ACCLIMATION OF ACTIVATED SLUDGES TO INDUSTRIAL WASTES

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by

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SCOPE AND CONTENTS: The procedures used to acclimate activated sludges for design criteria were reviewed. Experiments were performed to determine if cultures could be developed with the same characteristics as activated sludge from an actual treatment plant. Acclimation studies were performed with three refinery wastes. The resultant mixed cultures were compared, using removal rates, with activated sludge mixed cultures from the refinery's waste treatment plants. Soluble organic carbon was monitored for the removal rate curves. An acclimation procedure is proposed to be used in design studies so that a designer can place confidence limits on design data obtained from the batch reactor studies.

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CHAPTER 1

INTRODUCTION

Recent concern about abatement of water pollution has led to various proposals for design criteria for industrial waste treatment. The activated sludge process, suitable for reducing many organic wastes, has been widely accepted. Figure 1 shows a schematic of the activated sludge process. The raw waste enters a well mixed reactor, where it contacts the micro-organisms. The micro-organisms feed on the organic waste in their metabolic processes, thereby reducing the concentration of the waste. The activated sludge micro-organisms have the property of producing a gelatinous material, which agglomerates into flocculent suspensions that can be separated from the liquid by hydraulic separation processes. A gravity separator or settling tank is commonly used to separate these flocs (see Figure 1.)

In order to design a biological waste treatment process, the quality and quantity of the waste stream and the reaction kinetics of the biological system required to process this waste stream would have to be determined. The first step, chemical analysis, has been well outlined in Standard Methods (1). The second step requires detailed laboratory and possibly pilot plant studies to determine the process kinetics and information such as oxygen requirements, suspended solids (Bacteria) production and effluent-loading requirements, before the process plant design can be completed. Laboratory testing procedures have been laid out by Schultz (2), Busch (3) and Eckenfelder (4).

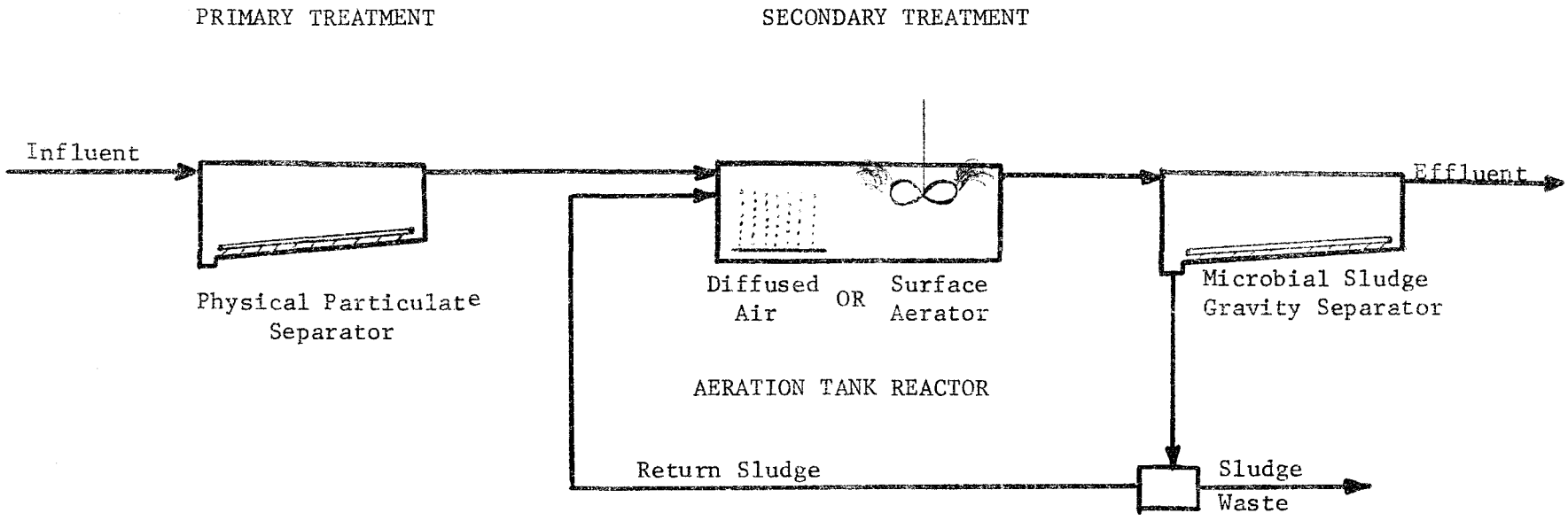

FIGURE 1

TYPICAL ACTIVATED SLUDGE WASTE TREATMENT PLANT

In brief, these procedures involve obtaining the waste to be treated, analyzing it for organic and non-organic constituents, and then setting up batch or continuous activated sludge bench or pilot scale aeration reactors. Each author stipulates that the mixed culture used in the aeration tank must be acclimated to the particular waste before test data can be obtained. The latter step involves many biochemical actions to be reviewed later. Generalizing, acclimation has been defined as a procedure involving a new environment (the new waste) which acts as a stimulant on the organisms (bacteria) to produce a new system of enzymes. These enzymes act as catalysts - hydrolyzing, transporting and degrading the organics. When enough bacteria have been grown to produce a desired system of enzymatic reactions, activated sludge mixed culture can be said to be acclimated to reduce the organic waste. The time period and detailed process for acclimation have not been well defined by researchers or designers. Generally five to ten days feeding in incremental steps has been the accepted procedure. No information has been presented to show whether this resultant bacterial culture would be representative of the activated sludge culture found in an existing plant.

The project was undertaken to study the acclimation of biological mixed cultures to phenolic wastes particularly from refineries. The refineries were chosen because they had existing activated sludge treatment plants, from which an actual mixed culture

processing the particular waste could be compared with a laboratory acclimated mixed culture. A detailed procedure for use in design has been proposed from the results of the empirical study.

CHAPTER 2

DESIGN

Design procedures are desired in order that one can determine with confidence that a specific treatment process will produce a desired effluent quality. Because of the complexity of modern industrial processes, industrial wastes may vary from plant to plant, making the wastes difficult to typify. Two industrial plants may produce an identical product but because of different processes and raw materials the waste streams will be different. Since handbooks are usually inadequate or unavailable, designers must rely on empirical data from laboratory testing methods to derive the process kinetics for biological treatment of industrial wastes.

In the early 1950's researchers began to make headway by describing the biochemical reactions of mixed cultures (activated sludge) in forms of kinetic expressions. Early studies followed work done by Monod (5) and Herbert (6) and their kinetic expressions for pure culture removal characteristics. Garrett and Sawyer (7), waste-water oriented, employed a mathematical form similar to the "Michaelis Menton" kinetic expression (described by Monod), by using data from existing waste treatment plants to derive reaction constants. The log growth curve associated with pure culture bacterial growth was found not to apply to waste-water conditions because of food limitations or low organic concentrations, Monod (5),

Hinshelwood and Dean (8). It is logical to assume that if an industrial waste has a high concentration of organic compounds from a product line, the producer should attempt to recover these organics for profit, rather than process them as a waste. Garrett and Sawyer, (7), by using similar rate equations found that reaction rates became progressively slower in going from pure culture bacteria to mixed cultures and finally to plant mixed culture (activated sludge). A simple explanation is that such things as inefficient bacteria - organic contact have a stronger influence on the system. Furthering the kinetic approach, Eckenfelder and Weston (9) used the first order equation of log growth modified to fit the organic limiting concentrations of waste-water. Whurman (10) has said that the fault with this general type of expression is that single substances are removed by a zero order reaction at very low concentrations but in a mixed waste the overall rate ranges from zero to second order. The ideal solution for a mixed waste would be to take each constituent of the waste, study its pure culture removal characteristics and then to sum all reactions and interreactions, to obtain the overall reaction rate. This latter approach points out the shortcomings of specific kinetic expressions and overall or unit rates applicable to all wastes.

In the 1960's, researchers such as Busch (3), (11), Gaudy (19) and McKinney (25) began using a rational approach to design, utilizing

laboratory kinetic studies. They emphasized the need to recognize that data obtained from laboratory studies were applicable only to the particular waste being studied. The design equations were formulated by taking mass balances around an ideal steady-state mixed reactor. The reaction rate or the substrate removal rate, was obtained from batch studies using acclimated activated sludge. Busch (11) stated that before the reaction rate could be obtained from a batch test the mixed culture should be acclimated in a continuous activated sludge reactor at a loading condition estimated to be necessary in the final treatment of the waste. Bennett (12) found similar reaction kinetics in mixed cultures kept on continuous and on batch feeding, however, the batch methods gave slightly lower reaction rates. The review of Busch (13) describes a typical industrial waste with sample calculations for a waste treatment system design.

The design of a waste treatment system is, therefore, heavily dependent on obtaining an acclimated mixed culture from which reaction rate data can be measured. The mixed culture developed for testing must be representative of the future mixed culture activated sludge which will be found in the actual treatment plant.

CHAPTER 3

ACCLIMATION

Acclimation can be defined as a process of adapting to a new environment. In the case of biochemical waste treatment, it is the mixed culture which must adjust to the new food (i.e., waste) environment. The process involved during this adjustment is called biodegradation, which can be defined as the "ability to reduce the complexity of a chemical compound by splitting off one or more groups or larger component parts."* To proceed through these biochemical pathways it has been accepted knowledge that activated sludge mixed bacterial cultures need periods of adjustment before some wastes can be degraded to final non-biodegradable end products. This period of acclimation has been documented by some researchers, Ludzack and Ettinger (14) and McKinney (15), as being exact time periods, while others, Gaudy (16), Eckenfelder (4) and Busch (3), claim a period of between five and ten days.

Since acclimation has been defined as a biochemical process examing the work performed by microbiologists on pure culture bacteria and related organisms should clarify the significance or the reason for certain inhibiting reactions. When studying the influences of temperature, pressure or drugs on cell physiology, any micro-organism culture must be acclimated before valid results

* Funk and Wagnalls, Standard College Dictionary, Longmans Canada Limited, Toronto, 1963.

can be obtained (Hinshelwood and Dean (8), Lamanna and Malette (17)).

The acclimation studies performed by waste-water researchers (Ludzack and Ettinger (14)) have been limited to pure compounds simply because reactions involving a mixed waste with a mixed bacterial culture necessitate too large a number of analyses to obtain exact answers. For design or biodegradation studies the designer's prime interest has been the reaction kinetics or final substrate concentrations and not how the bacteria has become accustomed to the waste. Acclimation, requiring between five to ten days, have been accepted as a necessary adaption period for most organic wastes.

3.1 MICROBIOLOGICAL APPROACH TO ACCLIMATION

Accepting the limitation that microbiologists employ pure culture bacteria, a basic understanding of influencing factors on a biochemical reaction can be obtained from biochemical literature.

A simple comparison with a chemical reaction shows how a biochemical reaction proceeds.

CHEMICAL

$$\underset{\text{Reactants}}{A + B} \xrightarrow{\text{Catalyst}} C + D \pm \text{Energy}$$

BIOCHEMICAL

$$\text{Organics+Nutrients} \xrightarrow[(\text{Enzymes})]{\text{Organisms}} CO_2 + H_2 + \text{New Organisms+Products+Stored Energy}$$

A chemical reaction rate or extent of reaction can be determined from the concentration of reactants along with temperature, pressure, etc. In a biochemical reaction, the reactants are the organic substrates (i.e., pollutant), and nutrients which combine using various enzymes as catalysts. Enzymes are defined as "a protein produced by cells having the power to initiate or accelerate specific chemical reactions in metabolic processes, i.e., acting as an organic catalyst."* Enzymes, therefore, make up one of the key items in biochemical reactions. Whether a reaction does or does not occur will depend to a large extent on the controlling enzymes and subsequently on the mechanisms which limit them.

In the text by Hinshelwood and Dean (8), five chapters have been devoted to the various aspects of acclimation which they refer to as adaption. The acclimation process is explained as being dependent on a very complex pattern of linked chemical actions, mediated by enzymes which, in time, build up as directed by information supplied by nucleic acids. The cell functions can be susceptible to interference in many ways and at many different points. Drugs are substances other than normal metabolic intermediates which can interfere. The effect of a drug substance before acclimation might be that of a protoplasmic poison, as an influence on an enzyme reaction or as an influence on nucleic acid replication. Another

* Funk and Wagnalls, Standard College Dictionary, Longmans Canada Limited, Toronto, 1963.

chapter discusses the effects of various drug concentrations on lag phase and growth rate before and after acclimation. Also described was "cross adaption" where bacteria grown on a substrate of a similar molecular structure were readily adapted and showed an increased growth rate with the new substrate. Hinshelwood and Dean propose that development of alternate mechanisms or enzymatic pathways occur in many adaptive steps.

In the text, "Basic Bacteriology" Lamanna and Malette (17) refer to an acclimation period as the adjustment phase or lag before the maximum growth. The following summarizes their modern view of acclimation.

(1) although subject to many variables the longer the generation time in lag growth phase, the longer the acclimation period.

(2) the age of the culture or average age of the micro-organisms from which the inoculum (i.e., bacterial culture) was derived will determine the lag phase.

(3) the length of the lag phase decreases with an increased inoculum and quantitatively tends to be a linear function of the logarithm of the number or organisms in the inoculum. The former can be exemplified in activated sludge which has many

millions of organisms acting as buffers when slugs of highly concentrated waste pollutant flow into a plant (i.e., shock loads). The latter point has not been defined in mixed cultures mathematically but the general trend has been accepted.

(4) whatever causes the phase adjustment does not affect the other phases of growth. The effect may be a scale-down in growth rate but in fact log growth, stationary and declining growth phases still occur.

(5) in the phase of adjustment or acclimation the rate of multiplication tends to lag behind the rate of growth. This results in a larger average size of organism than occurs during the other phases. It has been found that a higher rate of metabolic activity such as oxygen consumption, or carbon dioxide, ammonia and heat production occurs when it is expressed as activity per cell.

(6) the physical and chemical organization of bacteria cannot be fixed throughout the extent of the growth curve. Thus during the phase of adjustment and in

the early period of exponential growth, the organisms appear to be most permeable and most sensitive to sudden changes in environment. These organisms, often referred to as being in a state of "Physiological Youth" can be easily inactivated by heat and cold and by transfer into solutions of slightly higher salt concentrations (osmotic pressure change). This latter phenomena has been shown by Krishnan and Gaudy (18) in shock loading studies where they found young cells much more susceptible to shock loads than older cells. The performances of these two types of bacterial cells were observed in batch as well as continuous studies. The continuous reactor study reduced the possibility of an accumulated poison effect. The bacterial cells referred to as young cells represent a bacterial culture in which a high percentage of the cells are in the lag growth phase of their life cycle. Bacterial cultures referred to as older cells describes a culture in which the bacteria are in the stationary phase. In this latter phase cells have accumulated slime layers or waste products which cling to the bacteria acting as a buffer when a shock loading condition occurs.

In connection with enzyme activity, Lamanna and Malette (17) contend that two states of inhibition can occur either competitive or non-competitive. The former occurs when a chemical substance, which can take up a reactive site on the enzyme molecule but cannot provide the nutrient value nor fulfil the purpose of the competitive substrate, combines with the enzyme. The latter, a non-competitive chemical substance joins a reactive site somewhat removed from the desired substrate site, but in so doing physically blocks the site preventing the substrate from getting to the enzyme.

In a section on irritability of bacteria (capacity of a bacteria to modify its behaviour in response to changes in environment) Lamanna and Malette (17) state that much of the work performed has been of the descriptive nature. The authors have presented well documented ideas on how changing physical conditions stimulate bacteria.

3.2 ACCLIMATION - WASTE WATER

Acclimation views in this research area stem from two contrasting points of view expressed in Hinshelwood and Dean (8), where either all bacteria can develop from one species through mutations prompted by an environmental change stimulii or that different basic bacterial species have been found needed and can be found in a multi populus activated sludge from which natural

selection results in one predominanting species.

Employing the first idea of bacterial selection, researchers such as Gaudy (19) develop a mixed culture from low concentrations of bacteria already synthesising the waste material.

In a municipal waste the bacteria originate from human feces and earth bacteria. Bacteria utilizing industrial wastes can be obtained from the soil around ground spillage areas or from river beds receiving the untreated waste for some time period. Usually a fill and draw method has been employed to build up the concentrations of bacteria. The procedure follows:

(1) Feed waste and aerate 24 hours in vessel

(2) Remove 1/3 volume while mixing

(3) Settle remainder, remove 1/2 remaining volume as supernatant

(4) Replace 2/3 volume vessel with new waste.

This procedure can be repeated until a sludge mass is grown (i.e., zoogleal mass formed by bacteria accumulating to form flocs which will settle) to a concentration similar to a value derived in the full scale plant or until enough bacteria per unit volume are present to degrade the waste to a desired level. This method will develop bacteria which will treat the particular waste but, in general, a long time period will be required, two to six months, to

develop large masses of sludge. Specific baterial cultures have been found necessary to develop a mixed culture to treat a specific waste (Gaudy (27) and Eckenfelder (4)).

The second procedure most commonly used in design procedures involves obtaining the mixed culture from an existing municipal or industrial activated sludge plant. Laboratory study procedures have been outlined by Eckenfelder (4), Ludzack (24), Schultz (2) and Busch (3,11). Batch studies follow a program similar to the one listed by Gaudy for a period of acclimation and then the removal rate can be obtained with a final batch run. If a continuous reactor setup was employed, as the authors state, it would give more consistent results for design. Once an acclimated state was obtained, a batch run was then performed to obtain the removal rate.

No matter which system has been employed to get an acclimated sludge or which unit for feeding or aeration has been employed it is important to interpret data remembering the limitations of either the procedure or apparatus. It must be noted that acclimated mixed cultures must be subjected to similar hydraulic and food loadings required in actual plant operation.

A recent plant startup showed that a phenolic waste from a coke plant could not support growth of activated sludge obtained from a municipal plant but activated sludge obtained from a waste plant treating a somewhat similar phenolic influent was able to

grow. This would seem to support the idea that special cultures are required. A possible problem, nutrient deficiency, had been eliminated by adding phosphoric acid. Supporting the numerous adaption theory, Ludzack (14) states that oxydipropionitrile requires 100 days in river water, while only 1 week in activated sludge to degrade.

The following references (Eckenfelder (7), Gaudy (19), Busch (3)) point out in general terms that acclimation has occurred when the following conditions are satisfied:

(1) Organic (waste) removal satisfies government standards

(2) sludge settled to give a good sludge volume index

(3) nutrients sufficient for C:N:P ratio of 40:5:1

(4) buffering capacity so that pH ≈ 7.

The specified time period for acclimation varied between five and ten days.

Biodegradability studies have been performed to determine how stable various organic compounds, found in industrial waste effluents, are to biological oxidation. Interest in biodegradability has been noted in literature for over one hundred years (20). More recent works by Ludzack and Ettinger (14) and McKinney et al (15) show that workers have been interested in the new chemicals, particularly synthetics from the petro chemical field. Papers by Thompson (20) have proposed Biotreatability Indexes to describe the extent of reactions.

McKinney et al (15) in studies on treatability of industrial wastes proposed an acclimation procedure shown in Figure 2. The method necessitated an exhaustive study to identify the waste to be treated. In the case of an industry, the source of the waste machine or product washing was found. In a municipal waste any unusual waste sources were examined in case they entered the sewer or caused slugs of highly concentrated waste. The activated sludge culture was then grown in batch or continuous units (the latter if toxicity was likely to occur). The seed was obtained from sewage, soil or an activated sludge plant, and aerated until a floc was grown having a solids concentration between 1500 and 2000 ppm. The waste to be tested was then fed according to Figure 2. The authors (15) state that if all procedures on the chart failed a molecular pairing or substitute compound procedure could be employed. The alternative compound, which is biodegradable, could be acclimated to a bacterial culture after which the previously non-biodegradable substance could be then introduced in a stepwise procedure. Hinshelwood and Dean (8) in their section on Adaption to Drugs call this "cross adaption". This phenomenon was believed to occur when two substances of similar structure could be positioned at the same place in a metabolic chain and thus either could be used. In this case bacteria could adapt their metabolic pathways to use one substance more readily than the other, but once established both drugs or substances could be employed interchangably. McKinney further states that longer periods of time or lower concentrations of feed may be necessary for adaption when this type of waste is to be studied.

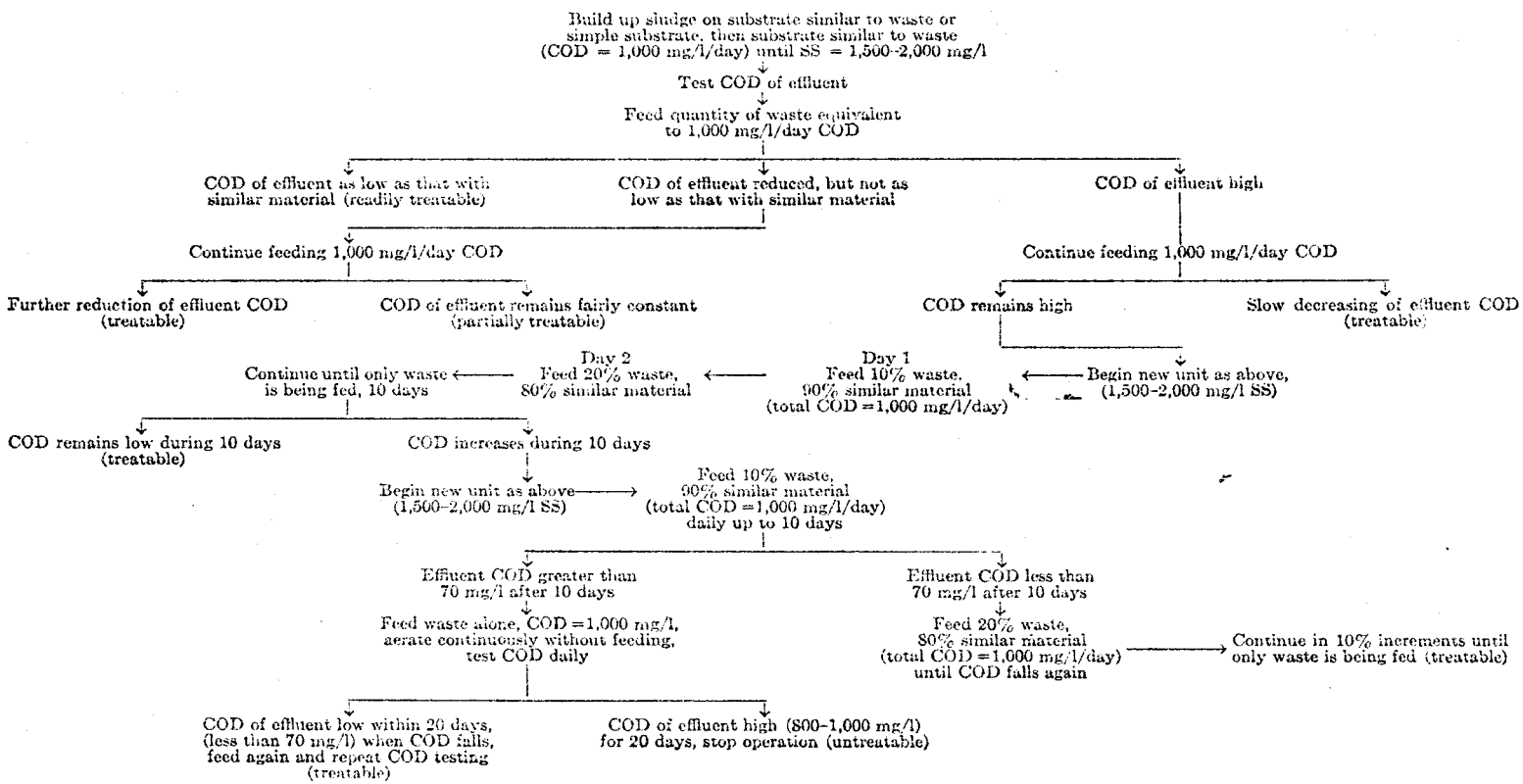

FIGURE 2.—Outline of acclimation procedures for the determination of biological treatability of industrial wastes.

CHAPTER 4

SCOPE OF INVESTIGATION

The chapters on design and acclimation have delineated a definite need for acclimation procedures from which one can predict realistic design information. The primary objective of this study is to determine when a state of acclimation has been attained in a test activated sludge. This mixed bacteria culture would have to have had similar properties to that of activated sludge reducing the same waste.

An acclimated activated sludge can be obtained through two processes described in section 3.2. The latter method was chosen as the most practical for design purposes, as activated sludge cultures are easily obtained and the method takes a shorter time period.

The variables to be studied involve the feeding technique and the period required for acclimation. The final state will be determined by a comparison of the removal rate curve with that of a mixed culture already treating the waste. The mixed culture source for the tests was a municipal activated sludge treatment plant. The wastes to be studied were chosen from local refineries with existing activated sludge treatment systems.

The primary object of the study was to determine the acclimation period such that the designer could state with confidence that the reaction rate derived from the laboratory

study would be the same as the reaction rate to be found with the actual activated sludge. The laboratory procedure followed was similar to that outlined by Busch (3) and Eckenfelder (4).

Excess nutrients were added so that the carbon source would be the only limiting nutrient. The mixed culture suspended solids concentration was maintained equal to that found in the refinery's treatment plant. As many of the discrepancies between the mixed culture and the refinery sludge as possible, were eliminated to improve the comparison between removal rates of the acclimated mixed culture and the existing plant sludge. This would then indicate whether laboratory studies could properly predict how future activated sludges would perform.

CHAPTER 5

INVESTIGATION

5.1 PROCEDURE

The method employed a batch reactor containing activated sludge obtained from a municipal sewage treatment plant. Successive runs were carried out, increasing the particular waste feed concentration each time, until the sludge could tolerate a full strength addition. The removal curve obtained from the final batch run was then compared with a removal curve from a batch study using the same waste feed and a mixed culture obtained from the activated sludge treatment plant reducing the same waste. To maintain a viable micro-organism culture the initial small percentage amounts of waste were supplemented by dextrose to keep the soluble organic carbon load on the mixed culture at a constant value. The tests were performed at room temperatures. If the particular waste ambient temperature will be significantly different, batch kinetic studies should be performed at these temperatures to insure biochemical reduction will occur. If temperature changes are inherent in the waste stream adaption to these changes should be with an acclimated mixed culture.

The procedure followed for acclimation is outlined below:

(1) a municipal activated sludge was obtained and aerated for one day prior to beginning the tests to stabilize the culture and dilute or concentrate it

to the desired suspended solids concentration (equal to the activated sludge from the refinery's waste treatment plant). Nutrients were also added.

(2) the waste and activated sludge were obtained from the refinery's treatment plant. The mixed culture was aerated for one day as in (1) and the batch run performed the next day.

(3) the acclimation study was begun by feeding the unacclimated sludge low concentrations of the waste and daily increasing the feed concentration. The percentage difference in soluble organic carbon was supplemented by dextrose. Nutrients were fed in prescribed amounts (listed later) and required trace elements were adequately supplied by the tap water used to make the feed (22).

(4) each batch run was monitored by soluble organic carbon sampling taken frequently to trace a kinetic reaction curve to a base soluble organic carbon level.

(5) The soluble organic carbon removal curve for the final batch run (i.e., using full strength feed) was compared with the removal curve for the refinery's activated sludge, to determine if acclimation had been effected.

(6) to compare an acclimated and unacclimated mixed culture, the latter was run during the testing period.

To prevent a buildup of toxic metabolic products or a supply of non-biodegradables in the reactor, the mixed cultures were settled to remove most of the supernatant. To prevent excessive waste source degradation, fresh supplies were obtained for each acclimation test run. It was not feasible to get new supplies each day.

Although the method mentioned above has proved successful one other acclimation procedure was examined, a continuous series of batch runs. The succeeding run would start when the soluble organic carbon reached a base line. The method was examined in the third series of experimental runs.

5.2 APPARATUS EMPLOYED

5.2.1 Reactors

The reactors used were 4 liter, 12 liter and 20 liter glass jugs along with 6 and 12 liter glass percolators. In preliminary investigations and during the first testing runs the glass percolators with sintered glass diffusers caused excessive foaming. A coarse air diffusion technique was used to limit foaming. Visual examination showed apparent complete mixing.

5.2.2 Air Supply

Air was supplied to the reactors at a rate sufficient to

keep the sludge flocs in suspension. This required air rate could be determined by visual inspection. The air was first bubbled through a water bath. This bath scrubbed out any oil and dust particles and saturated the air to limit evaporation from the reactor.

5.3 SAMPLING

To monitor the removal characteristics of the mixed cultures, samples for soluble organic carbon and mixed culture suspended solids were taken at 15 minute intervals.

5.3.1 Suspended Solids

A relative measure of the bacterial concentration was determined by first filtering the sample through a previously weighed membrane filter, drying the filter, and weighing again. The filters "Sartorius Membrane-filter" size .45 μ were of constant weight structure. The filtering apparatus using a 30 pound vacuum was a "Millipore Filter" type. The filters were placed in tagged aluminum dishes placed in an oven at 105°C for one hour, cooled for twenty minutes in a desicator and weighed on a Mettler Balance accurate to 0.1 mg.

5.3.2 Soluble Organic Carbon

The soluble organic carbon in the filtrate from the millipore filtration was measured on a Beckman infrared analyzer. Two possible sources of error in soluble organic carbon measurements

were corrected. The membrane filters have some residual organic carbon possibly from the resin or type of glue. Past researchers (Pollock (21)) have found that filtering 100 ml of distilled water removes this error. Bicarbonates and carbonates also oxidized to carbon dioxide in the carbon analyzer can be removed by acidifying to a pH of 2 and then sparging the sample with an inert gas for 5 minutes. The soluble organic carbon is then determined by injecting a 20 ml sample into the analyzer. A standard calibration curve was prepared using sodium oxalate as the carbon source.

5.3.3 pH Measurements

pH measurements were made with pH meters. Owing to the excess buffer added during each test and the buffering action of the activated sludges, the pH of the mixed cultures seldom varied from 7.

5.3.4 Dissolved Oxygen Concentration

Tests for dissolved oxygen were done by the Winkler Method (1). Oxygen concentration was measured periodically to insure an adequate amount existed at all times.

5.4 MIXED CULTURE SOURCE

The mixed cultures used in the following studies were obtained from the Dundas and Burlington Skyway Activated Sludge Waste Treatment Plants. The activated sludge was taken from the return sludge line from the Dundas plant to obtain a concentrated

mixed culture. The Burlington plant operates at a suspended solids concentration around 8000 mg/l, hence their was no need to concentrate the mixed culture.

5.4.1 Nutrients

To insure that a microbial culture will multiply, a proper supply of nutrients is necessary. In a previous study McLean (22) found that various compounds were necessary to maintain an excess nutrient supply sufficient to satisfy a ratio C:N:P: 40:6:1, (Eckenfelder and O'Conner (23)). The following solutions were prepared and added in indicated concentrations.

(1) Ferric Chloride $FeCl_3.6\ H_2O$ Solution 1.0 mg/ml
Feed ratio 1/4 ml $FeCl_3.\ 6H_2O$ to 100 mg soluble carbon/litre.

(2) Potassium phosphate KH_2PO_4 Solution 50 mg/ml
Feed ratio 1/2 ml KH_2PO_4 to 100 mg soluble carbon/litre.

(3) Magnesium sulphate $MgSO_4.7H_2O$ Solution 50 mg/ml
Feed ratio 1/2 ml $MgSO_4.7H_2O$ to 100 mg soluble carbon/litre.

Analysis of the Hamilton tap water used a a diluent showed elements in adequate amounts for microbial growth (22).

5.5 SOURCE OF TEST WASTE

To study acclimation procedures various sources of waste

were required which already had existing activated sludge waste treatment systems. Since all of the test runs were performed in McMaster's laboratory, the source of the waste had to be relatively close to the university. The waste had to be of the nature that upon storage for a week, degradation would not occur. The industrial waste source that met these requirements was a refinery type phenolic waste. Three sources were chosen and will be referred to as Refinery Waste A, Refinery Waste B and Refinery Waste C.

The pollution problem in this type of waste was phenol or similar aromatic compound variations centered around the benzene ring. Owing to the variations in the quality of crude oil being processed coupled with waste removal capacity of some refinery units, the waste water streams showed considerable variation in concentrations of phenolic compounds and other compounds such as hydrogen sulfide and ammonium sulfide. If either of the latter two were present biological action of activated sludge would be inhibited until the gases were stripped off by the aeration system.

In general, refinery waste water streams contain varying amounts of oil spill, phenol and phenolic compounds (the main concern of water pollution control commissions) and other compounds from the crude source such as cyanides, nitriles, mercaptans, ammonia, caustic etc. resulting from reforming processes. The oil can be removed by API separators, settling tanks with skimmers.

Volatile compounds such as H_2S, NH_3, HCN, can be removed by gas strippers. The phenol or phenolic compounds can be generally removed by biological treatment, namely the activated sludge process. Some bacteria readily utilize phenol as food although the exact metabolic pathway is presently in some dispute. As the concern of this report was not the actual waste characteristics but rather acclimation of the mixed cultures, soluble organic carbon was monitored. However, if monitoring of soluble organic carbon showed significant reduction and since phenol was the main carbon source, it follows that the biochemical reduction of the phenol was effected.

CHAPTER 6

EXPERIMENTAL RESULTS

The experiment was divided into a series of runs performed on the three sources of waste. Table 1 lists the runs performed, the waste type used, reactor volume used and length of acclimation period.

Following this are brief descriptions of each refinery waste source and the runs performed on the particular waste.

6.1 REFINERY WASTE A

The source of Waste A was from an equilization basin in the refinery's effluent treatment plant. This basin is positioned after the oil API separators to act as a buffer preventing large shock loading on the biological treatment plant. The waste contained a visable amount of suspended matter which was in a colloidal form. Chemical treatment would have been required to break the emulsion but operators had found this unnecessary. The soluble organic carbon remained relatively constant during the first two test runs (around 60 ppm). The lower value of soluble organic carbon in the last test run can be attributed to the fact that the influent was being pumped from the bottom of the equilization basin. A microbiologist examined the suspended matter in the latter waste supply and indicated that bacteria were present and were probably degrading the phenol. This would explain the lower soluble organic carbon value.

TABLE 1

LIST OF EXPERIMENTAL RUNS

RUN NO.	WASTE TYPE	REACTOR VALUE USED	ACCLIMATION PERIOD
1	A	6 Liter	10 days
1A	A	6 Liter	6 days
2	A	15 Liter	5 days
2A	A	15 Liter	10 days
3	A	4 Liter	(continuous ≈ 40 hours)
3A	A	15 Liter	10 days
4	B	6 Liter	No period req.'d
5	C	8 Liter	6 days
6	C	8 Liter	3 days

6.1.1 Test Run Numbers 1 and 1A

Refinery Waste Treated - Type A, separate sample for Number 1 and 1A soluble organic carbon 60 ppm

Reactor Used - 6 liter percolators, air hose aeration

Acclimation Study Time - (a) 10 days

(b) 6 days

(c) Refinery activated sludge for standard.

The results are tabulated on Table 2 and plotted on Figures 3 and 4.

The plot, Figure 3, indicates that a period of ten days was sufficient acclimation period for this waste. The test period was next reduced to six days for Run No. 1A as shown in Figure 4. Again the acclimated mixed culture showed a similar reduction curve to the refinery standard activated sludge. The time period required to reach a base soluble organic carbon level varied in the two runs from one and one-half (1-1/2) hours in the first, to three and one-half (3-1/2) hours in the second series. This could possibly indicate that although the waste was reduced to the same level the phenolic compounds in the Run 1A were more difficult to degrade and therefore took a longer time. Since the refinery's activated sludge standard curve changed, the change in period of acclimation cannot be suggested as an explanation in the time difference. The time period of six days can then be considered sufficient for the Waste A used in Run No. 1A.

TABLE 2

TEST RUN NUMBERS 1 AND 1A - WASTE A

TIME	10 DAY ACCLIMATION		REFINERY STANDARD		6 DAY ACCLIMATION		REFINERY STANDARD	
	Carbon	Solids	Carbon	Solids	Carbon	Solids	Carbon	Solids
0	44.3	1048	49.3	913	44.7	990	41.5	720
.25	-	-	-	-	40.3	990	39.5	727
.50	35.0	1070	35.5	937	37.2	1130	39.7	787
.75	-	-	-	-	37.2	963	35.6	707
1.00	27.0	1045	22.0	920	32.0	970	32.5	740
1.50	19.0	1077	14.0	923	30.0	960	29.3	740
2.00	14.0	1053	13.5	930	25.0	1033	24.2	767
2.50	14.7	1117	20.0	953	21.3	1030	21.2	750
3.00	12.5	1180	11.6	1050	14.7	990	16.2	730
3.50	-	-	-	-	12.3	997	13.0	737
4.00	-	-	-	-	11.0	960	12.0	727
5.00	13.5	1130	12.0	953	-	-	-	-

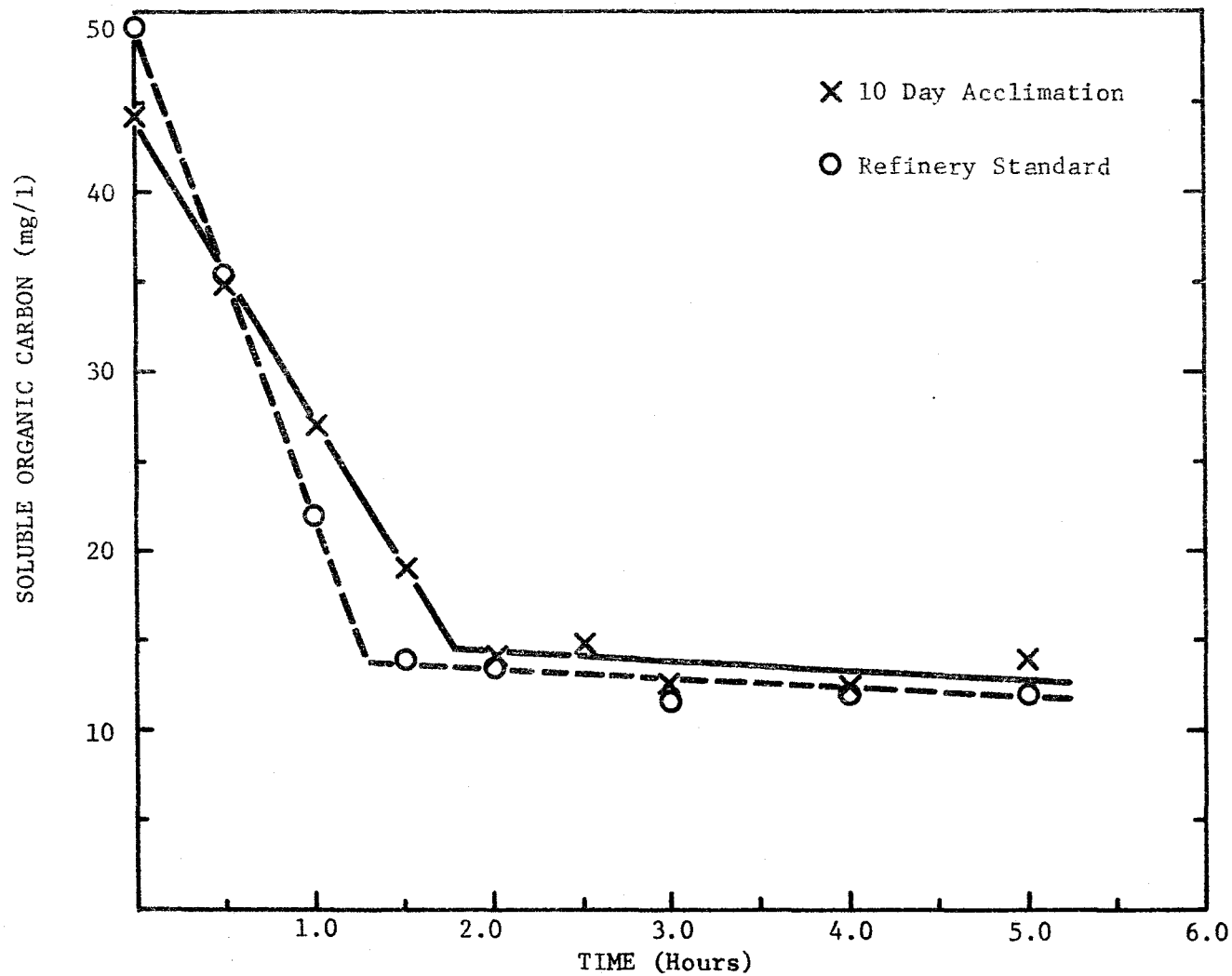

FIGURE 3

TEST RUN NUMBER 1 - WASTE A

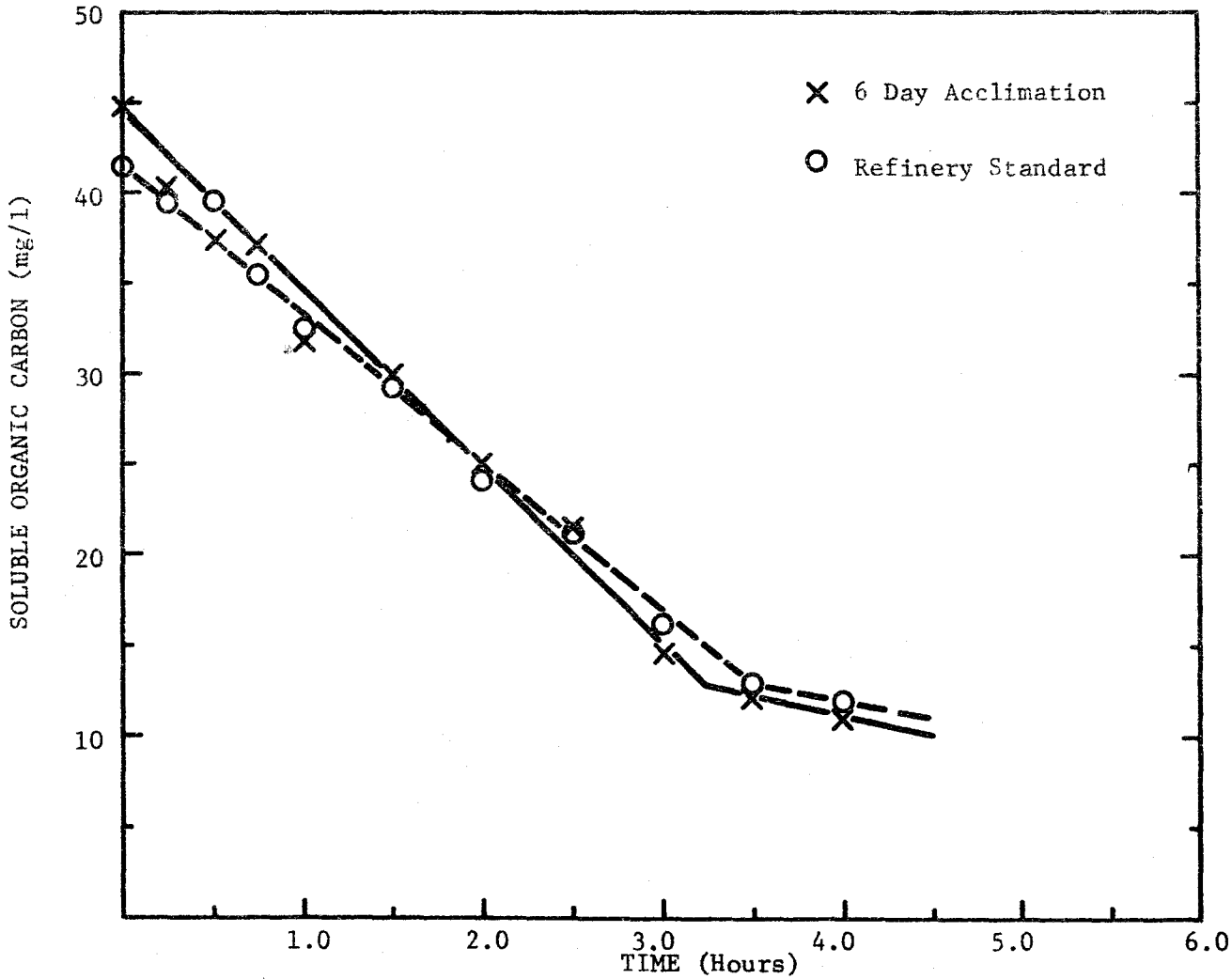

FIGURE 4

TEST RUN NUMBER 1A - WASTE A

6.1.2 Test Run Numbers 2 and 2A

To examine whether the size of the reactor vessel could have any detrimental effect on the batch study, a larger 20 liter vessel was employed. Work performed with batch reactors has indicated that in small reactors side growths of bacterial slime could affect reaction rates. They are comprised of a significant amount of bacteria although it is in an unaccountable form. This latter problem can be remedied by daily scraping the container walls. Improper mixing has caused some concern but visual inspection through a glass vessel can insure that this problem is kept under control.

Refinery Waste Tested - Type A

Soluble organic carbon, 60 ppm

Reactor Used - 20 liter vessel
filled to 15 liters

Acclimation Study - (a) test 5 day period

(b) test 10 day period, 9th day recorded to compare with 10th day

(c) test refinery's activated sludge for standard.

The results of Run No. 2 and 2A are tabulated in Table 3 and plotted in Figures 5 and 6.

A discussion with the refinery's Treatment Plant Operator revealed that the biological process was upset due possibly to a high concentration of toxic substance. The waste was noted to have a strong odor of

TABLE 3

TEST RUN NUMBERS 2 AND 2A - WASTE A

TIME	REFINERY STANDARD		5 DAY ACCLIMATION		10 DAY (9th Day Test)		10 DAY ACCLIMATION	
	Carbon	S.S.	Carbon	S.S.	Carbon	S.S.	Carbon	S.S.
0	47.5	1260	52.5	1017	52.8	1310	50.8	1045
.25	44.8	1217	47.0	1093	45.2	1265	48.0	1064
.5	42.3	1207	48.0	1157	45.7	1265	50.0	1060
.75	42.0	1195	45.0	1247	42.7	1285	46.3	1034
1.00	38.3	1243	48.0	1197	42.8	1265	45.0	1022
1.25	35.0	1205	47.0	1095	41.9	1310	46.5	1048
1.5	31.0	1175	49.0	1100	41.5	1310	45.0	1003
1.75	28.3	1215	45.0	1135	39.7	1300	42.2	999
2.00	24.5	1180	45.0	1105	38.4	1390	43.2	1019
2.25	23.0	1260	46.0	1175	36.8	1365	41.0	1053
2.5	20.5	1200	47.0	1255	34.0	1305	42.0	1032
2.75	19.7	1160	45.0	1145	30.0	1295	49.5	933
3.0	19.5	1125	47.0	1095	26.0	1320	39.2	984
3.25	19.5	1185	45.0	1120	23.0	1335	38.0	996
3.5	18.3	1280	44.0	1215	20.0	1320	37.0	1026
3.75	-	-	-	-	19.2	1320	34.5	1008
4.0	-	-	-	-	17.2	1325	31.8	1022
4.25	-	-	-	-	-	-	30.0	1032
4.75	-	-	-	-	-	-	23.0	1043
5.00	-	-	-	-	-	-	20.0	1093
5.25	-	-	-	-	-	-	16.7	1105
5.5	-	-	-	-	-	-	18.0	1050
5.75	-	-	-	-	-	-	15.5	1098
6.0	-	-	-	-	-	-	15.5	1083

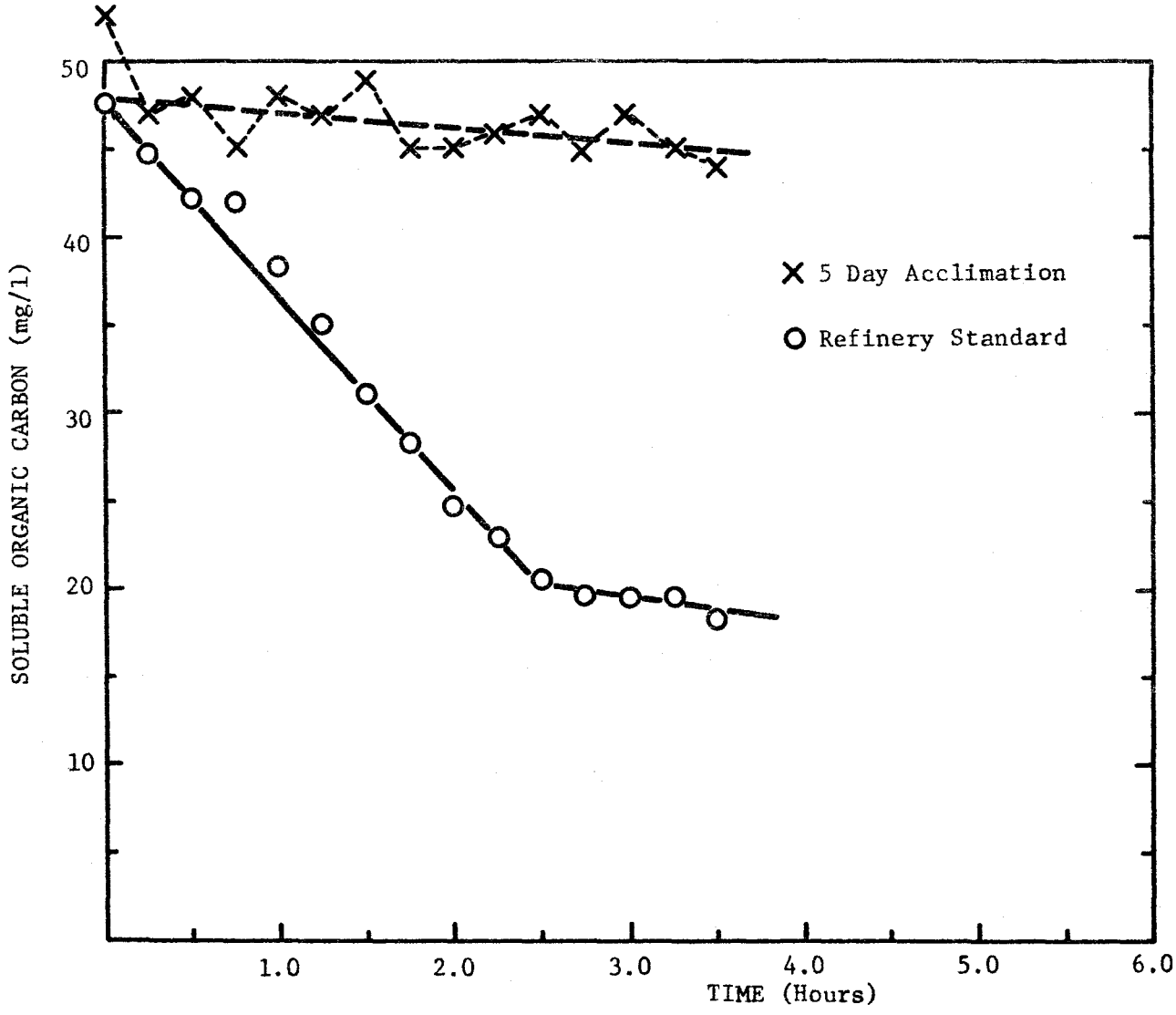

FIGURE 5

TEST RUN NUMBER 2 - WASTE A

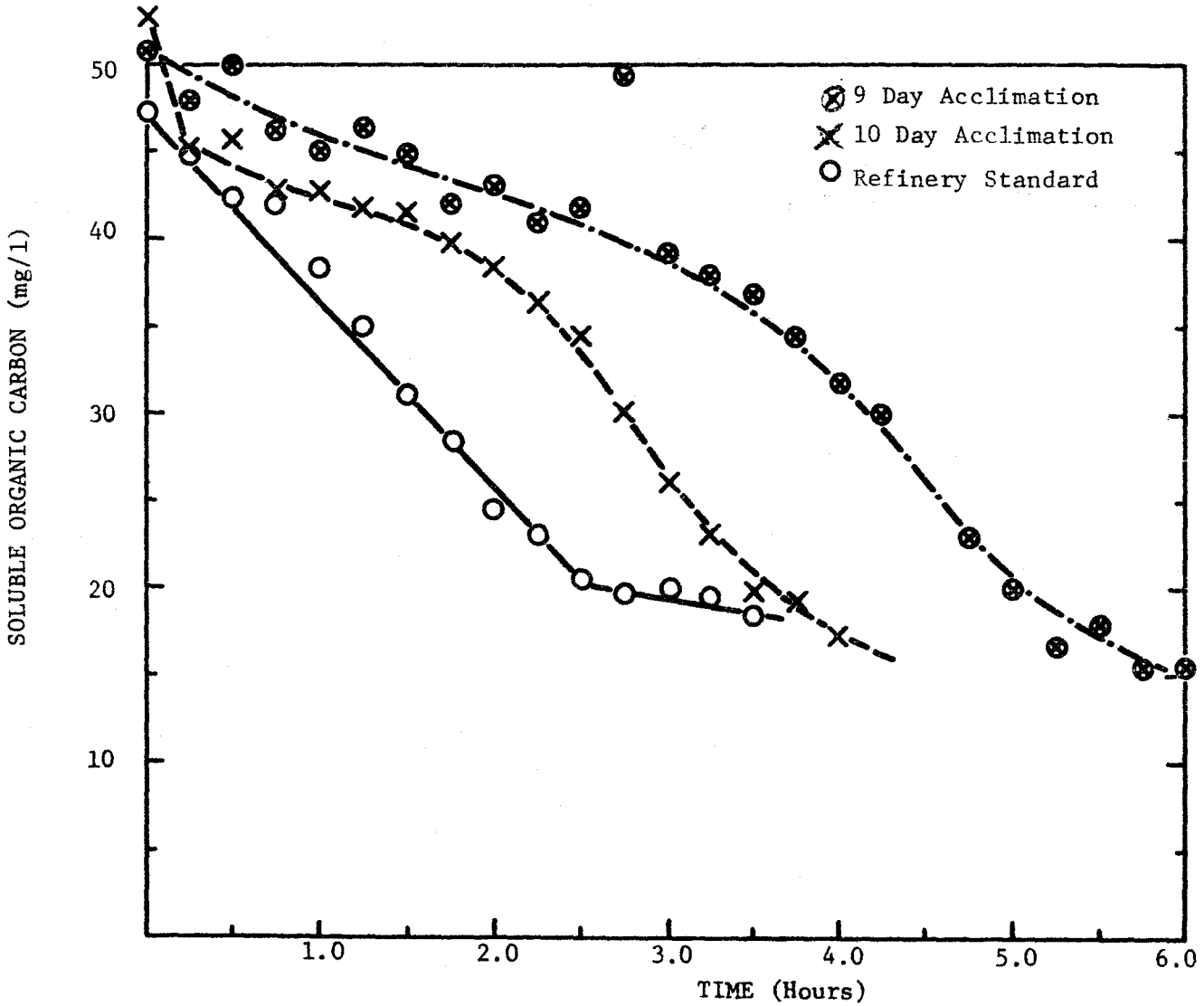

FIGURE 6

TEST RUN NUMBER 2A - WASTE A

hydrogen sulfide or ammonium sulfide. The results of the first run (No.2) Figure 5, show that acclimation had not occurred in 5 days but that the refinery's mixed culture reduced the waste. As a result a ten (10) day acclimation period was again studied as shown in Figure 6. The longer time period gave a reduction curve more closely approximating the standard. Comparing the 9th and 10th day reduction curves the mixed acclimated culture appeared to reduce the waste in a shorter period for the 10th day. The acclimation period required for this waste, with the apparent toxic substance, would then be greater than 10 days.

6.1.3 Test Run Numbers 3 and 3A

To decrease the time required for an acclimation study a continuous batch method was attempted. An unacclimated mixed culture was fed in a manner similar to the previous batch runs but when the organic carbon level reached the base value the mixed culture was fed the next incremental portion of waste. Assuming a similar reaction and 10 stages required the test should have taken between 30-40 hours. The method was then compared to a normal 10 day batch acclimation and a refinery activated sludge standard.

Refinery Waste Tested - Type A

Soluble organic carbon 45 ppm

Reactor Used - (1) Continuous batch study - 4 liter

(2) 20 liter for 10 day study

Acclimation Study - (a) test 10% incremental increase with continuous batch unit

(b) test 10 day period

(c) test Refinery Activated Sludge for Standard.

The results shown in Table 4 and plotted in Figure 7 show that both the 10 day period and the 10% incremental continuous batch studies were acclimated when a comparison was made with the refinery activated sludge standard. The 10 day period appeared to have had an equal reduction curve while the 10% continuous study showed a more rapid reduction of the waste. This more rapid removal rate in the later test can be explained by checking Table 4 and noting that the loading of lb. waste/lb. suspended solids was about 0.5 for the continuous test while about 0.35 for the standard and ten day test. The greater concentration of soluble organic carbon per amount of mixed culture could have stimulated the bacteria to reproduce more rapidly, thus taking up the soluble organic carbon at a higher rate.

Two observations are noted. The loading rate should be kept constant. This would mean accounting for volume of reactor contents removed for sampling when the succeeding feeding stage is added. The second observation concerns the waste type. Phenol appears to be quickly absorbed into the bacterial cell for energy and synthesis. If a colloidal or complex soluble waste were

TABLE 4

TEST RUN NUMBER 3 - WASTE A

(10% Incremental)

TIME	REFINERY STD.		10%		20%		30%		40%		50%	
	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid
0.00	33.2	1006	34.6	1169	42.2	1153	32.8	978	35.4	955	36.6	913
0.15	30.0		29.2		40.7				32.8		34.4	
0.30	28.3		26.0		33.8		29.4		31.6		34.3	
0.45	26.8		24.6		33.2		27.6		29.1		32.2	
1.00	26.4	928	24.0		26.5		26.5		27.6		28.8	
1.15	27.5		21.9				25.6		25.9		26.6	
1.30	23.7		20.9				22.1		24.0		21.2	
1.45	20.6		20.0				20.4		22.7		21.0	
2.00	18.3	975	18.9				18.6		20.4		18.6	
2.15	17.6		18.5				17.5		18.7		19.2	
2.30	16.8		16.3						16.9		18.1	
2.45	15.4											
3.00	17.5	1004	23.1		23.1							
3.15					21.5							
3.30					19.7							
3.45					17.2							

(Continued)

(Continuation)

TABLE 4

(10% Incremental)

TIME	REFINERY STD.		60%		70%		80%		90%		100%	
	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid
0.00	33.2	1006	37.2	793	37.5	810	42.0	823	36.9	788	39.0	818
0.15	30.0		33.5		33.7		32.3		30.7		34.3	
0.30	28.3		31.1		29.6		28.3		28.8		30.3	
0.45	26.8		30.5		26.5		26.8		25.6		26.6	
1.00	26.4	928	26.2		24.2		23.6		22.1		22.3	
1.15	27.5		24.7		23.3		20.7		19.8		20.7	
1.30	23.7		22.7		21.5		18.9		18.1		20.4	
1.45	20.6		21.3		21.5		17.8		17.8		18.1	793
2.00	18.3	975	19.5		18.5		15.8				17.8	
2.15	17.6		19.0		16.9							

(Continued)

(Continuation)

TABLE 4

TEST RUN NUMBER 3 - WASTE A

(10 Day Acclimation)

TIME	REFINERY STD.		1 DAY		2 DAY		3 DAY		4 DAY		5 DAY	
	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid
0.00	33.2	1006	26.5						30.5	1163	21.9	1193
0.15	30.0		25.0		Carbon Analyz-		Same as for		21.7		15.5	
0.30	28.3		23.4		er not funct-		2 Day		18.8		12.5	
0.45	26.8		23.1		ioning.				16.0		11.7	
1.00	26.4	928	23.6						12.3	1186	13.7	1115
1.15	27.5		20.7								13.7	
1.30	23.7		19.5						14.7		10.9	
1.45	20.6		18.2						15.0			
2.00	18.3	975	19.0									
2.15	17.6		16.5									
2.30	16.8											
2.45	15.4											
3.00	17.5	1004										

(Continued)

(Continuation)

TABLE 4

(10 Day Acclimation)

TIME	REFINERY STD.		6 DAY		7 DAY		8 DAY		9 DAY		10 DAY	
	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid	Carbon	Solid
0.00	33.2	1006	26.6	1186	27.1	998	25.4	1041	30.3	1164	34.9	1005
0.15	30.0		23.7		17.4		21.0		27.0		35.7	
0.30	28.3		16.9		18.1		18.9		25.7		31.8	
0.45	26.8		15.5		16.1		17.9		24.2		31.9	
1.00	26.4	928	15.2	1147	17.5	1010	18.5	1028	24.0	1121	29.1	980
1.15	27.5		14.9						21.9		27.0	
1.30	23.7		13.8						20.0		25.7	
1.45	20.6		14.1						18.7		24.4	
2.00	18.3	975							17.2		22.6	981
2.15	17.6										19.2	
2.30	16.8										19.1	
2.45	15.4										17.4	

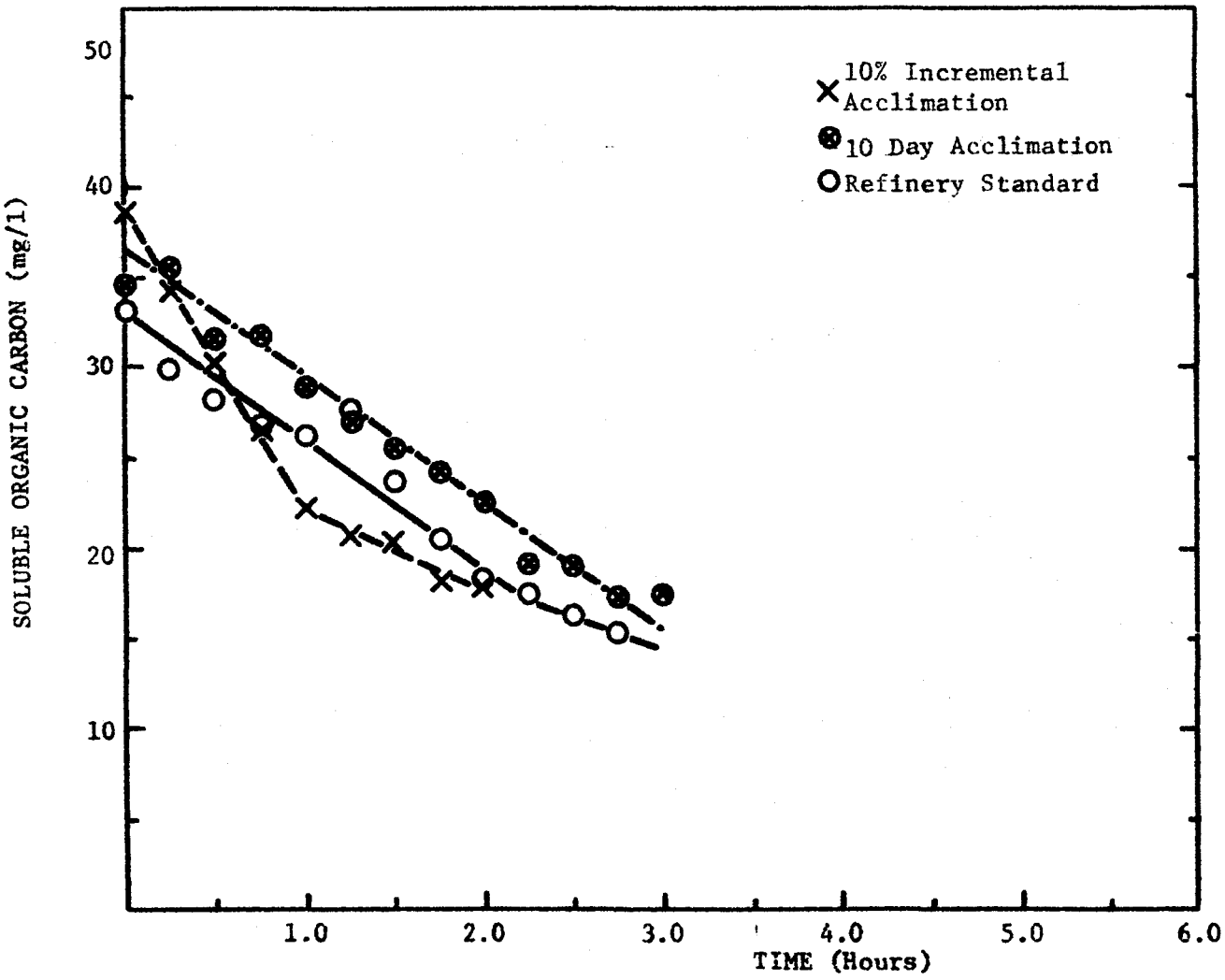

FIGURE 7

TEST RUN NUMBERS 3 AND 3A - WASTE A

acclimated an adjustment period would have been necessary for the enzyme reactions to occur. A slight toxicity might also necessitate a stabilization period for the mixed culture.

6.2 REFINERY WASTE B

The waste was obtained from the equalizing basins upstream of an air flotation unit and the activated sludge aeration tanks. The air flotation unit reduces suspended collodial oil which could possibly give a different characteristic to the resulting sludge. This discrepancy can be neglected since the oil adsorbs onto the bacterial floc and little reduction would occur due to the long complex chain hydrocarbon molecules. The unique characteristic of this refinery waste was a phenol concentration averaging between 25-35 ppm but which could fall to about 10 ppm. As a result the treatment plant operation was unique, in that provision had been made to feed a supplementary amount of phenol to maintain a load of 15 ppm, to prevent the biological sludge from starving.

6.2.1 Test Run Number 4

Noting that the waste was low in phenol concentration an unacclimated run was first attempted under the supposition that acclimation would not be necessary as long as a healthy mixed culture existed.

Refinery Waste Tested - Type B

Soluble organic carbon 35 ppm.

Reactor Used - 6 liter percolator

Acclimation Study Time - (a) unacclimated run

(b) refinery activated sludge for standard.

As shown in Table 5 and plotted in Figure 8 the mixed culture removed the soluble organic carbon in a similar way to the refinery activated sludge.

This would seem to indicate that if the substrate in question (i.e., phenol) is dilute enough, a mixed culture has a base level where substrate removal can occur immediately. This rapid acclimation can only occur if no inhibiting poisons or chemicals would be present in the waste stream. Under ideal conditions these inhibiting agents could be removed by air stripping or precipitation before biological treatment. One might argue that a strict adsorption was involved and not degradation but this could be refuted as the soluble organic carbon level 24 hours later was still at the base level of non-biodegradables.

6.3 REFINERY WASTE C

The raw waste was obtained from the feed line to the biological aeration tank. This feed was a blend of two waste stream holding tanks, one containing an average of 600 ppm phenol and the other very dilute. The blend results in a phenolic concentration between 75 - 100 ppm. This influent feed stream phenol concentration

TABLE 5

TEST RUN NUMBER 1 - WASTE B

TIME	STANDARD RUN		UNACCLIMITIZED	
	Carbon	Solids	Carbon	Solids
0	26.4	1380	23.4	1480
.25	24.8	1365	22.2	1495
.50	23.8	1335	20.3	1425
.75	23.2	1310	20.3	1475
1.00	22.7	1335	19.4	1445
1.25	20.4	1350	19.2	1480
1.50	21.2	1350	20.0	1455
1.75	20.4	1400	20.0	1410
2.00	20.4	1340	20.7	1380
2.25	20.7	1370	-	-
2.50	19.6	1390	-	-
2.75	19.8	1410	-	-
3.00	19.3	1360	-	-

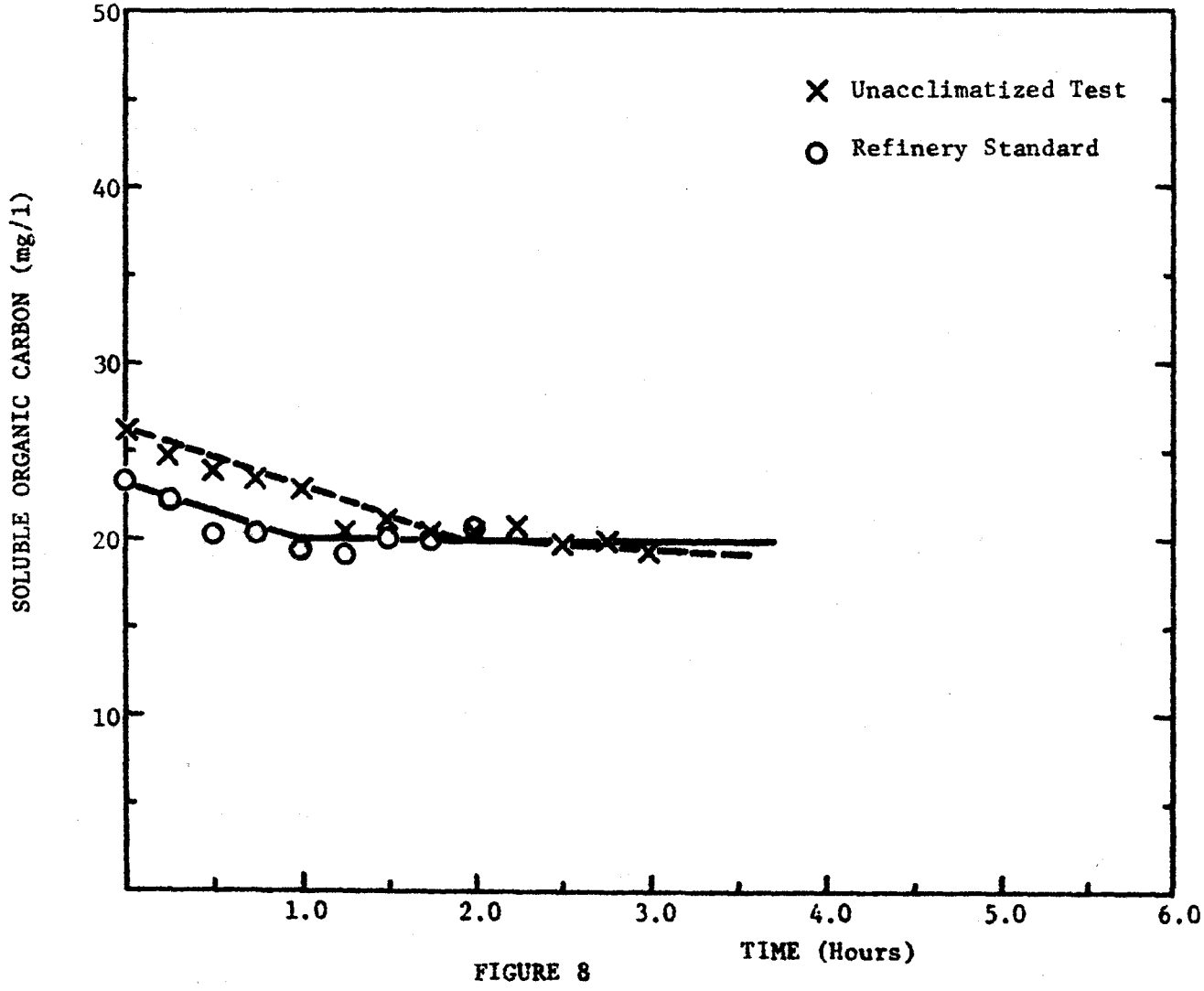

FIGURE 8

TEST RUN NUMBER 4 - WASTE B

could be varied depending on the immediate removal characteristics of the biological system. The loading concentration was, therefore, varied to maintain a desired effluent concentration.

6.3.1 Test Run Number 5

Refinery Raw Waste Tested - Type C

150 ppm C

Reactors - 10 litre vessel

8 litre reactants

Acclimation Study Time - (a) test six days on municipal sludge

(b) test refinery activated sludge standard.

The results are tabulated in Table 6 and plotted in Figure 9.

The results indicated that the refinery's activated sludge was not as well conditioned as the acclimated sludge. In this case comparing strict carbon values may lead to false conclusions. Observing the carbon values of the refinery's activated sludge (directly from plant) values around 75 ppm were recorded. Upon aerating this sludge for one day the soluble organic carbon value dropped to 45 ppm. This indicated that the existing treatment plant does not allow enough time for synthesis of the soluble organics once the organic carbon has been adsorbed. To more closely approximate the plant activated sludge a shorter acclimation period was prepared as in Run Number 6.

TABLE 6

TEST RUN NUMBER 1 - WASTE C

TIME	1 DAY Carbon	2 DAY Carbon	3 DAY Carbon	4 DAY Carbon	5 DAY Carbon	6 DAY Carbon	REFINERY STANDARD Carbon
0	88.7	109.1	95.0	100.1	116.8	106.0	100.1
.25	82.8	97.0	88.9	98.9	106.5	99.7	95.6
.50	85.7	89.2	78.9	86.7	99.6	91.3	90.7
.75	81.0	83.2	71.0	73.2	95.0	91.3	86.6
1.00	77.4	74.2	64.3	64.2	94.0	84.9	74.7
1.25	71.4	70.5	56.4	60.5	91.2	79.8	68.3
1.50	66.3	65.6	48.2	61.2	84.8	74.2	69.3
1.75	64.3	57.3	44.7	58.4	81.5	70.8	64.4
2.00	59.5	54.9	42.2	56.5	79.8	70.0	64.7
2.25	56.6	46.4	40.1	54.7	75.9	66.7	61.2
2.50	53.5	46.1	38.2	51.9	72.4	63.8	63.4
2.75	49.4	36.0	35.5	50.2	68.3	60.6	62.7
3.00	45.0	28.5	38.0	51.3	66.3	56.8	61.3
3.25	46.1	28.8	34.7	48.6	66.0	55.8	57.8
3.50	40.7	25.1	33.8	48.7	65.8	53.3	58.9
3.75	38.8	27.7	-	48.6	60.9	52.7	58.4
4.00	32.4	25.8	-	-	58.1	52.4	57.2
4.25	26.9	26.6	-	-	-	53.0	-
4.50	26.7	-	-	-	-	51.0	-
4.75	24.3	-	-	-	-	51.0	-
5.00	-	-	-	-	-	49.7	-
FINAL	17.1	23.5	34.3	41.6	40.8	38.6	53.9

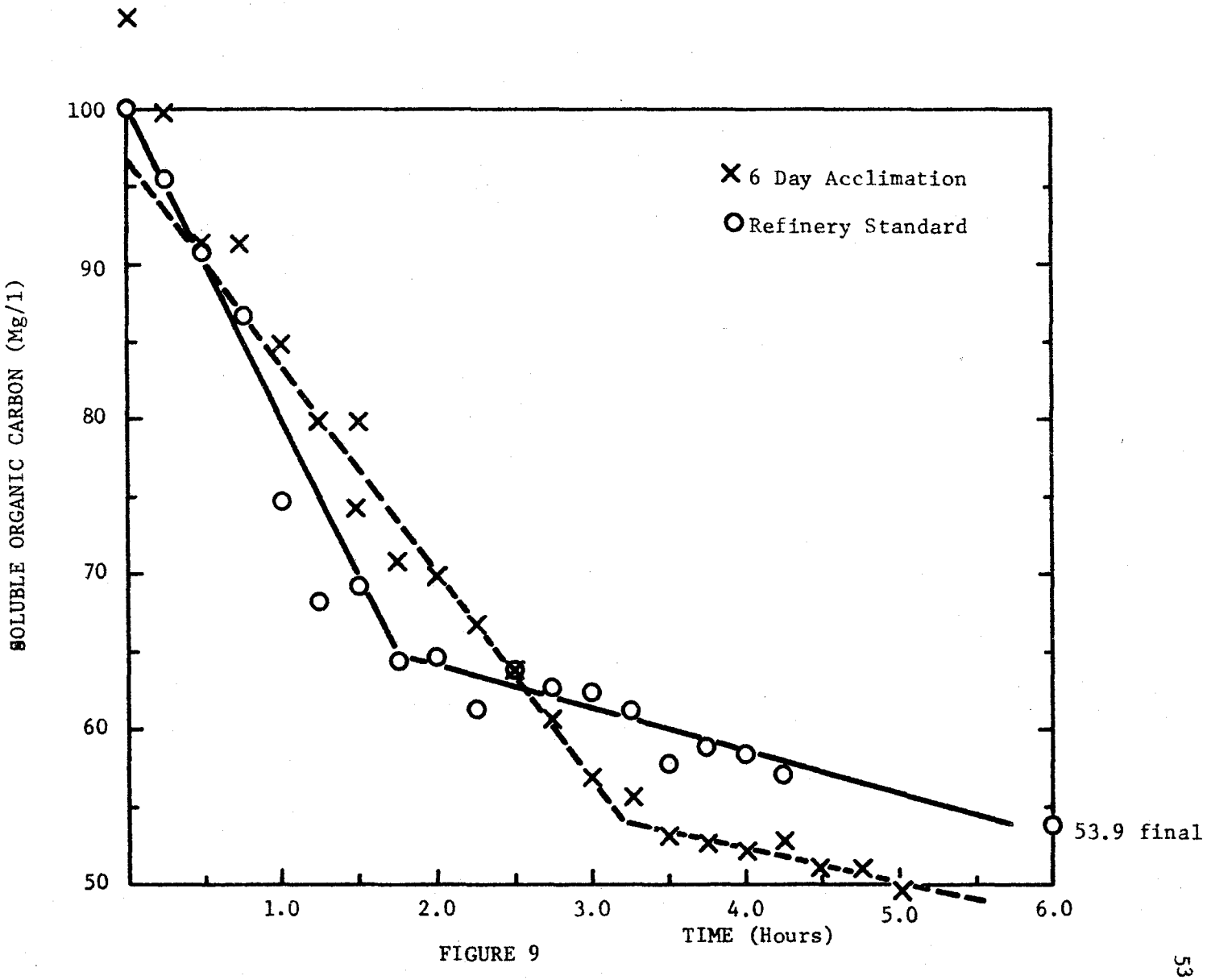

FIGURE 9

TEST RUN NUMBER 5 - WASTE C

6.3.2 Test Run Number 6

Raw Waste Tested - Type C

100 ppm C

Reactor - 10 litre vessel

Acclimation Study Time - (a) test three days acclimation

(b) test refinery activated sludge standard.

The results are tabulated in Table 7 and plotted in Figure 10.

The runs of increment feeding #1 and #2 were not followed due to the fact that the carbon analyzer required adjusting and results obtained were of little value during this time period.

The graphical plots again show that the acclimated mixed culture reduced the soluble organic carbon to a lower level than the refinery activated sludge but examining the batch run total carbon differences could explain the discrepancy. The mixed culture from the refinery's treatment plant could have had a higher concentration on non-biodegradable carbon than the municipal activated sludge. Possible interferences as listed in Chapter 3 could be affecting the plant mixed cultures. Acclimation was shown necessary as the unacclimated municipal sludge only reduced the carbon level about 8 ppm.

A possible explanation of the superior performance of the acclimated mixed culture could be given by Gaudy (26). The reference indicates that young cells tend to remove substances

TABLE 7

TEST RUN NUMBER 2 - WASTE C

TIME	REFINERY STANDARD		3 DAY ACCLIMATION		UNACCLIMITIZED OLD WASTE		UNACCLIMITIZED NEW WASTE	
	Carbon	Solids	Carbon	Solids	Carbon	Solids	Carbon	Solids
0	136	1750	111.2	2125	96.2	2750	119.0	1935
.25	122	1840	108.7	2170	87.5	2785	120.2	1840
.5	120.6	1930	103.6	2120	90.0	2870	117.0	1905
.75	-	-	104.5	2190	84.7	2855	118.6	1780
1.00	124.0	1955	101.4	2050	83.1	2835	114.1	1750
1.25	118.1	1870	95.8	2170	79.8	2670	100.2	1855
1.50	113.4	1910	90.0	2065	77.4	2675	102.5	1855
1.75	113.2	1925	92.0	2120	75.2	2670	111.6	1780
2.00	105.8	1960	95.8	2120	74.0	2665	110.7	1790
2.25	98.9	1845	94.7	2155	70.2	2710	112.5	1780
2.50	95.1	1865	89.3	2120	69.5	2695	105.2	1820
2.75	92.7	1955	87.6	2160	69.7	2615	108.1	1840
3.00	91.8	1995	88.0	2165	69.5	2715	111.0	1865
3.25	86.2	2015	90.9	2075	67.7	27.50	-	-
3.50	90.0	2010	84.4	2145	68.1	2735	-	-
3.75	-	1935	78.8	2150	68.1	2725	-	-
4.00	-	-	79.7	2130	-	-	-	-

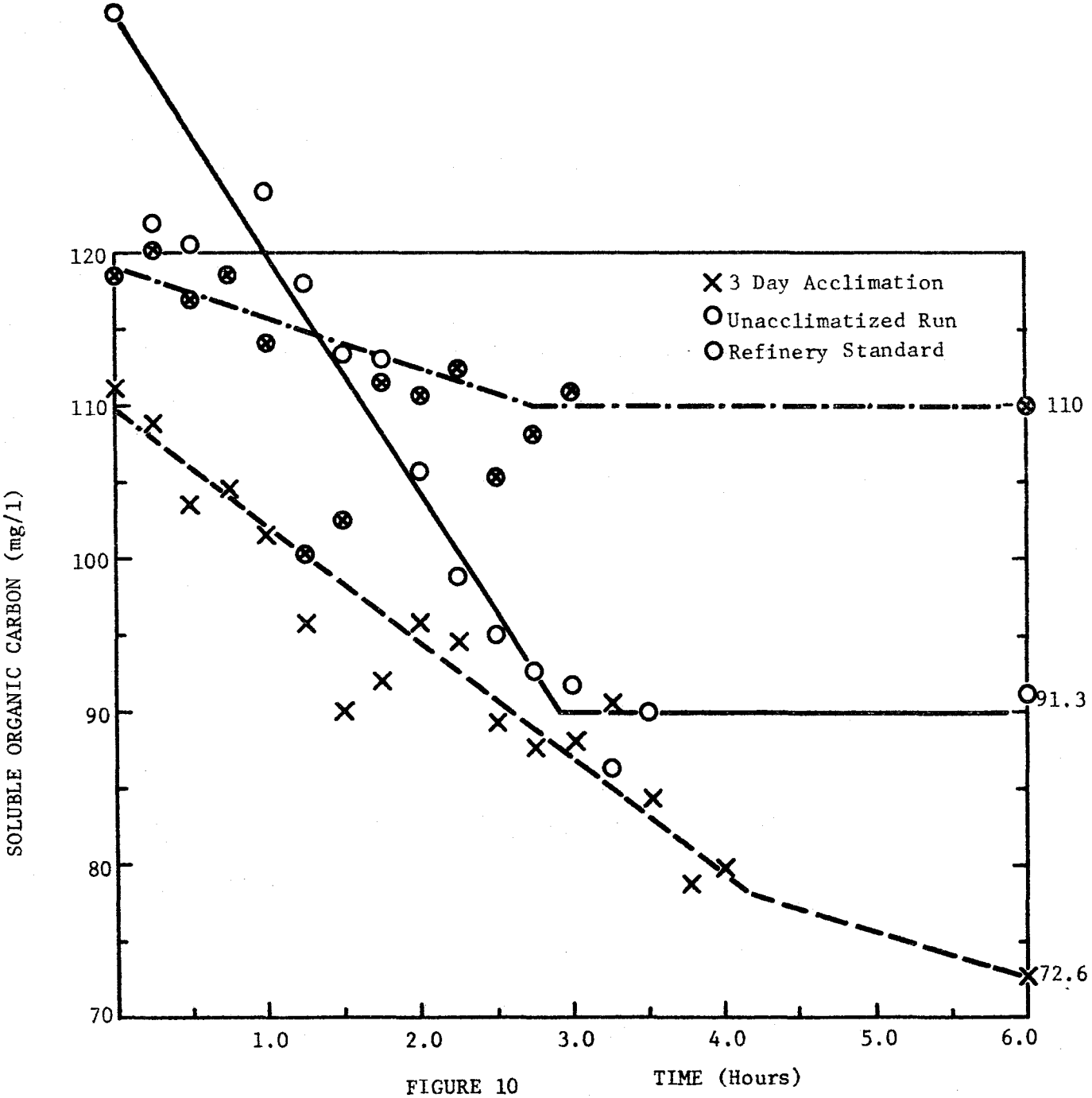

FIGURE 10

TEST RUN NUMBER 6 - WASTE C

sequentially in order of ease, while older cells remove substances simultaneously (the distinction between young cells and old cells is defined in the reference and on the page 13 in this thesis.) This would explain why the six (6) day acclimation period had a more rapid reduction than the three (3) day test. In a separate test the refinery activated sludge was aerated and after 24 hours, the soluble organic carbon level had dropped from 80 to 40 ppm. This would also explain why the refinery's treatment plant showed a poorer removal rate than the acclimated sludge.

CHAPTER 7

DISCUSSION OF RESULTS

The test mixed cultures achieved a state acclimation with the wastes examined that was equal or better than activated sludges treating each specific waste. This was verified by comparisons of the soluble organic carbon removal curves shown in Figures 3-10. Design studies could be performed in a similar manner to obtain biological reaction rates valid for process design. The variations in the phenol and inhibiting compounds concentrations in the three waste types as well as variation in individual wastes suggest that a series of acclimation studies need to be performed in order to place confidence limits on design data.

The variations in waste components and their concentrations both play decisive parts in determining the period of acclimation necessary. As shown in Waste Type B no acclimation period was necessary as the phenol was of a low concentration and there were no inhibiting substances in the waste. Continuous monitoring over a period of a month or even a year may show that at certain periods, inhibitors will be present in the waste stream and an acclimation period may be necessary. The effect of inhibiting substances was shown with Waste Type A. In Runs Number 2 and 2A a five (5) day period was not sufficient and nine (9) and ten (10) day acclimation periods showed two or three days more were required before a similar reduction curve to the refinery mixed culture

was obtained. A limitation to be noted for acclimation was that some organics could not be degraded as shown in Waste C. If the non-biodegradable substances were to be in the treatment plant's feed stream, acclimation must be performed with these substances unless their spillage or entrance to the feed stream could be limited to an insignificant value. As noted in Chapter 3, these compounds can stop enzymatic reactions by taking up or blocking reaction sites.

One of the critical steps to be performed before the actual mixed culture acclimation procedures are begun is then the determination of the chemical composition of the waste stream variation over the day, week, month and year. By obtaining samples from the lowest, average and worst conditions, mixed cultures could be acclimated to each. Noting in Figures 3 and 7 that although a longer than necessary acclimation period of 10 days was used, the reduction curves were similar to the plant activated sludge. The designer could then be confident of data if the two longest acclimation periods were equal or had similar reduction curves.

CHAPTER 8

RECOMMENDATIONS FOR DESIGN ACCLIMATION PROCEDURE

(1) A mixed culture can be obtained from any well-operated activated sludge plant. If the plant does not have primary sedimentation equipment, inorganics may be present in the sludge. The sludge should be settled to separate the activated sludge from heavy matter such as grit. The activated sludge should next be settled for one hour and the supernatant decanted to insure that no excess on non-biodegradables remain in solution to hinder the reaction.

It has been noted that some organic compounds require special types of bacterial cultures. If this case arises two alternatives are available: (a) Obtain a biodegradable compound similar in structure to the desired organic pollutant and acclimate a mixed culture to the former switching to the latter in a stepwise feeding process, or (b) Obtain a mixed culture from an existing activated sludge plant treating a similar waste.

(2) Sampling should be similar to investigation procedures outlined in Chapter 5 of this report. A mixed culture is considered to be acclimated when the organic pollutant has been removed to the desired or lowest concentration obtainable. Analysis should include settling characteristics, oxygen uptake rates, suspended solids concentration and possible buildup of suspended solids.

(3) The acclimation study should begin with a batch run using the mixed culture and the waste added at a conventional loading rate. If the waste is not reduced as indicated in point 2, a series of test runs should be performed over various time periods. For example, three, five and ten day periods, the wastes added in incremental amounts, of 1/3. 1/5 and 1/10 respectively, each day.

If the waste is not removed a special acclimation procedure may be required. For example, a highly concentrated waste may require two stage biological oxidation and therefore, two acclimation stages. An inhibiting compound may be inherent in the waste stream and therefore demand acclimation in a continuous reactor. The industrial plant may have a daily variation of product and so acclimation may be necessary to each product and also the interactions of one product waste on an activated sludge acclimated to another product.

(4) The designer could then select the necessary acclimation period which required the shortest period to achieve similar removal rates as succeeding longer acclimation periods. Once the time period has been determined, additional test runs could be performed around it. The final design data could then be evaluated using statistical tests on the experimental results obtained from the final series of test runs.

CHAPTER 9

CONCLUSIONS

(1) The acclimation procedure described herein on refinery wastes predicts an effluent quality similar to that found in existing refinery waste treatment plants.

(2) In general, the procedure for acclimation depends on the type of waste, the strength of the particular pollutant, the occurence of non-biodegradables and toxic substances and the periodic variation in the above parameters.

(3) This acclimation procedure can be used for any organic waste, allowing for modifications as in Chapter 8, point 3. The shortest period acclimation run showing results comparable to longer times should be selected as necessary acclimation period to obtain design data.

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