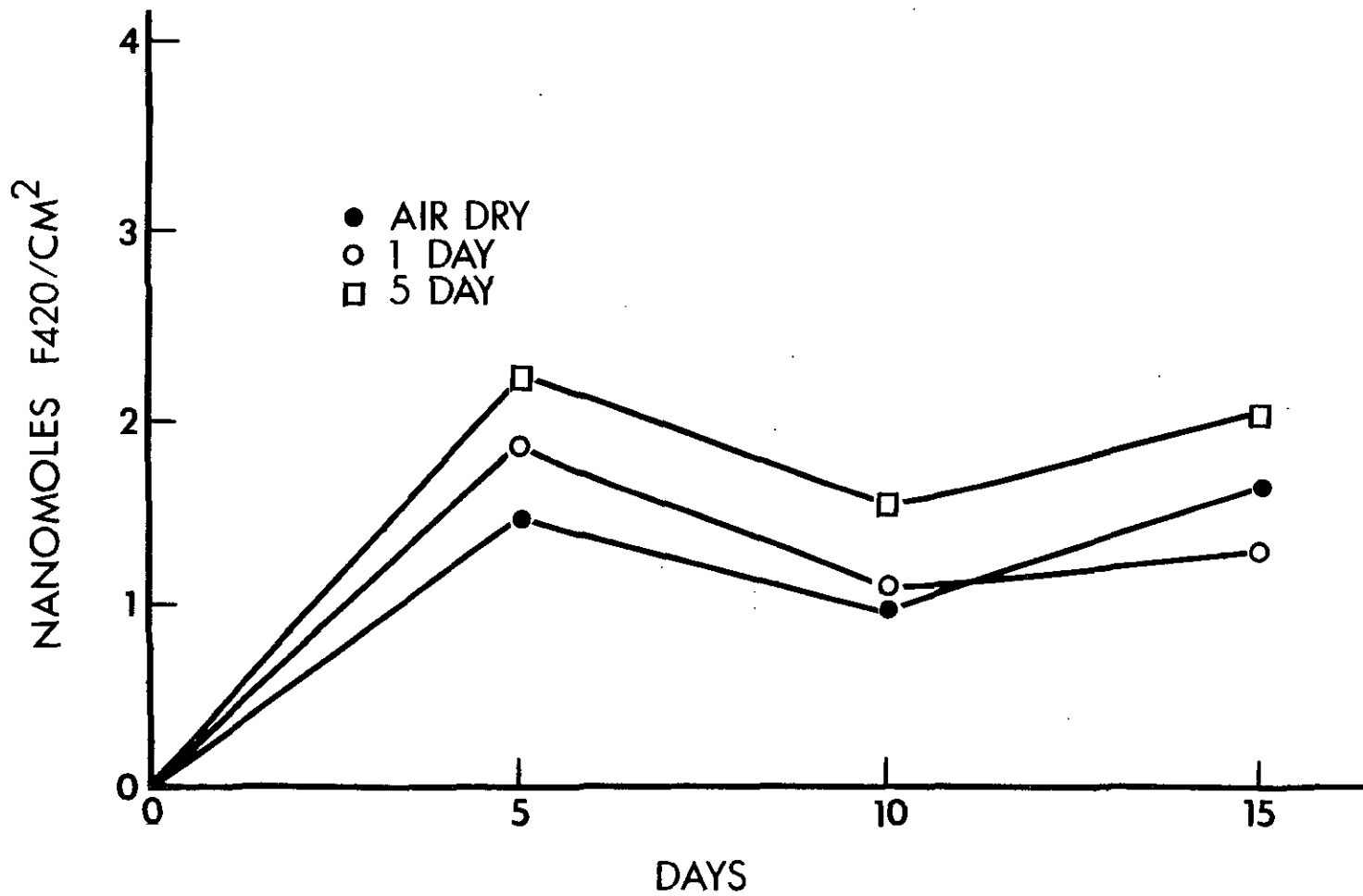


Figure 12. Precoated PVC (nanomoles F₄₂₀) vs. time (days).

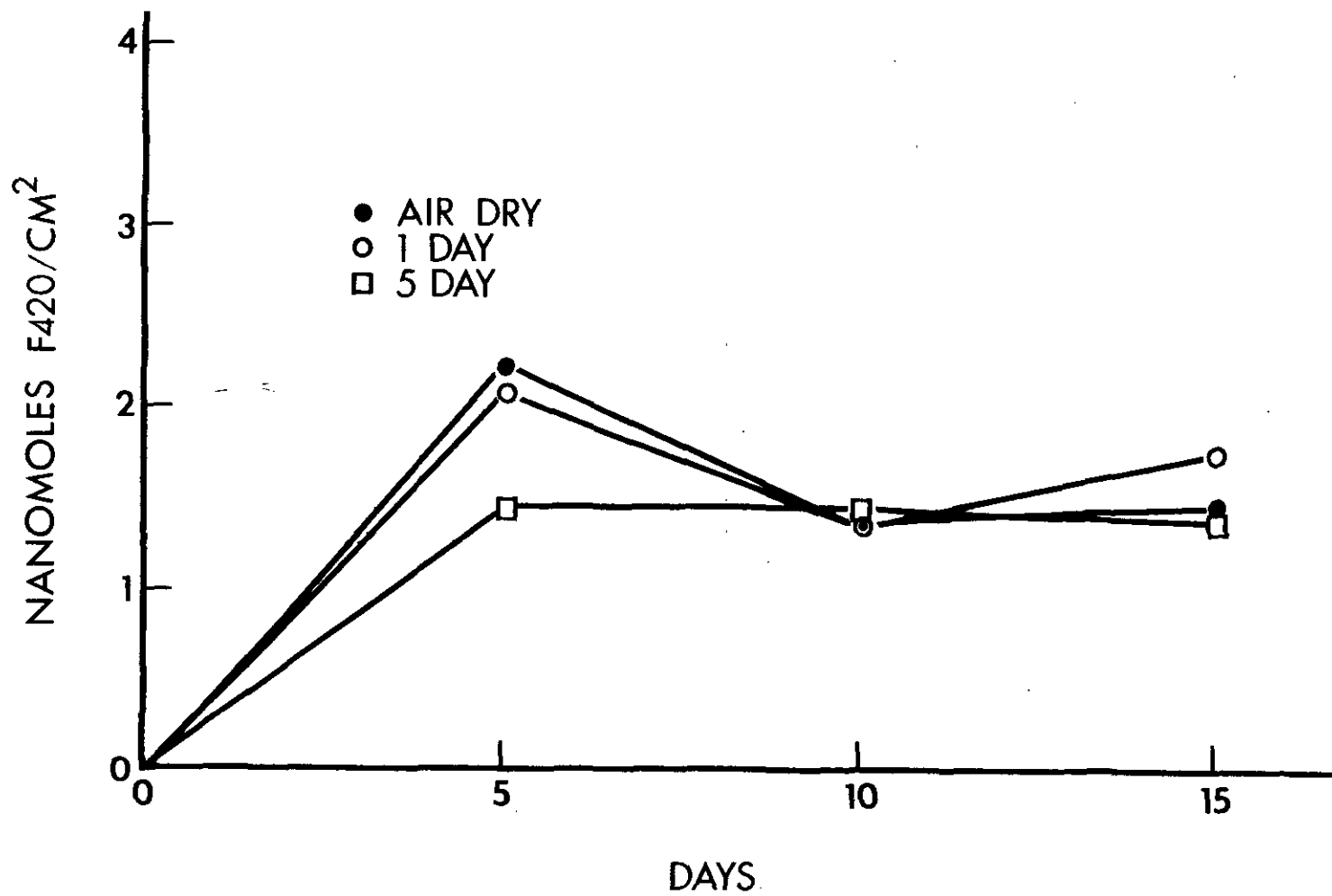


Figure 13. Precoated aluminum (nanomoles F₄₂₀) vs. time (days).

Table 5. Anaerobic and Methanogenic Biofilm Accumulation Data
(Precoated Materials)

MATERIAL/LENGTH OF PRECOATING	ANAEROBIC ACCUMULATION $\mu\text{g COD}/\text{cm}^2$			METHANOGENIC ACCUMULATION nanomoles F-420/ cm^2		
	5d	10d	15d	5d	10d	15d
PVC/DIPPED-AIR DRIED	28	109	255	1.47	0.94	1.60
PVC/1 DAY	37	141	274	1.86	1.08	1.26
PVC/5 DAY	43	193	411	2.23	1.52	2.02
ALUMINUM/DIPPED- AIR DRIED	41	186	255	2.23	1.34	1.44
ALUMINUM/1 DAY	3	177	382	2.08	1.34	1.73
ALUMINUM/5 DAY	28	207	377	1.44	1.45	1.37

CHAPTER V

DISCUSSION

5.1. Interpretation of Anaerobic and Methanogen Biofilm Accumulation Data for Uncoated Support Materials. The design of the first phase of this experiment considered the effect of support media variations on biofilm accumulation. Initial rates of anaerobic accumulation were determined through graphical calculation of the linear slope of data from zero to 15 days. It has been shown that, under certain circumstances, anaerobic biofilm accumulation data past the 15 day period began to level off, or diverge from this linear trend (38). Values of anaerobic biofilm accumulation rates for each material are presented in Table 6.

By inspecting biofilm COD's and accumulation rates, it appears that the anaerobic attachment process is faster for the materials stainless steel and teflon, and slower for PVC and aluminum. Roughly speaking, a 57 percent increase in the rate of anaerobic biofilm accumulation was exhibited when stainless steel, rather than aluminum, was used as a support media. Relative increases and decreases in attachment rates for various materials are presented in Table 7.

In order to better analyze anaerobic biofilm accumulation data, an analysis of variance was performed. The influence of support material

Table 6. Anaerobic Biofilm Accumulation Rates
(Uncoated Materials)

<u>Material</u>	<u>Rate of Attachment</u> <u>($\mu\text{g COD}/\text{cm}^2 \cdot \text{d}$)</u>
Teflon	29.6
PVC	23.1
Stainless Steel	30.4
Aluminum	19.4

Table 7. Percent change in Anaerobic Biofilm Accumulation Rates between Support Materials Tested (Uncoated Materials)

Material Preferred Over	MATERIAL USED			
	Stainless Steel	Teflon	PVC	Aluminum
Stainless Steel	-	-3	-24	-36
Teflon	+3	-	-22	-34
PVC	+32	+28	-	-16
Aluminum	+57	+53	+19	-

$$\% \text{ change} = \frac{\text{accumulation rate of material used} - \text{accumulation rate of material preferred over}}{\text{accumulation rate of material preferred over}} \times 100$$

(Note: accumulation rates are given in Table 6)

on $\mu\text{g COD/cm}^2$ levels at five, ten, and 15 days was analyzed. A summary of these analyses is shown in Table 8.

In examining Table 8 there exists a significant difference among the four sets of $\mu\text{g COD/cm}^2$ data at the ten day interval ($F = 11.28 > F_{.05} = 3.10$), and the 15 day interval ($F = 5.95 > F_{.05} = 3.10$). At the five day interval there was no significant difference among data sets ($F = .80 < F_{.05} = 3.10$). This statistically indicates that the type of support material used has a marked influence on the initial rate of anaerobic biofilm accumulation during the five to 15 day period. This is the period before steady state biofilm thickness is achieved.

Methanogen accumulation data are more difficult to analyze. This is most likely due to the fact that F-420 fluorescence levels vary with SRT variations. The methanogens present in the anaerobic slurry were metabolizing close to their critical washout rate, at five days SRT, and thus were in a very active state. Near critical washout, it has been shown that F-420 concentrations approach their maximum levels for a given system (35). As bacteria begin to attach and grow to a support surface, their retention times may increase by an order of magnitude from that of cells in the bulk slurry. With an increase in retention time for the attached cells, it is possible that a decrease in their level of activity will take place. In examining the F-420 levels exhibited by the four support materials in Figure 9, it can be seen that the levels increased rapidly from zero to ten days and dropped off

Table 8. Analysis of Variance of Anaerobic Attachment Data
(Uncoated Materials)

	<u>5 day ($\mu\text{g COD/cm}^2$)</u>	
Teflon	101,142,81,142,142,162	
PVC	67,162,81,108,243,270	DF(3,20)
Stainless steel	107,209,230,230,67,67	
Aluminum	94,67,107,121,121,134	F = 0.80
	<u>10 day ($\mu\text{g COD/cm}^2$)</u>	
Teflon	243,364,587,385,425,364	
PVC	135,175,216,202,270,162	DF(3,20)
Stainless Steel	342,245,294,504,375,310	
Aluminum	148,229,175,202,202,175	F = 11.28
	<u>15 day ($\mu\text{g/COD/cm}^2$)</u>	
Teflon	364,466,506,364,466,364	DF(3,20)
PVC	364,391,297,364,337,267	
Stainless Steel	213,472,423,472,391,439	F = 5.95
Aluminum	283,323,310,256,188,283	

Critical $F_{0.05}$ (3,20) = 3.10

quite drastically from ten to 15 days. An explanation for this behavior might be the previously described effect of microbial activity reduction after attachment has taken place.

Although no definite conclusions may be made in regards to actual numbers of methanogens attaching from F-420 data, the data is at least consistent. F-420 levels for stainless steel were always highest followed by teflon, aluminum and PVC. This agrees fairly well with the anaerobic biofilm (COD) accumulation data, as higher levels were exhibited by teflon and stainless steel, with lower levels for PVC and aluminum. These findings back up the earlier investigations of Marchand and Le Duy in regards to stainless steel's ability to decrease anaerobic fixed film reactor start-up time(30).

If support materials with low and high critical surface tensions exhibit better bioadhesive properties, as the findings of Dexter (13), Baier (1), and Pringle and Fletcher show(36), then the levels of attachment exhibited by teflon and stainless steel in this study indicate that this may also be the case for anaerobic systems. However, PVC and aluminum, both with high critical surface tensions, exhibited low levels of attachment. It was observed during the course of experimentation that aluminum corroded in the anaerobic environment. It is possible that PVC could exert a toxic effect on the methanogens (13). Perhaps PVC leaches chlorinated monomers into the biofilm. For this reason, the ability of a material to resist corrosion and chemical attack may be of greater significance to initial anaerobic biofilm

development than critical surface tension. These physical properties would be of particular interest for future attachment research.

5.2. Interpretation of Anaerobic and Methanogen Biofilm Accumulation

Data for Precoated Support Materials. In the second phase of this study, biological precoating of aluminum and PVC support media was investigated. Three durations of precoating using denitrifying bacteria were examined with each material to test this effect on initial anaerobic biofilm development. Initial rates for anaerobic attachment were determined by graphical calculation of the slope of $\mu\text{g COD}/\text{cm}^2$ data with respect to time. These rates are presented in Table 9 and are the results of COD data taken at zero, five, ten, and 15 days of anaerobic exposure. COD test at zero days provided base line data for future measurements.

It would appear from the plots of Figures 10 and 11 that quicker anaerobic biofilm accumulation rates were obtained for those materials which were allowed to accumulate a denitrifying precoat layer for five days. In order to verify this, a statistical analysis of variance was performed on precoating data. The effect of duration of precoating support materials in a denitrifying slurry for five days, one day, and dip and air dry was analyzed at five, ten and 15 days of anaerobic slurry exposure. Table 10 is a summary of this analysis.

In examining Figure 10 and Table 10, significant statistical variation existed between precoating durations for PVC at ten and 15

Table 9. Anaerobic Biofilm Accumulation Rates
(Precoated Materials)

<u>Material</u>	<u>Duration of Precoating</u>	<u>Rate of Attachment</u> ($\mu\text{g COD}/\text{cm}^2 \cdot \text{d}$)
PVC	DIP/AIR DRY	13.0
	1 DAY	15.0
	5 DAY	23.0
ALUMINUM	DIP/AIR DRY	16.5
	1 DAY	19.0
	5 DAY	19.5

Table 10. Analysis of Variance of Anaerobic Attachment Data
(Precoated PVC and Aluminum)

	<u>5 day ($\mu\text{g COD}/\text{cm}^2$)</u>	
PVC(dip/air dry)	16,30,30,30,30,30	
PVC(1 day)	51,51,23,37,23,37	} F = 3.68
PVC(5 day)	30,43,57,30,43,57	
Aluminum(dip/air dry)	28,41,55,28,41,55	
Aluminum(1 day)	0,3,3,3,3,3	} F = 20.72
Aluminum(5 day)	12,12,25,39,39,39	
	<u>10 day ($\mu\text{g COD}/\text{cm}^2$)</u>	
PVC(dip/air dry)	58,127,113,113,127,113	
PVC(1 day)	106,120,106,189,175,147	} F = 10.99
PVC(5 day)	154,168,236,181,195,223	
Aluminum(dip/air dry)	179,221,152,193,179,193	
Aluminum(1 day)	152,110,124,221,207,248	} F = 1.11
Aluminum(5 day)	205,191,205,205,205,232	
	<u>15 day ($\mu\text{g COD}/\text{cm}^2$)</u>	
PVC(dip/air dry)	264,251,196,306,264,251	
PVC(1 day)	244,244,382,189,285,299	} F = 7.10
PVC(5 day)	554,223,471,361,402,457	
Aluminum(dip/air dry)	262,221,248,290,262,248	
Aluminum(1 day)	455,290,441,359,414,331	} F = 15.14
Aluminum(5 day)	370,370,384,315,425,398	

Critical $F_{0.05} (2,15) = 3.68$

days, while there was no significant variation between data points at five days. This would indicate that past the five day interval, PVC support material exposed to the longer five day precoating duration obtained better rates of anaerobic biofilm attachment than PVC exposed to lower precoating durations.

Analysis of variance results between precoating durations for the aluminum support material did not lend themselves to interpretation as easily as PVC. The largest statistical variation for aluminum precoated support media occurred at five days ($F = 20.72 > F_{.05(2,15)} = 3.68$). No definite conclusion could be drawn between rates of attachment for precoated aluminum due to the stray in data at the ten day interval. This stray in data may be due in part to the previously mentioned fact that aluminum was observed to exhibit surface degradation in the anaerobic environment. It may also be due to extreme experimental error in COD measurements for this ten day test group. Surface corrosion may have interfered with the ability of anaerobic bacteria to remain attached to the aluminum support media.

Methanogen accumulation data for both precoated PVC and aluminum, Figures 12 and 13, indicate that methanogenic activity remains constant after the five day interval. The range of F-420 fluorescence levels for both PVC and aluminum at all precoating durations was only 0.94 nanomoles F-420/cm² to 2.23 nanomoles F-420/cm². It is difficult to determine if these results significant, as the methanogens adsorbing to the precoated media are subjected to a reduction in activity with the

increase in retention time from attachment. Since no definite peak F-420 level was displayed by any precoated material, it may be assumed that the attached methanogen population was never in a highly active state when denitrifying biological precoating was practiced.

5.3. Comparison of Uncoated and Precoated Support Media Biofilm

Accumulation Data. Anaerobic biofilm accumulation data for uncoated PVC and aluminum were compared to those data for precoated PVC and aluminum. In order to ascertain the effect of denitrifying precoating on anaerobic biofilm attachment, ratios of precoated to uncoated materials were calculated. The results of this analysis are listed in Table 11. Figures 14 and 15 display comparisons of attachment data for uncoated and precoated materials at five, ten and 15 days.

Anaerobic biofilm accumulation values for both precoated PVC and aluminum were less than uncoated PVC and aluminum values from zero to ten days. At ten days, air dried and one day precoated materials approached the attachment values for uncoated materials. Five day precoated PVC and aluminum displayed equal and larger attachment levels than uncoated PVC and aluminum. At the 15 day interval precoated attachment data exceed or are still approaching those levels for uncoated materials.

These results are quite different from results obtained for precoated materials subjected to aerobic processes (24). La Motta and Hickey found that initial rates of attachment for aerobic and nitrifying

Table 11. Comparison of Precoated and Uncoated Materials

Day of Measurement	Material	Uncoated	Precoated			Ratio AD	<u>precoated</u> <u>uncoated</u>		
			AD	1D	5D		1D	5D	
5 day	Aluminum	107	41	3	28	0.38	0.03	0.26	
	PVC	155	28	37	43	0.18	0.24	0.28	
10 day	Aluminum	189	186	177	207	0.98	0.94	1.09	
	PVC	193	109	141	193	0.56	0.73	1.00	
15 day	Aluminum	274	255	382	377	0.93	1.39	1.37	
	PVC	337	255	274	411	0.76	0.81	1.22	

Uncoated units are micrograms COD/cm²

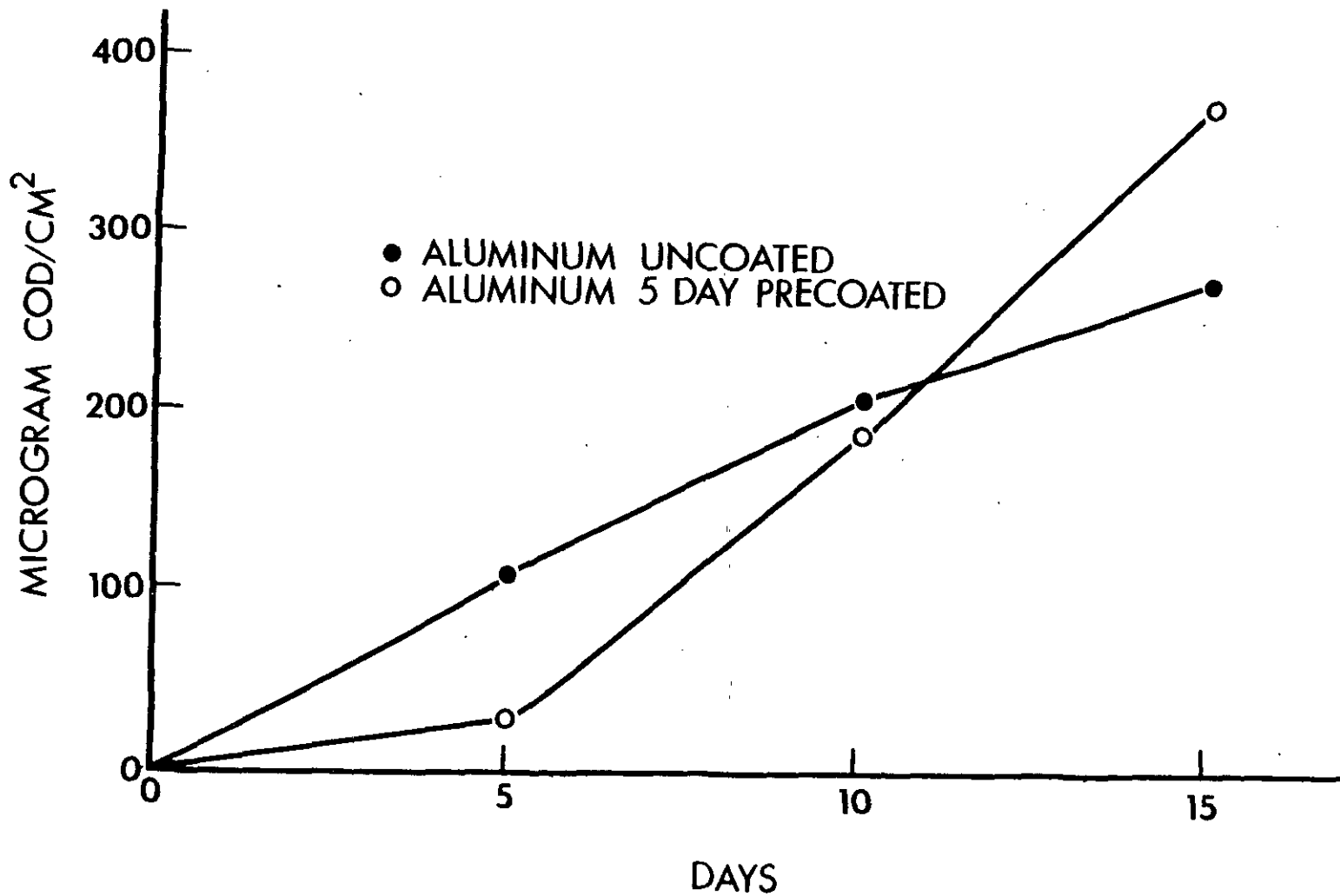


Figure 14. Uncoated aluminum and 5 day precoated aluminum ($\mu\text{g COD/cm}^2$) vs. time (days).

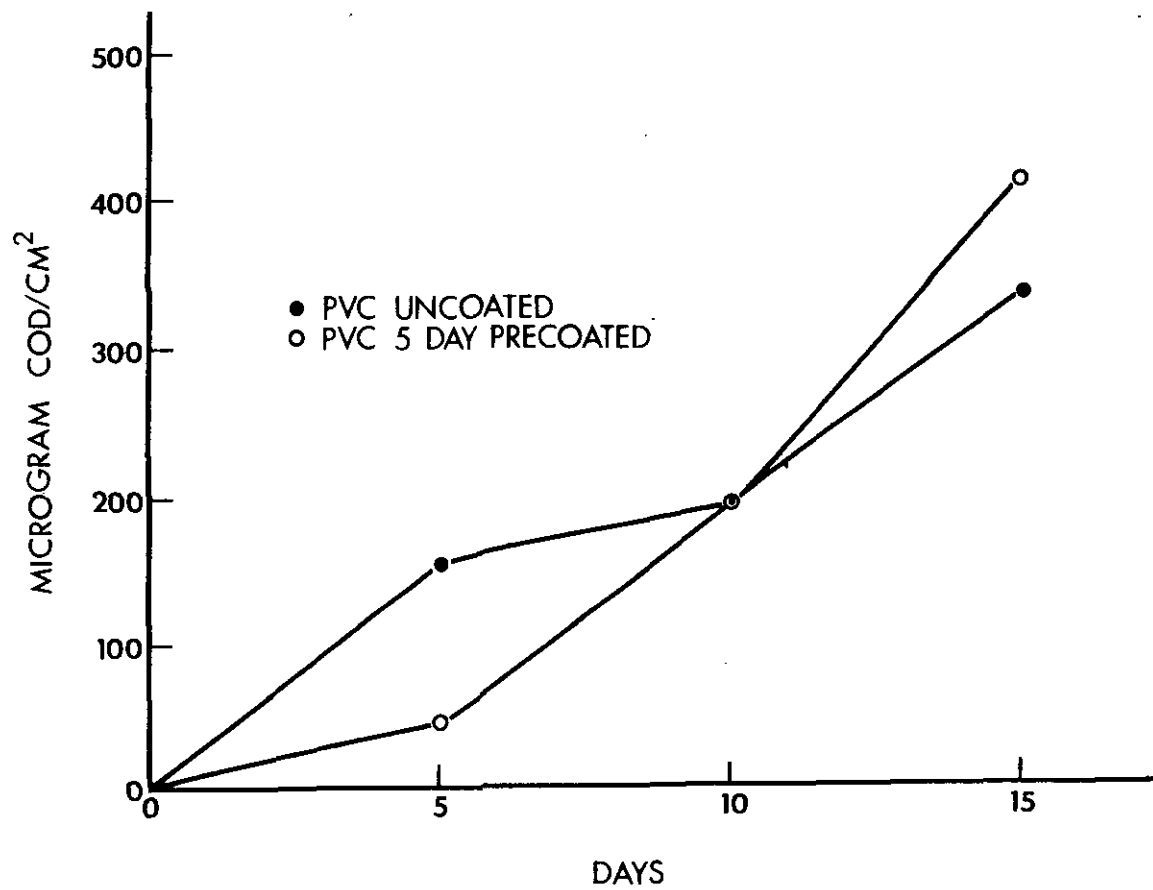


Figure 15. Uncoated PVC and 5 day precoated PVC ($\mu\text{g COD/cm}^2$) vs. time (days).

bacteria were enhanced through the application of synthetic polymers, but that final levels of biofilm thickness remained unchanged between precoated and uncoated materials (24). Denitrifying bacterial precoating in anaerobic systems may retard initial biofilm accumulations. It appears however that past the five day interval, attachment levels for the precoated materials begin to approach those for uncoated materials.

A plausible explanation of these results may be the initial effect on the precoat film when exposed to the anaerobic environment. Anaerobic bacteria may adsorb and grow on the denitrifying layer as quickly as they would to clean surfaces. However, it is likely that the denitrifying layer is dying and sloughing-off of the rods once inside the anaerobic reactor. This would account for initially low attachment levels among precoated media. As the denitrifying layer sloughs-off, more surface area is exposed for anaerobic bacteria to adhere to.

This finding bears significance in regards to its implication on anaerobic fixed film reactor start-up. Denitrifying precoating appears to slow down early attachment, while the overall effect on final attachment levels are about the same. Further research into the effect of precoating support materials on anaerobic biofilm development, in particular utilizing other bacterial cultures for precoating would be significant to this area of research.

C H A P T E R V I

SUMMARY

The objectives of this study were to investigate the possibility of reducing anaerobic fixed film reactor start-up times through the use of various support materials and by utilizing denitrifying bacterial precoating as a means of enhancing initial anaerobic biofilm development.

An anaerobic slurry reactor was maintained at five day SRT and fed a nutrient broth/sucrose feed to which a number of nutrient salts were added. Feeding was performed on a draw-and-fill daily schedule. Support media test sections were placed into the reactor and served as the surface upon which the methanogen and anaerobic biofilm developed. The material extracted from the wall of the tubing was used to measure the increase of anaerobic biofilm (as $\mu\text{g COD}/\text{cm}^2$) and the relative increase of methanogenic bacteria in the biofilm (nanomoles F-420/ cm^2). Experiments were conducted for 15 days, with measurements at five day intervals.

Results of the first phase of the experiment, support material variation, demonstrated that support material had an influence on the initial rate of biofilm accumulation. Anaerobic and methanogenic biofilm accumulation data were highest for stainless steel and teflon. Teflon and stainless steel represent materials from the low and high ranges of

critical surface tension, and suggest that these types of materials may provide faster anaerobic biofilm development and a reduction of process start-up time. PVC, with a critical surface tension more indicative of materials between the high and low ranges, produced lower anaerobic and methanogenic biofilm accumulation data.

Aside from critical surface tension, data from the first phase also indicated that other unique surface physio-chemical properties of a material may influence anaerobic and methanogenic biofilm accumulation. Aluminum, which was observed to corrode in the anaerobic environment, produced the lowest anaerobic accumulation data. Teflon and stainless steel are rather inert materials, as both resist corrosion. Such physical-chemical properties as corrosivity, roughness, and chemical composition are important areas of study for further anaerobic biofilm development research.

The effect of precoating support materials with biofilms of denitrifying bacteria prior to the anaerobic process, phase 2 of this experiment, indicated 1) that materials precoated by five days of denitrifying growth exhibited better rates of anaerobic biofilm accumulation than materials precoated for one day and dipped and air-dried, and 2) that denitrifying bacterial precoating causes anaerobic accumulation data to lag uncoated data up to ten days, but final rates of attachment are generally the same for uncoated and precoated materials averaged over the 15 day start-up period.

Methanogenic biofilm accumulation data, measured as F-420 concentration, proved somewhat inconclusive in this study. It appears that the increase of methanogen SRT due to the process of attachment causes a marked decrease in the levels of F-420 present in the methanogen population in the biofilm. Studies of this type carried out at longer SRT's for the slurry system, may provide more conclusive information regarding the methanogenic population in the biofilm.

For reduction of anaerobic fixed film reactor start-up times, it may prove favorable to precoat support materials with synthetic polymers. Precoating materials with bacterial films which degrade in the anaerobic environment appears to reduce immediate anaerobic biofilm development (zero to ten days). Precoating support materials with synthetic polymers may provide more stable surfaces to which anaerobic bacteria will attach. This area of research is of particular interest in the study of start-up reduction.

C H A P T E R V I I

CONCLUSIONS

Based on the results of this study, the following can be concluded.

1. Materials with very high or low critical surface tensions (e.g., Teflon and stainless steel) provide faster rates of biofilm attachment in anaerobic fixed film systems.
2. Materials which corrode in the anaerobic environment, or perhaps exhibit toxic effects on anaerobic and methanogenic bacteria (e.g., PVC and aluminum), are unsuitable for optimizing anaerobic biofilm attachment in fixed film systems.
3. Rates of anaerobic biofilm attachment for clean materials and denitrifier precoated materials are virtually the same at 15 days, but less at 0-10 days for precoated.
4. The apparatus used in this experiment was simple to construct and easy to work with. A similar design is suggested for further research of this type.
5. COD analysis of materials proved effective for investigative purposes and greatly aided the study of anaerobic biofilm accumulation. F-420 analyses were, for the most part, inconclusive for this study. Longer reactor SRTs than that used in this experiment (> five day) might help make F-420

analysis a useful analytical tool for this work. Other analytical techniques (e.g., total protein analysis) are suggested for future research.

6. Anaerobic fixed film reactor start-up times may be reduced by constructing reactors which utilize materials with very high and low critical surface tensions and which exhibit no toxic effects or surface degradation in the anaerobic environment. These materials must be low in cost to justify their use.

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APPENDIX - GLOSSARY

AEB	-	anaerobic expanded bed
AF	-	anaerobic filter
ALUM	-	aluminum
COD	-	chemical oxygen demand
DSFF	-	downflow stationary fixed film
F	-	F distribution
F-420	-	factor 420
PVC	-	polyvinylchloride
S_b	-	bulk substrate concentration
S_s	-	surface substrate concentration
SRT	-	solids retention time
S.S.	-	stainless steel
UASB	-	upflow anaerobic sludge blanket
VSS	-	volatile suspended solids
Y_{MAX}	-	maximum yield
γ_c	-	critical surface tension
γ_{LV}°	-	surface tension at liquid/vapor interface
γ_{SL}	-	surface tension at solid/liquid interface
γ_{SV}°	-	surface tension at solid/vapor interface
μ_{MAX}	-	maximum specific growth rate
θ	-	contact angle