EFFECT OF BIOCATALYSTS ON WASTEWATER TREATMENT

By

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TREATMENT

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CHAPTER I

INTRODUCTION

For years, man has been increasing his technology to provide necessities and luxuries for an ever increasing population. Until recently, man had little concern as to how his increase in population and his production of millions of products each year was polluting his once abundant water supply. Now, man has realized that he must produce new technology and methods to treat the wastewater he produces.

Engineers have increased their technology of wastewater treatment through research and application of recently developed ideas and methods. One of the methods proposed to increase the rate of removal of pollution from our wastewater is the addition of biocatalysts for use in inline treatment, aeration, sedimentation, lagoons, sludge digestion, oil spills, and others.

Like many new technological ideas, biocatalysts production and availability has preceded the research and technological information necessary to evaluate its value and proper use as a wastewater treatment method. The information necessary for operators and engineers to apply the biocatalysts to a wastewater treatment process in an economical manner can be gathered only through research and publication of methods of research and results.

Microorganisms are the basic units for all biological treatment processes in removing organic material from wastewater. Stabilization and reduction of the organic material in the wastewater is dependent on these microorganisms. The microorganisms produce and use various enzymes to perform the many processes involved in biochemical reactions necessary for the breakdown of organic material to produce energy and organic products for cell growth.

One must keep in mind the interrelationship of the wastewater, the microorganisms and their enzymes, and the major factors which affect the ability of the microorganisms to use organic materials at the high rates necessary for efficient wastewater treatment. These factors are wastewater characteristics, microorganism characteristics, concentration of substrate, concentration of enzymes, concentration of end products, pH, temperature, oxidation-reduction potential and inhibiting substances.

This investigation was conducted to study the effect of biocatalysts on wastewater treatment. Five biocatalysts were investigated. Studies were conducted with various concentrations of biocatalysts on a synthetic wastewater and various mixtures of municipal sewage and slaughterhouse wastewater. An activated sludge system was used as the unit process to treat the wastewater biologically.

CHAPTER II

LITERATURE REVIEW

A. Historical

The idea of the addition of bacteria preparations or enzymes to wastewater is not new. Schloesing and Muntz (1) conceived the idea of applying biocatalysts, which they recognized as "organized fermenters," to sewage while they were making studies on the purification of sewage by filtration through a column of sand and limestone. Little work was done on the aspect of using enzymes in relationship to biological treatment processes for the thirty-five years following Schloesing and Muntz' studies.

In 1908, Wilson (1) suspected that the slime formed on the surface of material in an active filter might contain enzymes which could clot the colloidal material in fresh sewage. Some of the slime was removed from the filter medium, and when added to fresh sewage and shaken, a certain amount of purification took place. Buchner (1), Harden (1), Sorenson (1), Michaelis (1), and Wilstatter (1) also studied the concept of enzymes.

In 1912, Fowler (1) suggested that the colloids of sewage could be flocculated by certain enzymes of a certain bacterium in the presence of traces of iron salts. Since 1912, several studies have been conducted on the effect of biocatalysts on biological treatment and on enzymes and bacteria activities in activated sludge, sludge from septic tanks, filter material, and sludge digestion.

B. Manufacturers' Claims

Biocatalysts have been produced for twenty years or longer. There have been several investigations on the value of biocatalysts in wastewater treatment in the past, but few investigations have been made in the last fifteen years. Manufacturers and several product users indicate the addition of biocatalysts is the solution to many problems encountered in biological treatment units.

In 1954, McKee, Benas, Henderson, Kennedy, and Pearson (2) made a survey of advertising literature and questionnaires from members of the California Sewage and Industrial Waste Association. This survey revealed the following claims made by manufacturers of several biocatalysts.

1. In collector sewers and pumping plants, the biocatalyst will reduce odors and hydrogen sulfide concentrations, and that one substance will "remove dead organic solids from sewer lines."

2. In primary settling tanks, that one substance "liquifies a large portion of the colloidal solids," and the carriers used "serve as a flocculating agent, giving greater clarification;" that another substance "improves flocculation through the effect of its colloidal electrolytes" and improves oxidation "by increasing aerobic bacteria activity in the sedimentation;" that one substance "positively liquidizes sewage by enzyme action;" and that another has the "specific ability to flocculate sewage" and "to produce rapid settling."

3. In trickling filters that "one material increases the operating efficiency;" that another "promotes healthy algae growth and minimizes ponding;" and that a third "reduces the suspended solids and

BOD in the effluent from a trickling filter plant."

4. In the activated sludge process, that one substance favors the "rapid production of floc to accelerate settling rate," and "reduces the suspended solids and BOD in the effluent."

5. In lagoons or oxidation ponds, that "an additive eliminated sludge blankets on lagoons, gave increased percolation rate, improved the dissolved oxygen, and produced only a paper-thin sludge in dried-up lagoons."

6. In digestion tanks, that "one substance improves disintegration by accelerating the breakdown of solids in digesters. It is capable of increasing gas production as much as 300 percent;" that it "reduces scum formation and stabilizes the pH in digesters and Imhoff tanks;" that it "increases gas production by 18 to 35 percent--in fact, 'there will always be an increase in gas production;'" that it "reduces the volume of digested sludge and its volatile content with reduced solids in the supernatant;" and that "another additive improves the pH and increases gas production in digesters with less solids, odor, and BOD in the supernatant."

7. In sludge drying, that "one additive reduces sludge drying time by 50 percent, reduces sludge odors, and favors free-flowing sludge and easier sludge action of sludge lagoons."

8. In a miscellaneous category, that "one material contains many enzymes that do not occur in normal sewage treatment," and that it "is capable of digesting sterile sewage, doing so in less time than it is accomplished in properly designed sewage digestion plants;" that "operation of a new plant with another is a definite advantage and "you won't experience starting trouble because the seeding of your plant

will take place with the most efficient organisms for each process;" and finally, in a modest note, that "one additive is not a cure-all."

Enzymatic (8) and Bionetic (9)(10) were two of the biocatalysts used and investigated in the early 1950s. These biocatalysts were claimed to be effective in reducing BOD, suspended solids, increasing digestion, and grease reduction.

Recent literature on present preparations such as Bac-zymes, DBC plus, and Biogen, indicate the use of these biocatalysts to give beneficial results on various units such as inline treatment, digesters, sedimentation, trickling filters, activated sludge, septic tanks, lagoons, and pertroleum wastewater treatment (3)(4)(5)(6)(7).

C. Previous Investigations

McKinney (11) made a study regarding inline waste treatment. He reported that the oxygen available in the line is very limited, and is depleted quickly during biological activity in the sewage line resulting in anaerobic metabolism. If the wastewater is contained in the line for a considerable length of time, the anaerobic biological activity becomes quite extensive, resulting in H_2S production. The addition of bacteria to wastewater of low DO content would increase the biological activity and result in odorous wastewater. McKinney reported that the addition of bactericidal agents, when added early in the collection system, would reduce the biological activity in the sewage line, and would aid in reducing anaerobic conditions in long sewage lines.

McKinney and Poliakoff (12) studied the effect of biocatalysts on activated sludge. Activated sludge units were used in accordance with accepted biological principles for activated sludge. Three substrates were tested: a synthetic sewage, a raw sewage, and a synthetic industrial waste, for a wide range of conditions and loadings. They concluded that the biocatalysts did not alter the normal biological action in any of the three series of experiments. The rate of BOD removal was not increased by the use of biocatalysts. Since the biocatalysts failed to show a beneficial effect on the treatment of sewage in activated sludge units, there was no need for a study of economics on the biocatalysts.

Heukelekian and Berger (16) studied the effect of biocatalysts on digestion. Results showed an increase in the BOD of the supernatant liquid after a 12-hour incubation period with each of five biocatalysts on sterilized fresh solids. Studies on unsterilized fresh solids showed no significant increase in BOD of the supernatant after an 18-hour contact. Studies on one biocatalyst indicated no increase in the rate of oxygen utilization resulted from the addition of the biocatalysts to the sludge.

McKinney (16) stated that the major portion of the biocatalysts would be passed through a septic tank before they could act on the sewage, and that it would be more practical to leave some sludge in the tank when it is cleaned instead of removing all of the sludge and adding biocatalysts. A process to enhance biological conditions in septic tanks was patented by Santo De Lucia (14). The process used weighted pellets of biocatalysts which were glued with various glues to allow the slow release of the biocatalyst into the unit for aid in biological action.

A special committee on enzymes and biocatalysts, California Sewage and Industrial Waste Association (2), investigated literature, and questionnaires received from operators, and tested the use of five biocatalysts. The cost of the dried biological preparations ranged from three to five dollars per pound.

Substance C was tested on total sulfide removal. A careful record of total sulfides in the sewage arriving at the control point was made, and at no time was any significant reduction in sulfides noted. Tests were conducted on oxidation ponds, trickling filters, activated sludge, sludge digestion, scum blankets, and grease cloggings, and aerobic stabilization.

In response to the questionnaires sent to users of biocatalysts, twelve replied that they did not plan to continue using the substance. One operator gave an affirmative reply, and one operator wished to continue experimentation of the substance if his budget allowed it.

The following observations were advanced by the committee until more reliable experimental results were available to confirm or disprove the beneficial use of biocatalysts.

1. "The new biocatalytic additives apparently contain numerous enzymes of the type which are capable of assisting or promoting biochemical decomposition, providing that they are not already present in the process."

2. "These same enzymes are normally present in waste treatment processes in concentrations far greater than the recommended dosage of additives would provide, inasmuch as the enzymes are produced by the bacteria indigenous to the process. Hence, the addition of biocatalytic substances in properly designed and operated plants will produce no benefits."

3. "Additional enzymes are not induced for means of reducing

odors and H_2S formation. This purpose can be accomplished best by adequate aeration or by bactericidal agents."

4. "The majority of reliable evidence indicates that biocatalytic additives will not increase gas production, improve pH, or alter the alkalinity and volatile acids in the digesters, nor will they change the quality of the supernatant liquor."

5. "It is possible that the enzymes in these substances may exert beneficial action on scum blankets or clogged grease, especially if the normal enzymes in the waste treatment process do not have access to the scum or grease. Future research in this respect is needed."

6. "There is also a possibility that biocatalytic additives may improve the performance of percolation and oxidation ponds used for seasonal organic industrial waste; however, this possibility should be investigated by reliable controlled experiments."

7. "Many of the claims advertised by the promoters of the substances appear to be grossly overstated and misleading. Some of the claims as quoted hereinbefore, border on the ridiculous and display an ignorance of the fundamental processes of waste treatment."

Similar conclusions were made by a second committee on enzymes and biocatalysts, California Sewage and Industrial Waste Association.

D. Slaughterhouse Wastewater Treatment

Dart (18) indicated the chief sources of polluting matter in slaughterhouse wastewater were urine and feces, blood, washings from carcasses, floors, and utensils, and the undigested food from the paunch of the slaughtered animal. In meat processing waste from cooking, curing, and pickling, might also be present in the wastewater. Dart stated that slaughterhouse wastewater could be treated successfully with domestic sewage, or alone by usual aerobic methods. The slaughterhouse wastewaters are generally more readily oxidized than is domestic sewage, and fairly high rates of treatment are possible.

Hopkins and Dutterer (17) explain a method of treating liquid waste from a slaughterhouse. The analysis of the waste disclosed a 5-day BOD of 1700 mg/l, suspended solids of 1090 mg/l, grease of 90 mg/l, and pH value of 6.6.

The use of extended aeration and chlorination gave the effluent a quality adequate to be put into a stream. The effluent contained an average BOD of 10.1 mg/l, DO of 8.1 mg/l, pH of 6.4, and free chlorine residual of 0.3.

Saruta, Ishinishi, Itto, and Inoue (19) studied the effluent and biological characteristics of wastewater containing slaughterhouse wastewater and domestic wastewater. They concluded that the use of mixed slaughterhouse and domestic wastewater activated sludge produced a predominant microorganism which allowed efficient wastewater treatment.

CHAPTER III

MATERIALS AND METHODS

A. Biological Reactor

The reactor used for this study is illustrated in Figure 1. The reactor is rectangular, and is constructed of one-fourth inch thick plexiglass with the following dimensions: length, 25 3/4 inches; width, eight inches, depth, eight inches.

The reactor consisted of six compartments or units, each 4" x 8" x 8", providing for a three-liter volume for each unit and freeboard to prevent foaming from mixing contents of the units. A 12 1/4-inch depth plexiglass back was put on the reactor. The top four inches were used for the attachment of airflow meters.

B. Temperature Control System

The reactor was placed in a water bath unit manufactured by Lab-Line Instruments, Inc. This water bath allowed water, which was cooled or heated to the desired temperature, to be circulated around the reactor for constant temperature control.

C. Airflow Control

A five-liter/min Gelman airflow rate meter for each unit was attached to the plexiglass back, above the container water level. Each meter was attached to the same air line for simplicity and

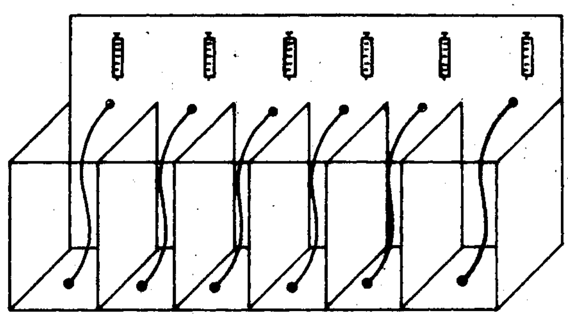


Figure 1. Biological Reactor

convenience in disconnecting the air line when the reactor was removed from the water bath for cleaning and feeding the units.

D. Wastewater

1. Synthetic

A synthetic wastewater was made and used in the unacclimated wastewater study to obtain basic information on the performance of dried bacterial enzyme cultures on a simple carbohydrate feed. The synthetic wastewater consisted of glucose to provide a carbon source, a salt solution to provide the necessary salt requirements, ammonium sulfate to provide a nitrogen source, and a phosphate buffer solution consisting of KH_2PO_4 and K_2HPO_4 to provide a phosphorus source and buffering action. The synthetic waste is the standard glucose minimal medium used in the bioenvironmental engineering laboratores of Oklahoma State University. This feed had the following makeup:

Constituents	Concentration
$(NH_4)_2\ SO_4$	250 mg/l
$MgSO_4 \cdot 7H_2O$	50 mg/l
$MnSO_4 \cdot 7H_2O$	5 mg/l
$FeCl_3 \cdot 7H_2O$	.25 mg/l
CaCl	3.75 mg/l
Glucose	500 mg/l

The phosphate buffer solution containing 107 gm/l of K_2HPO_4 and 52.7 gm/l of KH_2PO_4 used for pH control and phosphorus requirement. Ten ml of the solution was added per liter of activated sludge in each batch unit.

2. Municipal Wastewater

A municipal wastewater was used both as a wastewater and as a heterogeneous microbial seed. This wastewater was collected from the preaeration unit of the Stillwater, Oklahoma municipal wastewater treatment plant.

3. Slaughterhouse Wastewater

A slaughterhouse wastewater was used in the acclimated and unacclimated studies for a high chemical oxidation demand wastewater and a wastewater which was high in protein. The wastewater was obtained from Ralph's Slaughterhouse & Meat Packing Plant, Perkins, Oklahoma.

E. Bacteria and Enzyme Prepared Cultures

Biocatalysts provided by a manufacturer were used as an additive to the wastewater used to investigate a portion of their effectiveness in treatment of industrial wastewater. The following two biocatalysts were provided and designated as having a specific use as indicated by the following:

Sample No.	Purpose
8	sink drain and septic tank treatment
7	grease treatment

Other samples of various enzymatic and biological mixtures were indicated by the manufacturer as having possible application to biological loading. The following general information was submitted for each of the other samples provided:

Sample No. 1: This sample would approach basically a carbo-hydro sugar conceptional area, with little or no emphasis in the grease or protein system.

Sample No. 2: A balance through the carbo-hydro system, sugars, alcohols, and cellular structure, with some emphasis on grease.

Sample No. 3: Carbohydrate liquefaction, with secondary emphasis on grease and protein.

Sample No. 4: Heavy in the cellulase and carbohydrate area, with some emphasis in grease control, but none in protein.

Sample No. 5: Heavy in grease, with secondary effect in carbohydrate.

Sample No. 6: A balance in carbohydrates, grease, and cellulase structures, with a secondary emphasis on protein.

Sample No. 9: A product from another company was prepared material recommended for use as a treatment of septic tanks.

The actual content as to type of bacteria and/or enzyme of these materials was not provided, and no attempt was made to identify them.

F. Experimental Methods

1. Unacclimated Wastewater Study

a. Synthetic Wastewater

The water bath was filled and allowed to reach the equilibrium temperature of 23^{o}C. This temperature was chosen as the control temperature throughout the study and was controlled within a fraction of a degree by the temperature control of the Lab-Line water bath unit.

Measurement of quantities of dried bacterial cultures were made on Grami-Atic balance manufactured by Fisher Scientific Company. The dried bacteria were mixed for one hour by means of a magnetic stirrer in a liter of distilled water to a concentration of three mg/ml, and then the bacteria enzyme mixture was measured in ml. The addition of 20 ml of the three mg/l mixture in the 3-liter units would produce a biocatalyst concentration of 20 mg/l.

Synthetic wastewater was prepared and refrigerated for use as needed. The glucose feed was fed at a rate of 500 mg/l glucose, producing a wastewater with an initial COD of approximately 530 mg/l.

Thirty ml of municipal sewage from the preaeration unit at the Stillwater sewage treatment plant was added to each unit, giving a concentration of 10 ml/l of sewage seed. The seed was applied to the units containing biocatalyst, as well as to the control unit. The units were filled with the wastewater and seed, and biocatalysts were placed into the unit. The reactor was placed into the water bath with a temperature of 23^{o}C; air was applied at a rate of three liters/min for the 3-liter units. Samples were collected throughout the experiment, filtered through 0.45 μ membrane filters, and the COD of the samples was obtained.

b. Slaughterhouse and Sewage Wastewater

The reactor was set up in the same manner as in the synthetic wastewater study with respect to water bath, temperature, airflow rate, and biocatalyst preparations. Slaughterhouse wastewater varied with concentration of blood and carbohydrate material, thus each experiment had different initial COD values. The ratio of sewage wastewater to slaughterhouse wastewater varied with different experimental studies.

2. Acclimated Slaughterhouse and Sewage Wastewater

The units were filled initially with a wastewater and seeded with biocatalyst as indicated in Table I. The units were aerated until the filtered COD of the wastewater was reduced to a low level. After this level was reached, the solids were allowed to settle and the supernatant was removed to a level of 500 ml of solids and supernatant in each unit. Units one through five were then fed 2500 ml of slaughterhouse wastewater, and unit six was fed 2500 ml of municipal sewage. The units were again aerated, and the above procedure repeated each time the COD was reduced to a low COD.

Ten mg/l of biocatalyst sample No. 3 was added to units four and five on the fifth day of aeration. On the seventh day, the reactor was removed from the water bath, and the wastewaters from units one, four, five, and six were placed in 4000 ml containers and aeration was continued. The 4000-ml containers were not placed in the water bath, but were at room temperature (22^{o}C). The units were filled with wastewater and constituents as indicated in Table II.

On the 13th day of the study, the activated sludge which was initially seeded with 2000 mg/l of biocatalyst sample No. 3, was fed 50 mg/l of biocatalyst sample No. 3. On the 14th day, the units were filled with the contents indicated in Table III, and aerated. The wastewaters were sampled periodically, centrifuged, filtered through 0.45 μ membrane filters, and COD analyses performed as outlined in Standard Methods (20).

TABLE I

CONTENTS OF WASTEWATER ON THE 1st DAY

Unit		Biocatalyst Added
1A	1/3 sewage, 2/3 slaughterhouse wastewater	none
2A	1/3 sewage, 2/3 slaughterhouse wastewater	80 mg/l No. 3
3A	1/3 sewage, 2/3 slaughterhouse wastewater	160 mg/l No. 3
4A	1/3 sewage, 2/3 slaughterhouse wastewater	240 mg/l No. 3
5A	slaughterhouse wastewater	2000 mg/l No. 3
6A	sewage	none

TABLE II

CONTENTS OF WASTEWATER ON THE 7th DAY

Unit	Wastewater (Slaughterhouse Waste and)
1B	500 ml of acclimated seed from Unit 1A
2B	500 ml of acclimated seed from Unit 4A
3B	125 ml of acclimated seed from Unit 5A
4B	250 ml of acclimated seed from Unit 5A
5B	500 ml of acclimated seed from Unit 5A
6B	500 ml of acclimated seed from Unit 6A

TABLE III

CONTENTS OF WASTEWATER ON THE 14th DAY

Unit	Wastewater (Slaughterhouse Wastewater and)
1	200 ml of acclimated seed from Unit 1A
2	400 ml of acclimated seed from Unit 1A
3	100 ml of acclimated seed from Unit 5A
4	200 ml of acclimated seed from Unit 5A
5	400 ml of acclimated seed from Unit 5A
6	500 ml of acclimated seed from Unit 6A

CHAPTER IV

RESULTS

A. Unacclimated Wastewater Study

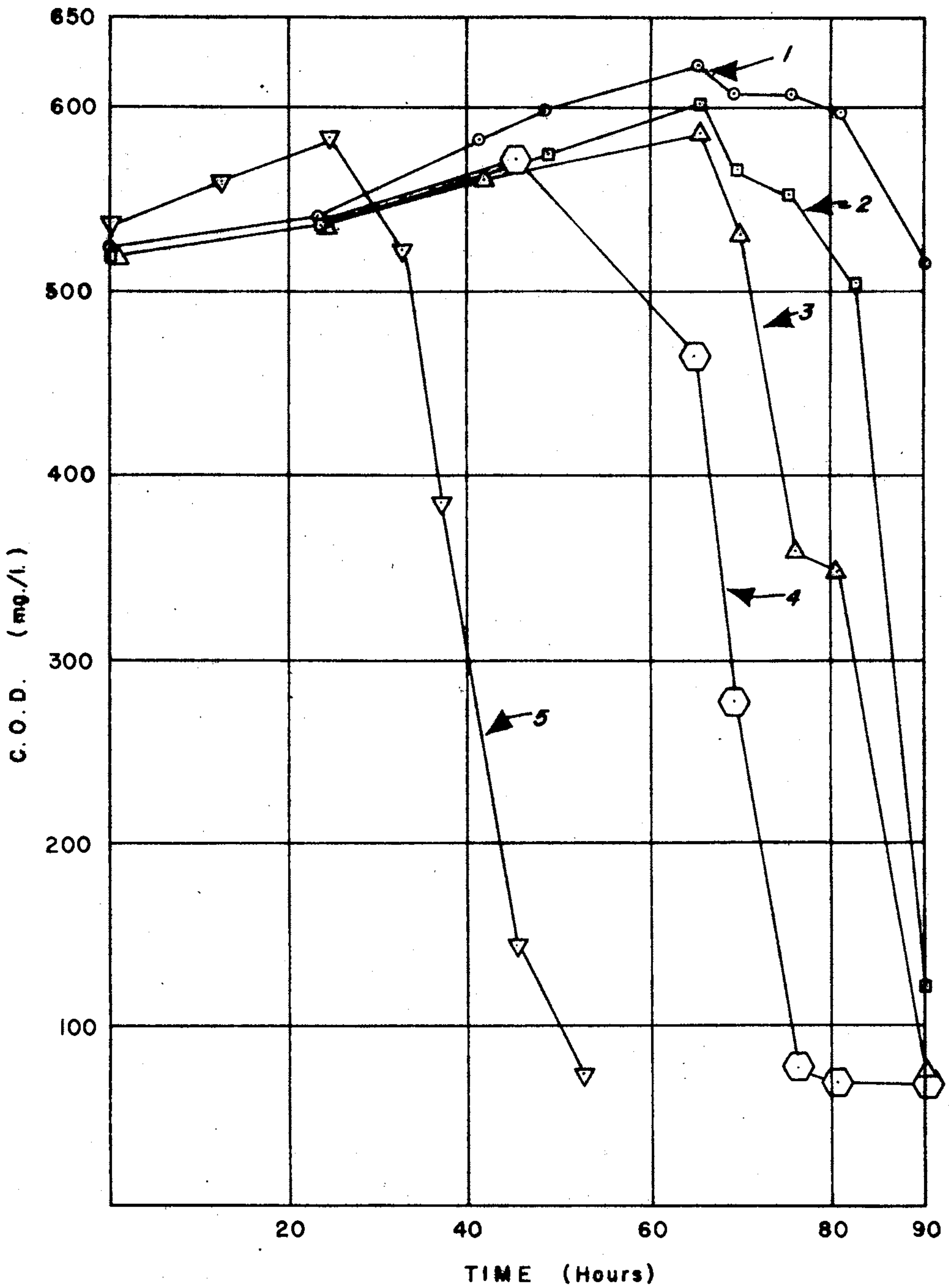
1. Synthetic Wastewater

Figure 2 shows the effect of the application of 10 mg/l, 20 mg/l, 40 mg/l, and 80 mg/l of biocatalyst sample No. 8 on the chemical oxygen demand (COD) of the standard glucose minimal medium used in the bio-environmental engineering laboratories at Oklahoma State University. The control unit indicated by (1) was seeded with 30 ml of municipal sewage for a heterogeneous microorganism population. Each of the other units was also seeded with 30 ml of sewage seed to avoid a variation from the control unit caused by seeding.

Figure 2 indicates that the biocatalyst had a significant role in decreasing the COD of the synthetic wastewater. The unit containing 80 mg/l of biocatalyst sample No. 8 had 50 percent removal in 42 hours, and 86 percent removal in 52 hours. The unit containing 40 mg/l had 50 percent removal in 70 hours, and 86 percent removal in 75 hours.

The COD of the wastewater increased gradually from the initial COD until the wastewater became a greenish color, indicating a high microbial population. The COD of the wastewater was reduced rapidly after the wastewater became greenish. The rise in COD cannot be explained from the data collected.

Figure 2. Relationship of COD Removal Between Synthetic Wastewater Containing (1) no Biocatalyst, (2) 10 mg/l Biocatalyst No. 8, (3) 20 mg/l Biocatalyst No. 8, (4) 40 mg/l Biocatalyst No. 8, and (5) 80 mg/l Biocatalyst No. 8


650
600
500
400
300
200
100
C.O.D. (mg./l.)
20
40
60
80
90
TIME (Hours)
1
2
3
4
5

It is interesting to note that the COD reduction rate was approximately equal for each unit after the COD began to decrease. The increase in concentration of the biocatalyst decreased the acclimation period, but did not increase the reduction rate; therefore, it is believed that the bacterial population of the biocatalyst attributed to the reduced acclimation time.

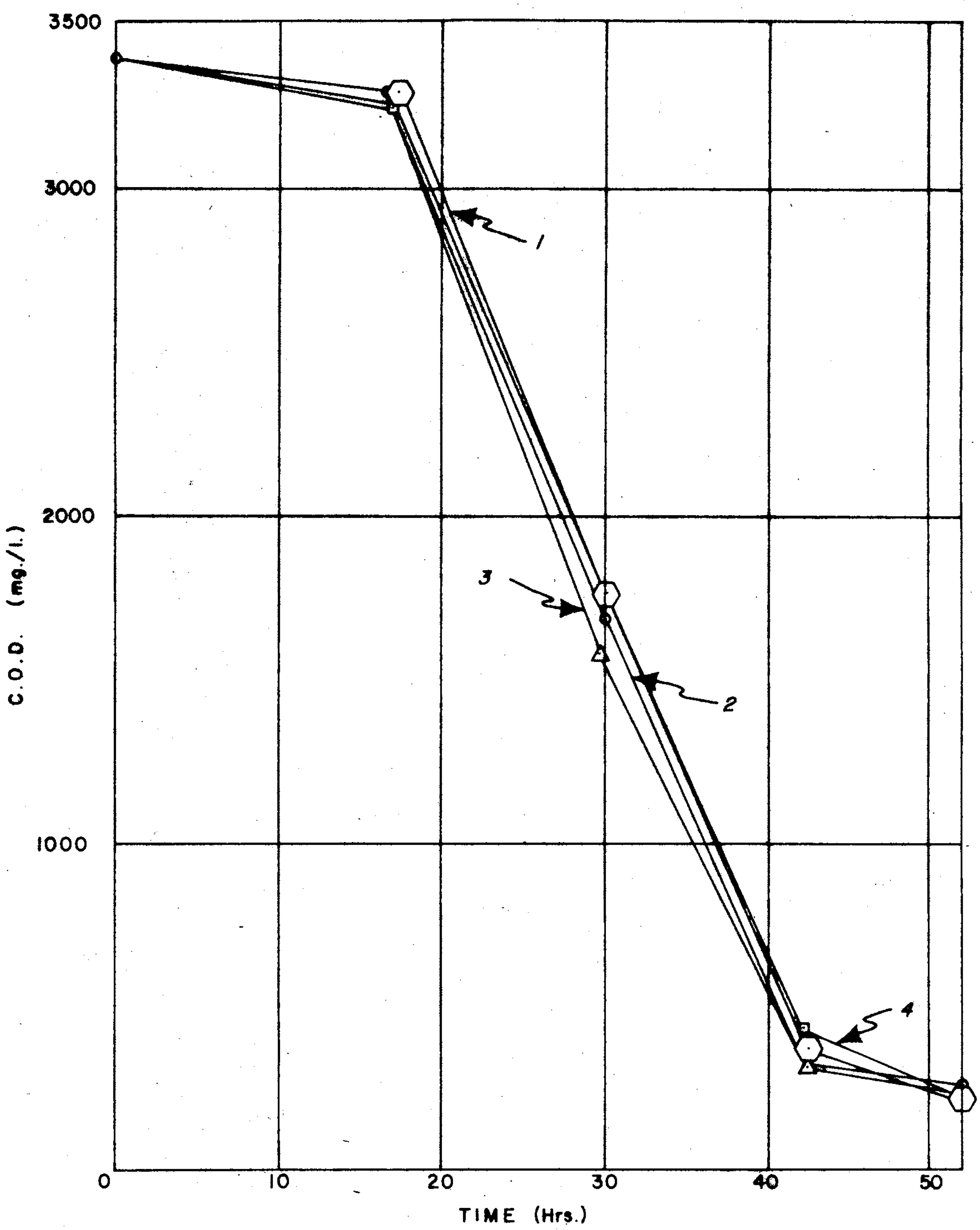
2. Slaughterhouse and Sewage Wastewater

The simplicity of the synthetic wastewater eliminated the need of enzymes to break down large compounds; therefore, to study the effect of biocatalysts in breaking down large compounds, a slaughterhouse wastewater was selected for the study, because of its complexity and local availability. The blood in the slaughterhouse wastewater had a high protein content, and enzymes were required to break down the blood cells.

Figure 3 shows the relationship of COD removal between wastewaters containing 1/6 slaughterhouse wastewater and 5/6 municipal sewage, with 0 mg/l, 20 mg/l, 40 mg/l, and 80 mg/l of biocatalyst sample No. 3. The addition of the biocatalyst of the above concentrations did not have any effect on increasing the COD reduction rate. COD removal was 92 percent after aeration of the wastewater for 52 hours.

Some manufacturers of biocatalysts have recommended the use of their products for inline waste treatment. They suggest that the biocatalyst be placed into the sewage line at or near the place where the wastewater enters the line. An experiment was conducted to study the effect of biocatalysts on inline waste treatment. The reactor was removed from the water bath and a magnetic stirrer was placed under each unit, thus the water was mixed in a manner to simulate inline mixing.

Figure 3. Relationship of COD Removal Between Wastewater Containing 1/6 Slaughterhouse Wastewater and 5/6 Sewage Wastewater With (1) 0 mg/l, (2) 20 mg/l, (3) 40 mg/l, (4) 80 mg/l of Sample No. 3 Biocatalyst


3500
3000
2000
1000
C.O.D. (mg./l.)
0
10
20
30
40
50
TIME (Hrs.)
1
2
3
4

Figures 4 and 5 show the relationship of COD removal between the 1/3 municipal sewage and 2/3 slaughterhouse wastewater stirred without air diffusion, and with (a) 80 mg/l of biocatalyst sample No. 9, (b) 80 mg/l of biocatalyst sample No. 3, (c) 80 mg/l of biocatalyst sample No. 6, (d) 80 mg/l biocatalyst sample No. 7, and (e) without biocatalyst. The results of the study show that the addition of biocatalyst did not have any beneficial effect in reducing the COD of the wastewater beyond the reduction obtained in the unit containing wastewater without a biocatalyst. After 62 hours, the COD removal ranged from 45 percent to 69 percent.

It is interesting to note that the slower removal of COD was in units containing sample Nos. 6 and 7. Figure 5 shows that these two units may have been inhibited by the biocatalysts.

A study was made to determine the relationship of COD removal on wastewaters containing five of the biocatalyst samples with the same concentration of 80 mg/l of each biocatalyst to determine what effect each had on the municipal and slaughterhouse wastewater mixture, and the relationship of the removal of each biocatalyst to each other. Figures 6, 7, and 8 show the results of the study. Figure 6 shows the relationship between the COD removal of the units containing biocatalyst sample No. 6, biocatalyst sample No. 3, and the control unit without biocatalyst addition. It is interesting to note that the wastewater without biocatalyst had a faster COD reduction rate than the wastewater containing biocatalyst samples Nos. 3 and 6. These two biocatalysts were recommended as having secondary emphasis on protein.

Unfiltered COD values indicate a rise or plateau between 15 hours and 25 hours, while the filtered COD does not show any rise or plateau.

Figure 4. Relationship of COD Removal Between 1/3 Sewage and 2/3 Slaughterhouse Wastewater Without Air, Stirred by Magnetic Stirrers and With (1) 80 mg/l Biocatalyst Sample No. 9, (2) 80 mg/l Biocatalyst Sample No. 3, and (3) Without Bacteria Added

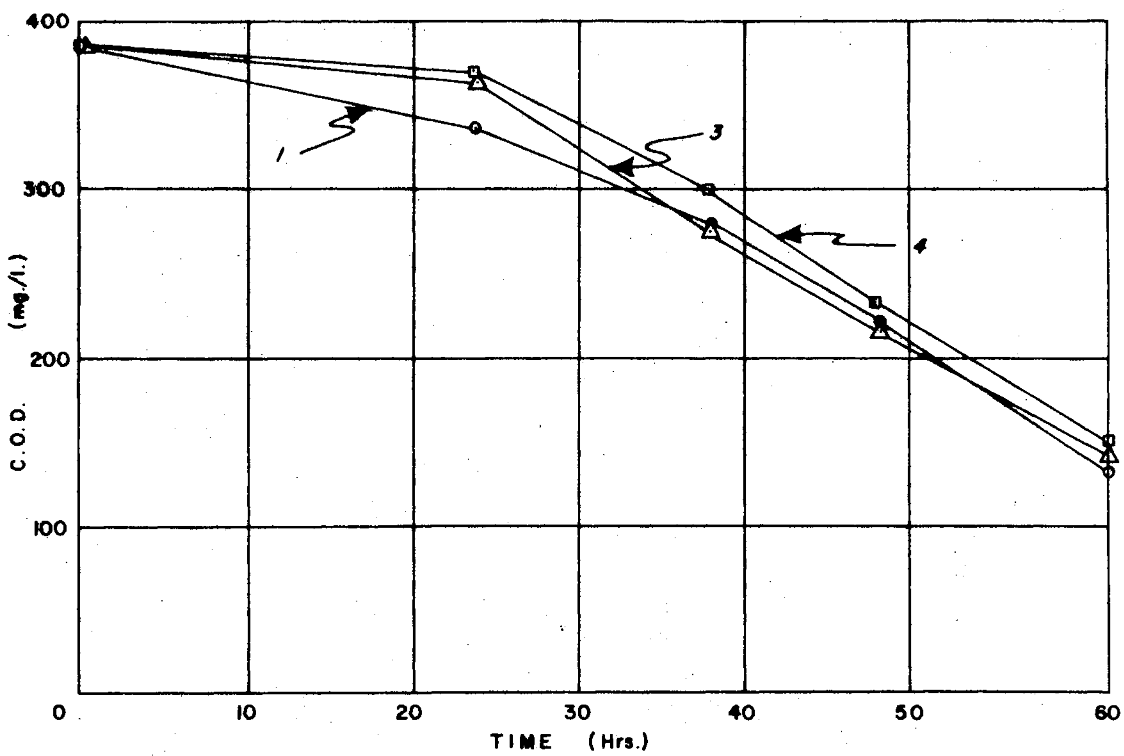

400
300
200
100
0
10
20
30
40
50
60
C.O.D. (mg./l.)
TIME (Hrs.)
1
3
4

Figure 5. Relationship of COD Removal Between 1/3 Sewage and 2/3 Slaughterhouse Wastewater Without Air, Stirred by Magnetic Stirrers, and With (1) 80 mg/l Biocatalyst Sample No. 7, (2) 80 mg/l Biocatalyst Sample No. 6, and (3) Without Bacteria Added

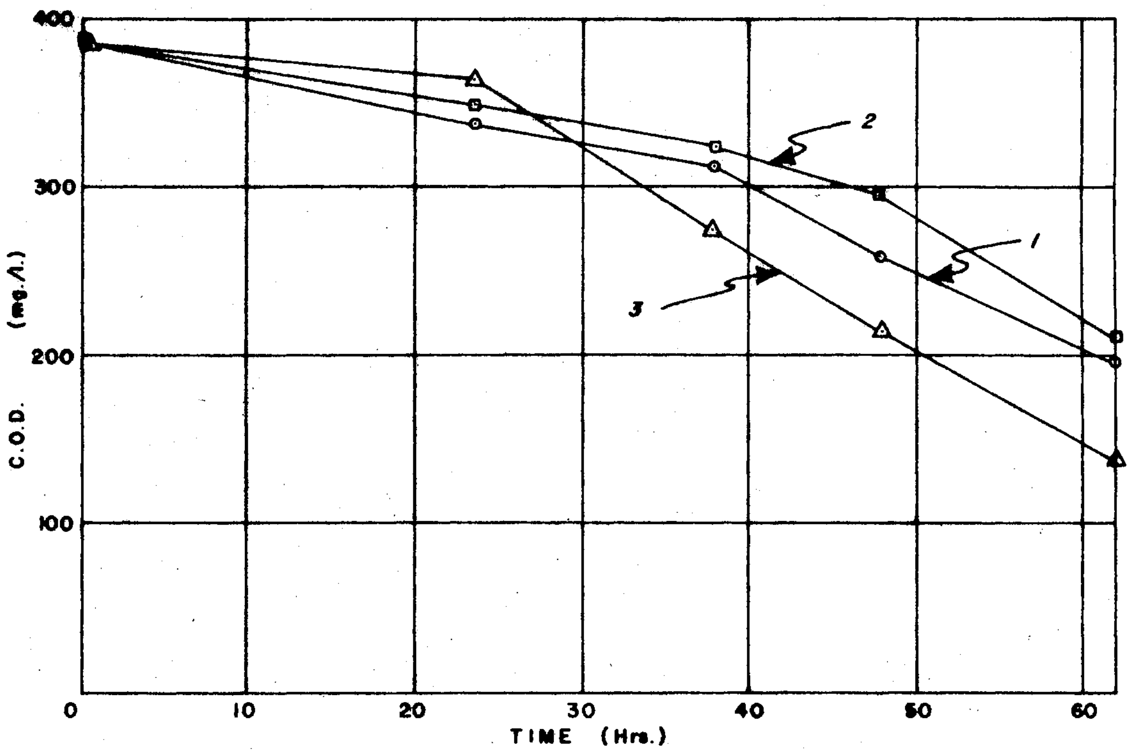

400
300
200
100
C.O.D. (mg./l.)
0
10
20
30
40
50
60
TIME (Hrs.)
1
2
3

Figure 6. Relationship of COD Removal Between 1/3 Sewage and 2/3 Slaughterhouse Wastewater With (1) 80 mg/l Biocatalyst Sample No. 3, (2) 80 mg/l Biocatalyst Sample No. 6, and (3) Without Biocatalyst. Dashed Lines are Unfiltered COD Values.

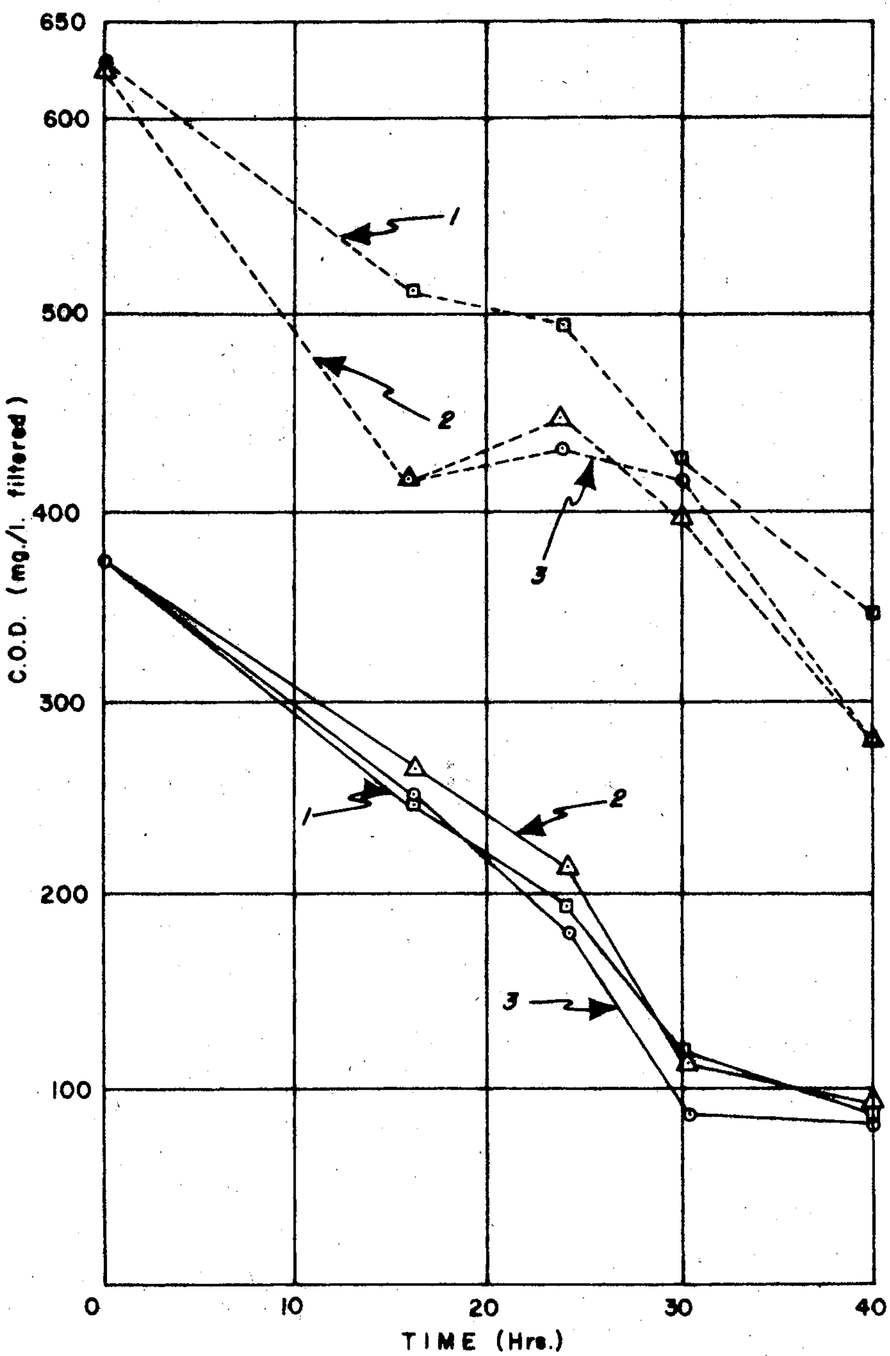

650
600
500
400
300
200
100
0
10
20
30
40
TIME (Hrs.)
C.O.D. (mg./l. filtered)
1
2
3
1
2
3

Figure 7. Relationship of COD Removal Between 1/3 Sewage and 2/3 Slaughterhouse Wastewater With (1) no Biocatalyst, (2) 80 mg/l Biocatalyst Sample No. 8, and (3) 80 mg/l Biocatalyst Sample No. 7. Dashed Lines are Unfiltered COD Values

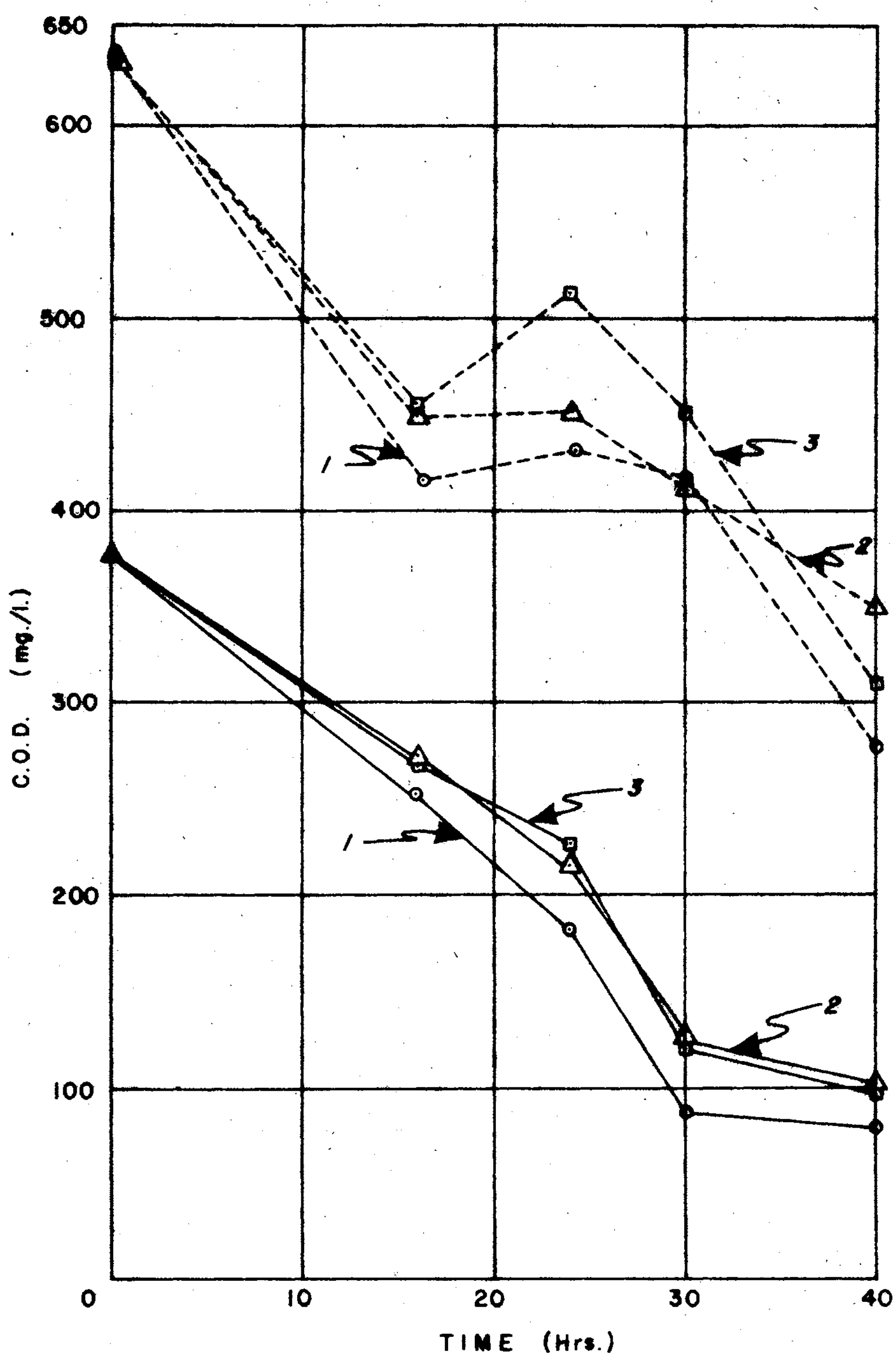
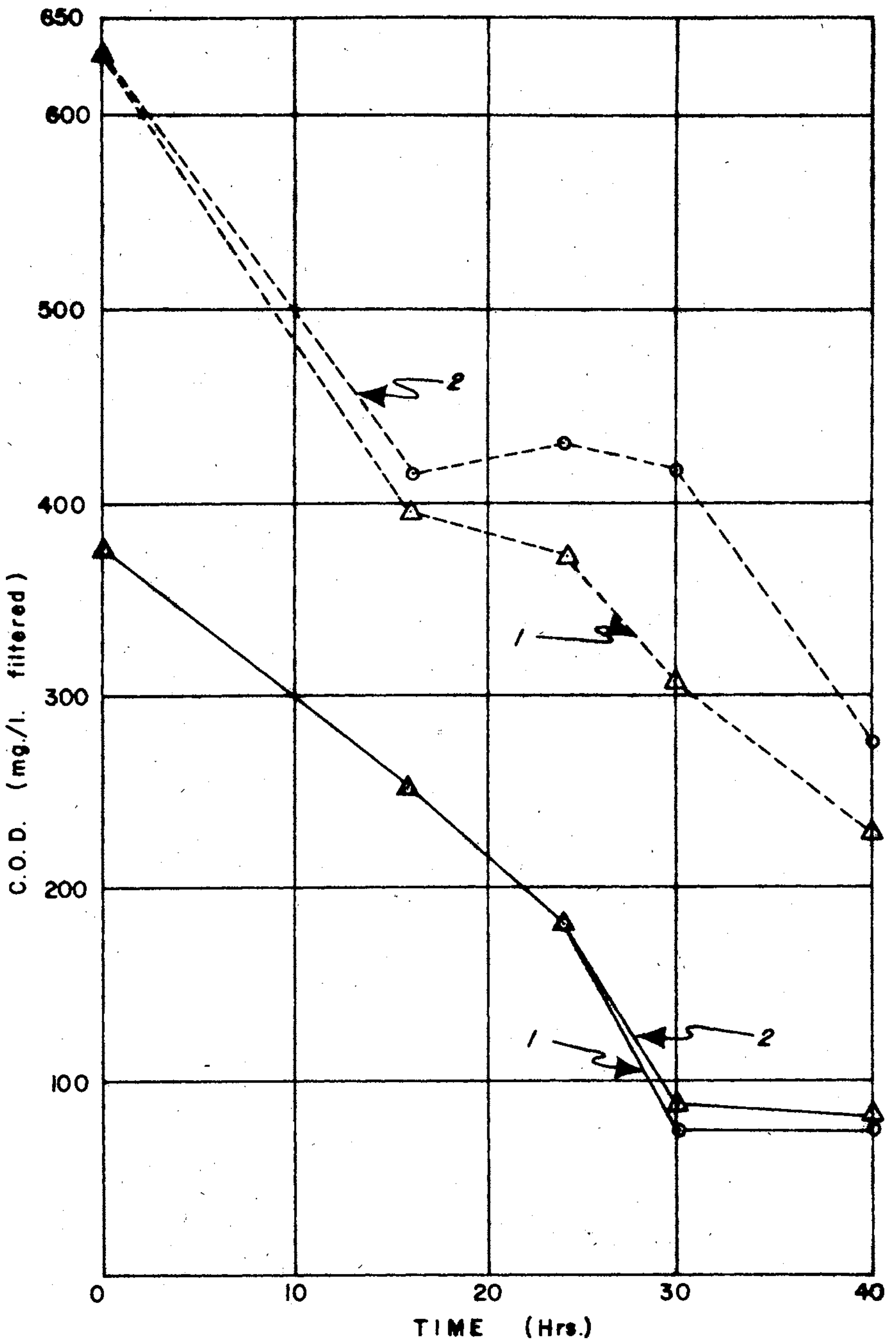

650
600
500
400
300
200
100
0
10
20
30
40
C.O.D. (mg./l.)
TIME (Hrs.)
1
2
3
1
2
3

Figure 8. Relationship of COD Removal Between 1/3 Sewage and 2/3 Slaughterhouse Wastewater With (1) 80 mg/l Biocatalyst Sample No. 9, and (2) Without Bacteria Added. Dashed Lines are Unfiltered COD Values


650
600
500
400
300
200
100
0
10
20
30
40
C.O.D. (mg./l. filtered)
TIME (Hrs.)
1
2
1
2

A filtered effluent of 85 mg/l COD was obtained in 40 hours with a COD removal of 80 percent. The unfiltered wastewater had a 280 mg/l COD, and a COD removal of 56 percent after 40 hours of aeration.

Figure 7 shows the relationship of COD differences between 1/3 sewage and 2/3 slaughterhouse wastewater using biocatalyst samples No. 8 and No. 7. The wastewater without biocatalyst showed a faster removal of COD than did the wastewater containing the biocatalyst. Unfiltered COD shows a rise at 15 hours to 25 hours. Biocatalyst samples Nos. 3, 6, 7, and 8 had similar effects on the wastewater.

Figure 8 shows the relationship of COD difference between wastewater containing biocatalyst sample No. 9 and wastewater containing no biocatalyst. The filtered wastewater from the unit containing sample No. 9 had a slightly lower effluent COD than the effluent COD of the control unit after 40 hours of aeration. Unfiltered COD of the unit containing sample No. 9 was also lower than the unfiltered COD of the control unit. At 30 hours, both units had a filtered COD of approximately 85 mg/l, which stayed constant for the next ten hours, while the unfiltered COD of both units continued to be reduced, indicating endogenous respiration during this period. Unfiltered COD removal from the control unit and the unit containing sample No. 9 were 57 percent and 64 percent, respectively, after 40 hours of aeration.

B. Acclimated Wastewater Study

In the preceding study, the biocatalysts were added to the wastewater after being mixed for one hour in distilled water, thus simulating the addition of the unacclimated biocatalyst to the wastewater at a slaughterhouse plant. The preceding study indicated the addition of biocatalyst to the wastewater in the above manner required 40 to 60 hours for the wastewater COD reduction rate to be reduced to a minimum.

A study was made on the application of biocatalysts to the slaughterhouse and sewage mixture containing biological solids from an activated sludge unit containing the same wastewater mixture. A portion of the acclimated biological solids was added to the raw wastewater to give the wastewater a microbial seed population acclimated to the wastewater, thus aiding in the reduction of the removal time.

Figures 9, 10, and 11 show the results of COD removal from units containing the wastewater and constituents indicated by Table II.

Figure 9 shows the relationship of COD removal between wastewaters from unit 6 with acclimated sewage seed, and unit 3 with acclimated slaughterhouse wastewater seed with biocatalyst sample No. 3. The 500 ml of sewage seed added to unit 6 had a solids content of 568 mg/l. The 125 ml of slaughterhouse wastewater seed had a solids content of 1693 mg/l. Figure 9 shows the wastewater containing sewage seed having a COD of 50 mg/l after 20 hours of aeration, and the wastewater containing slaughterhouse seed having a COD of 80 mg/l after 20 hours of aeration. The faster removal rate of the sewage seed wastewater can be explained by the fact that unit 6 contained 283 mg of solids in the sewage seed, and the slaughterhouse seed fed contained 212 mg of solids. The higher microbial population in the sewage wastewater allowed faster removal.

Figure 10 shows the COD relationship of units 1, 2, and 5. Each unit shows the same rate of removal and an effluent COD of approximately 50 mg/l, with a removal of 88 percent of the COD in 15 hours.

Figure 11 shows the effect of different quantities of acclimated slaughterhouse seed on the removal rates of COD from wastewater in

Figure 9. Relationship of COD Removal Between Slaughterhouse Wastewater (1) With 42 mg/l Acclimated Slaughterhouse Wastewater Seed and 10 mg/l Biocatalyst Added 48 hr. Before t_o, (2) With 167 mg/l Acclimated Sewage Wastewater Seed, Without Biocatalyst

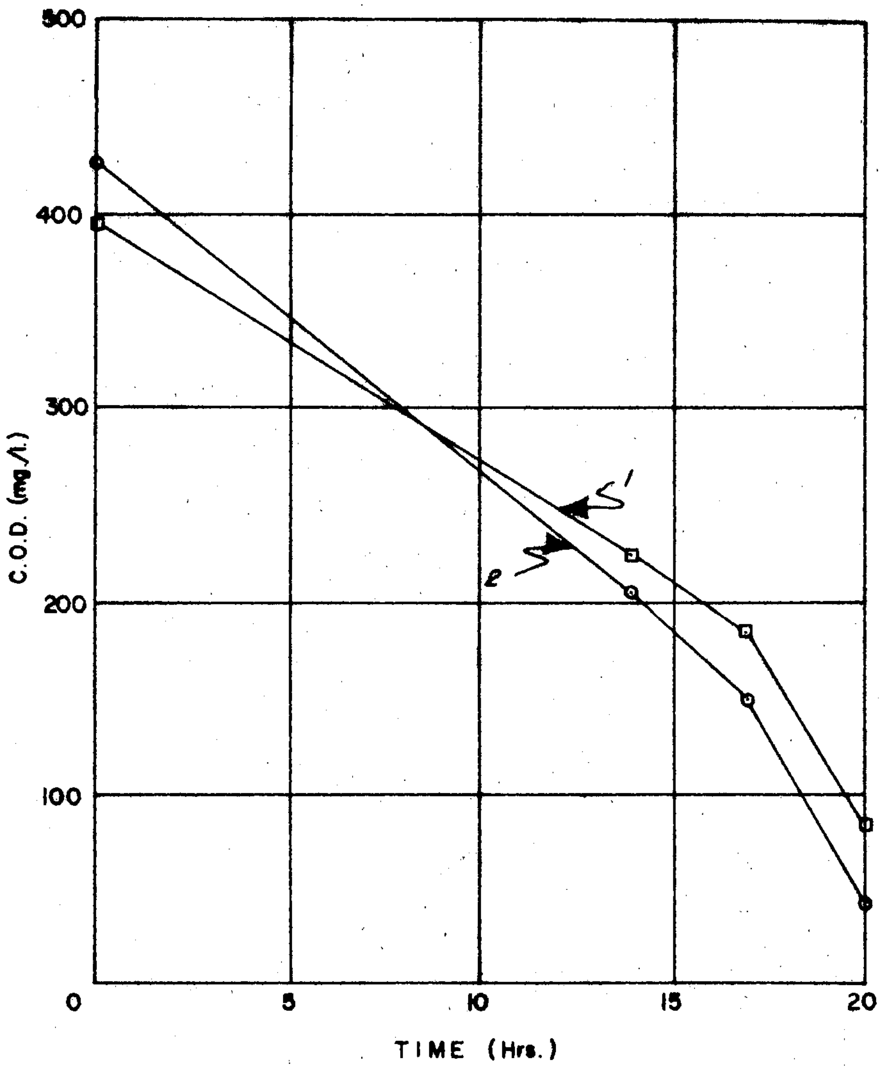

500
400
300
200
100
0
5
10
15
20
C.O.D. (mg./l.)
TIME (Hrs.)
S'
ℓ

Figure 10. Relationship of COD Removal Between Slaughterhouse Wastewaters (1) With 167 mg/l Acclimated Slaughterhouse Wastewater Seed and 10 mg/l Biocatalyst Added 48 hr before t_o, (2) With 167 mg/l Initial Sewage Seed Acclimated Slaughterhouse Wastewater Seed and 10 mg/l Biocatalyst Added 48 hr before t_o, and (3) With 167 mg/l Initial Sewage Seed Acclimated Slaughterhouse Seed Without Biocatalyst

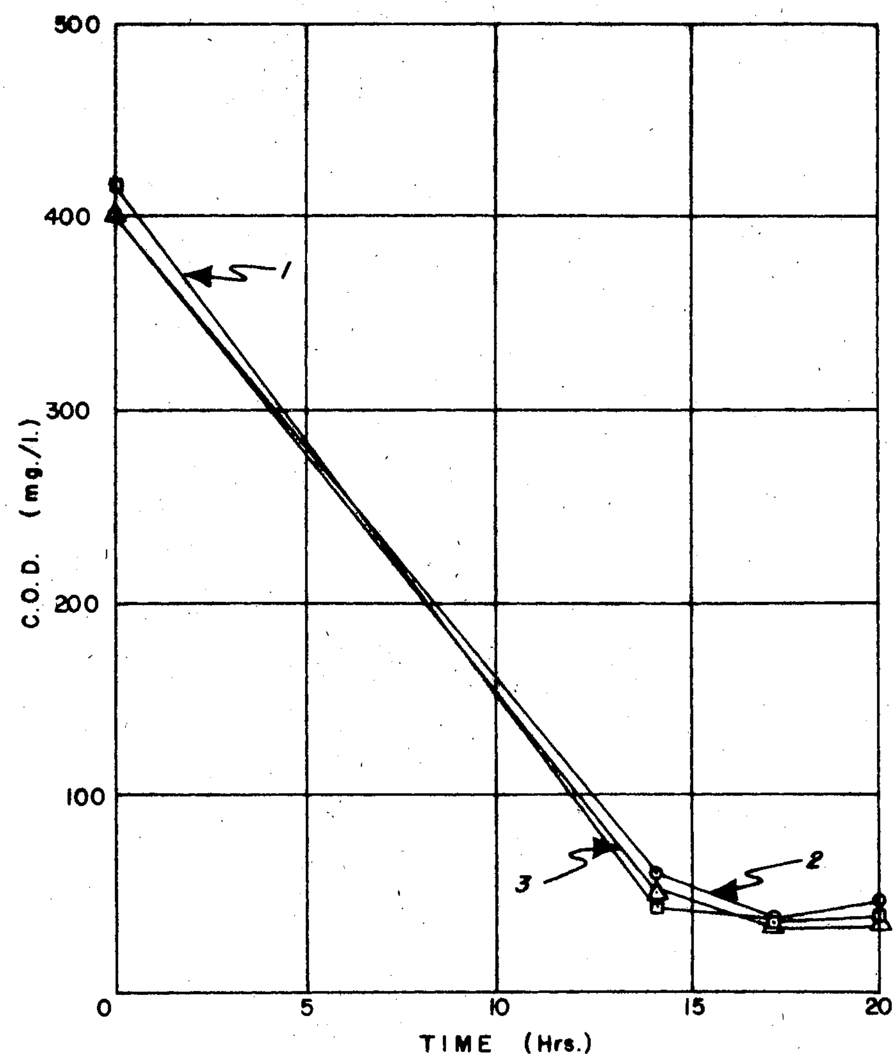
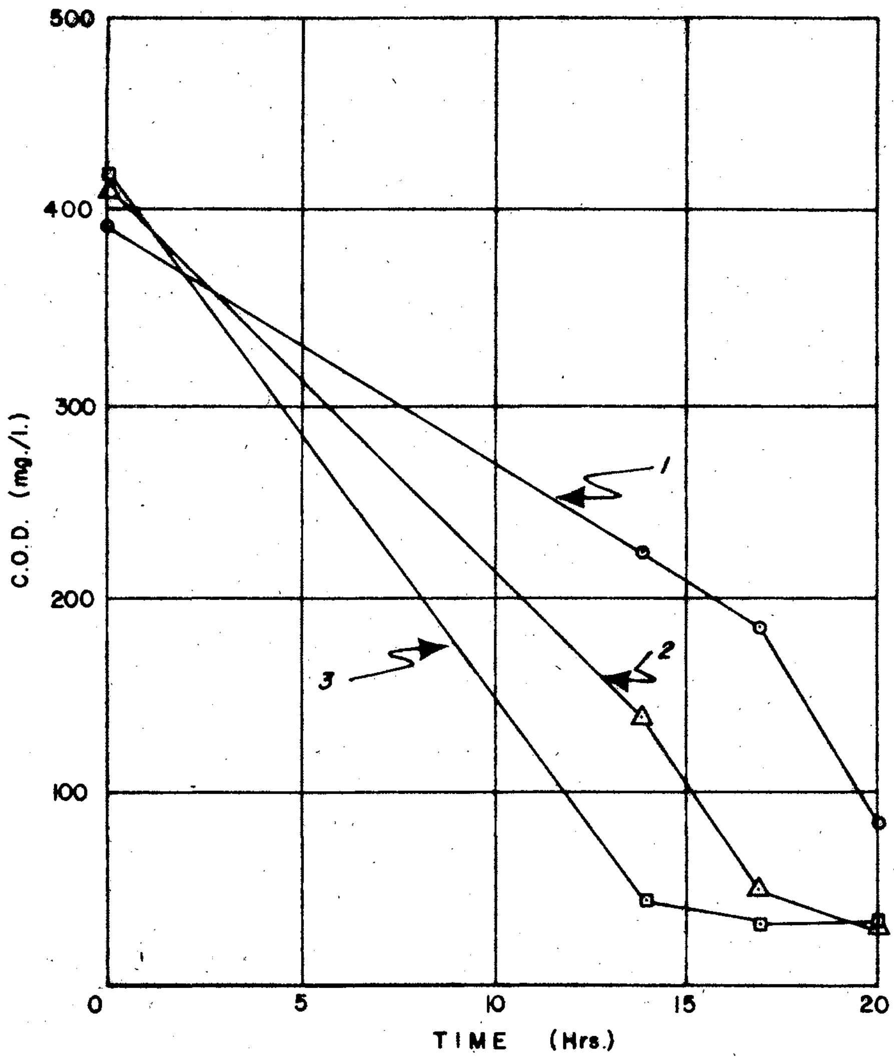

500
400
300
200
100
C.O.D. (mg./l.)
0
5
10
15
20
TIME (Hrs.)
1
2
3

Figure 11. Relationship of COD Removal Between Slaughterhouse Wastewater Containing 10 mg/l Biocatalyst and (1) 42 mg/l, (2) 83 mg/l, and (3) 167 mg/l of Acclimated Slaughterhouse Wastewater Seed


500
400
300
200
100
C.O.D. (mg./l.)
0
5
10
15
20
TIME (Hrs.)
1
2
3

units 3, 4, and 5. On the 14th day of the study, the units were filled with the contents indicated in Table III, and aerated.

The results obtained from the study are shown in Figures 12, 13, 14, and 15. Figure 12 shows the relationship of COD differences between acclimated slaughterhouse wastewater with biocatalyst sample No. 3 and 34 mg/l, 67 mg/l, and 133 mg/l of acclimated seed. The solids content of the acclimated seed was 2513 mg/l. As in Figure 11, Figure 12 shows that with an increase in acclimated seed there is an increase in the COD removal rate. The unit containing 133 mg/l of acclimated seed had a removal rate of 45 mg/l of filtered COD/hr during the period from five hours to 15 hours after beginning aeration. The unit had a 94 percent COD removal at 18 hours, reducing the COD from 800 mg/l to 50 mg/l.

Figure 13 shows the COD relationship of units 1 and 6. The solids content of the seeds for units 1 and 6 were 2270 mg/l and 386 mg/l, respectively, giving 455 mg of seed solids for unit one, and 194 mg of seed solids for unit 6. Unit 6 had a higher rate of COD removal, but both units had a 95 percent COD removal after 25 hours of aeration.

Figure 14 shows the rate of COD removal from wastewater in units 2 and 5 to be the same. Unit 2 contained no biocatalyst, and unit 5 contained biocatalyst sample No. 3. Both units contained approximately the same amounts of solids, each having 133 mg/l of acclimated seed.

Figure 15 shows the rate of COD removal from units 1 and 4 are the same. Unit 1 contains no biocatalyst, and unit 4 contains biocatalyst sample No. 3. As in Figure 14, both units contained approximately the same amount of solids. Each unit contained 67 mg/l of acclimated seed.

Figure 12. Relationship of COD Removal Between Acclimated Slaughterhouse Waste With Biocatalyst With 34 mg/l, 67 mg/l, and 133 mg/l of Acclimated Seed

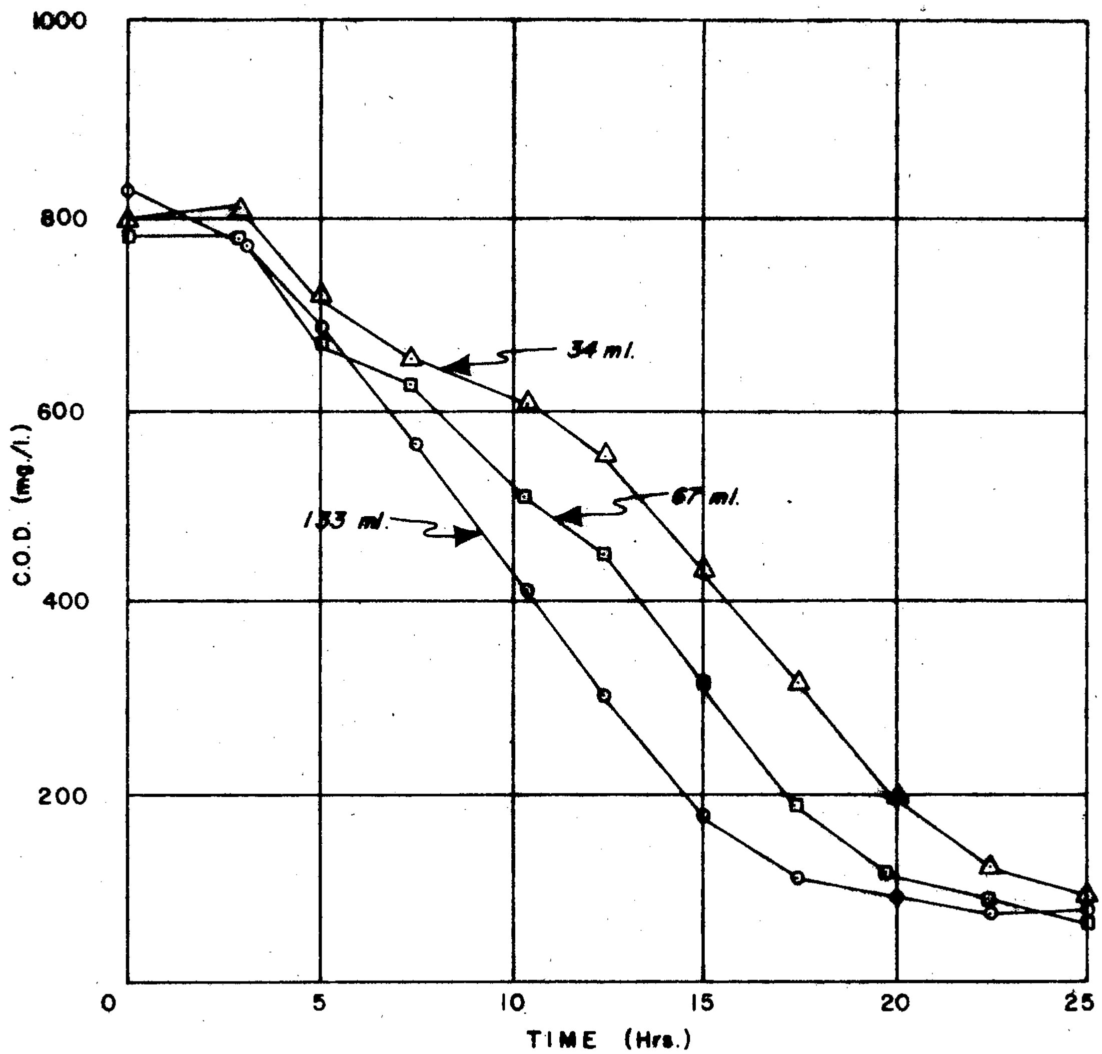

1000
800
600
400
200
C.O.D. (mg./l.)
0
5
10
15
20
25
TIME (Hrs.)
34 ml.
67 ml.
133 ml.

Figure 13. Relationship of COD Removal Between Wastewater, Without Biocatalyst With (1) 67 mg/l Acclimated Slaughterhouse Wastewater Seed, and (2) 167 mg/l Acclimated Sewage Seed

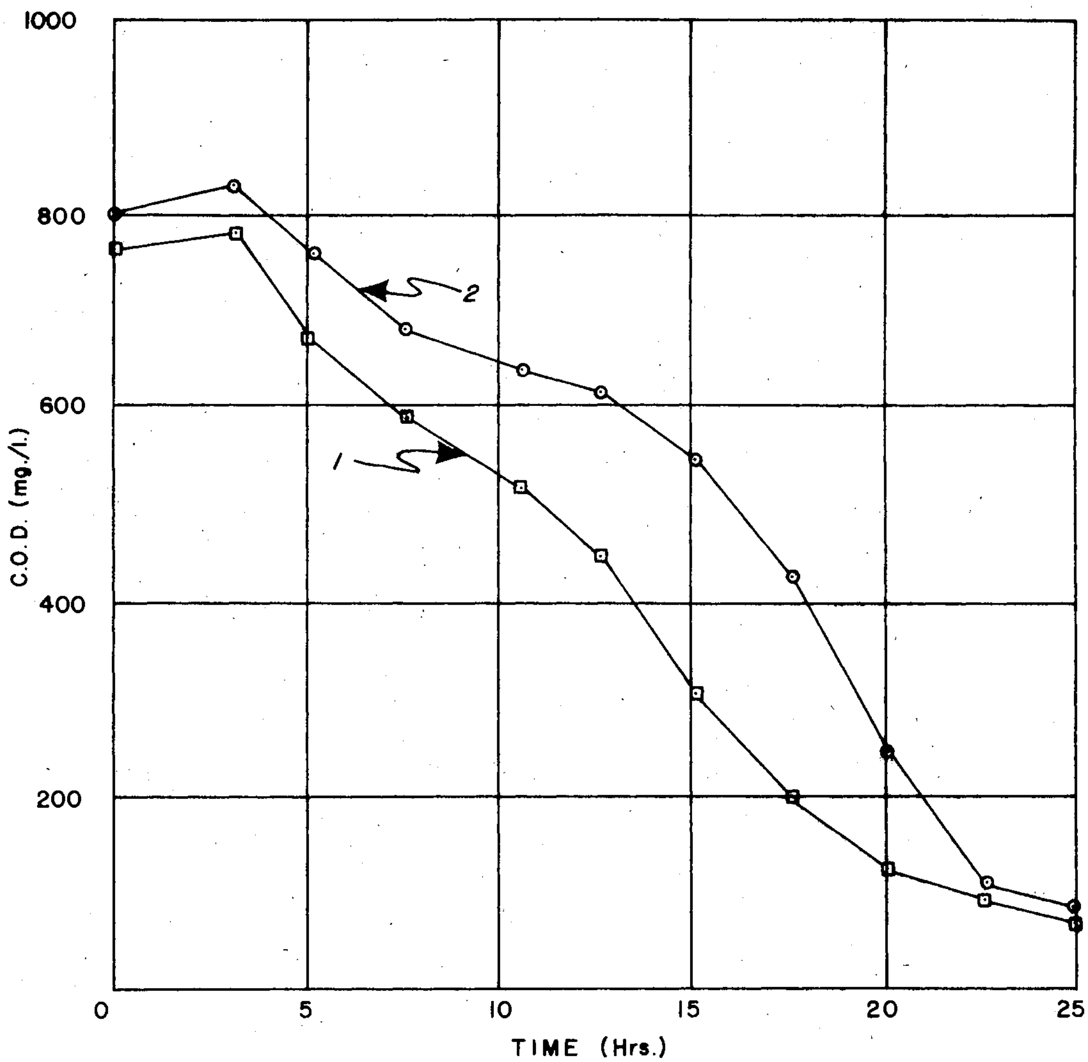
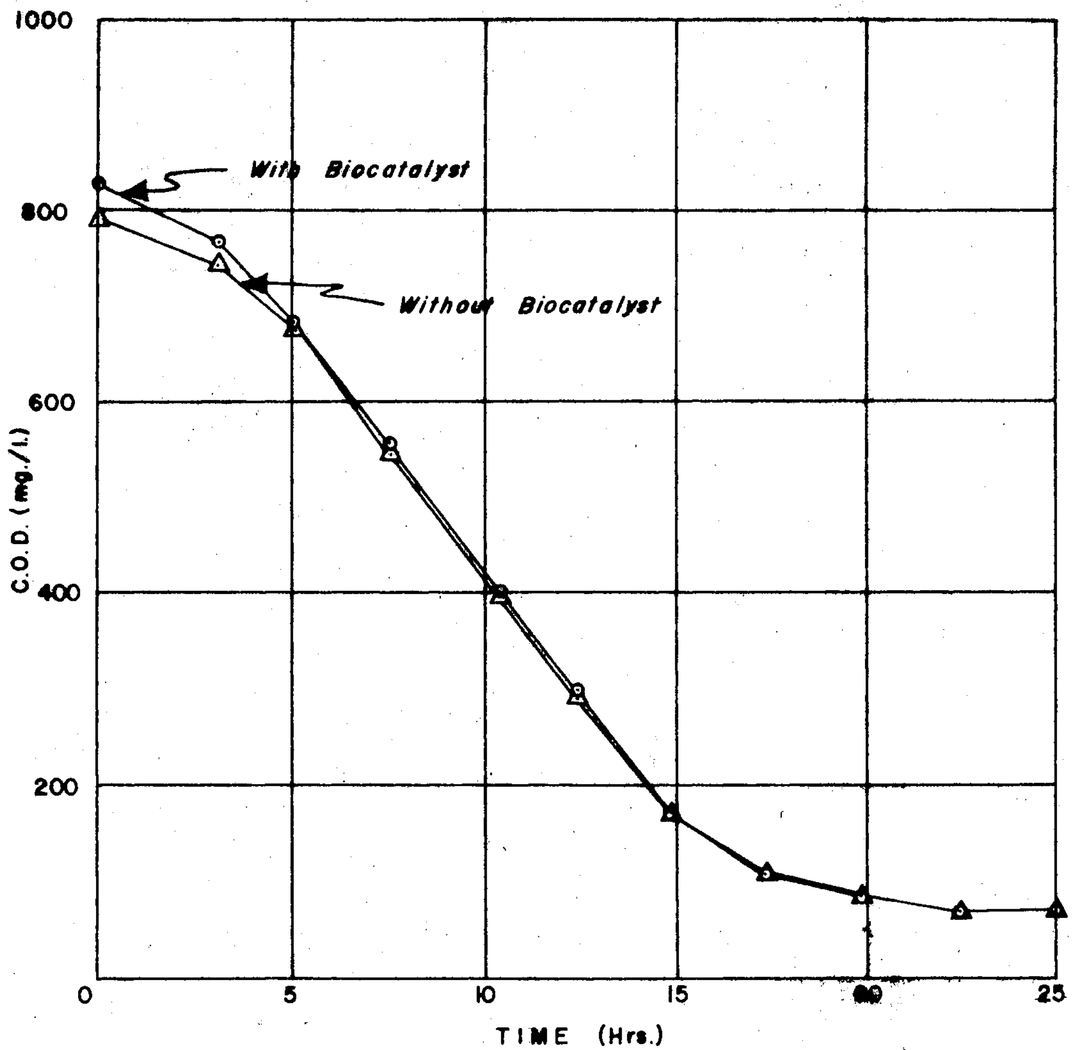
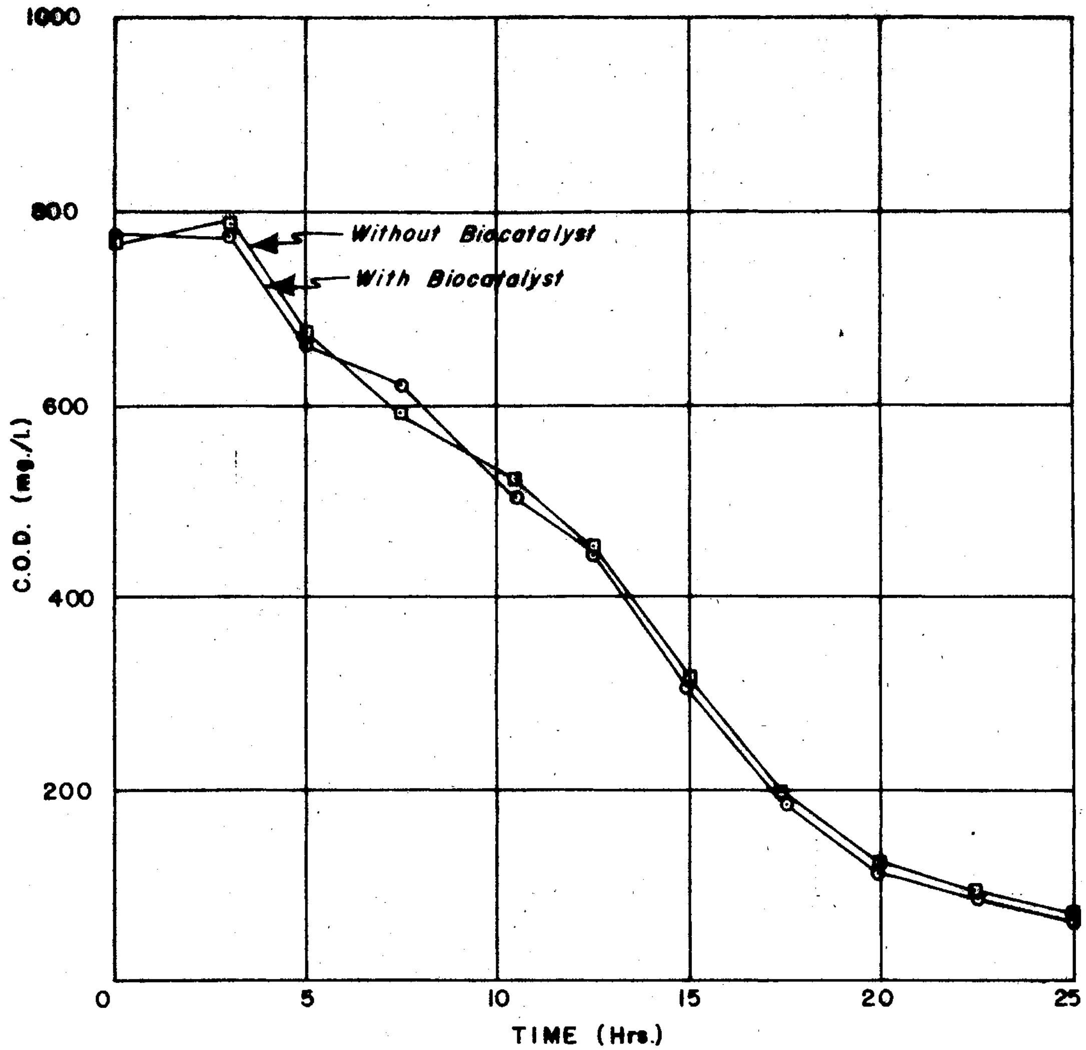

1000
800
600
400
200
C.O.D. (mg./l.)
0
5
10
15
20
25
TIME (Hrs.)
1
2

Figure 14. Relationship of COD Removal Between Acclimated Slaughterhouse Waste With and Without Biocatalyst With 133 mg/l Acclimated Seed



1000
800
600
400
200
0
5
10
15
20
25
C.O.D. (mg./l.)
TIME (Hrs.)
With Biocatalyst
Without Biocatalyst

Figure 15. Relationship of COD Removal Between Acclimated Slaughterhouse Waste With and Without Biocatalyst With 67 mg/l Acclimated Seed



1000
800
600
400
200
0
5
10
15
20
25
C.O.D. (mg./L)
TIME (Hrs.)
Without Biocatalyst
With Biocatalyst

CHAPTER V

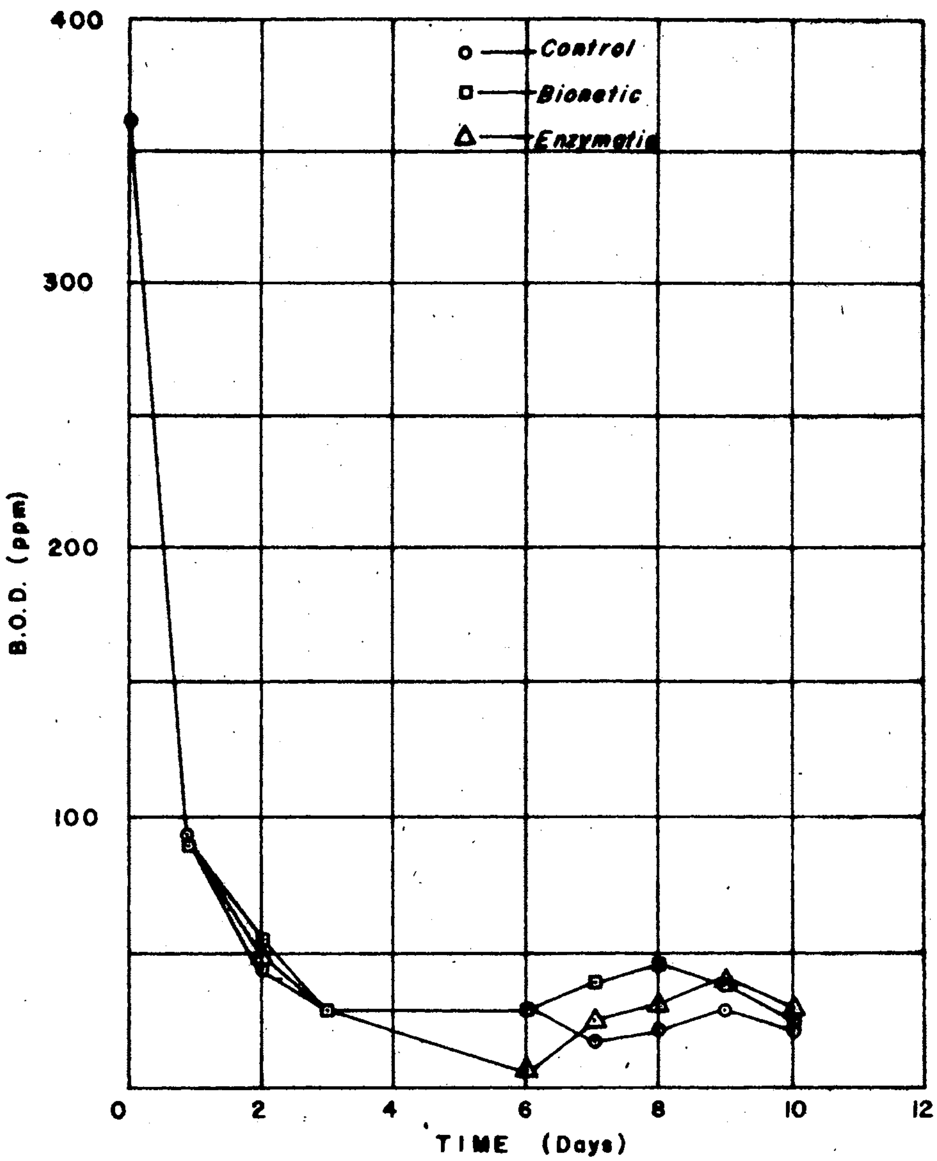
DISCUSSION

This investigation was conducted to study the effect of biocatalysts on wastewater treatment. Five biocatalysts were investigated. Studies were conducted with various concentrations of biocatalysts on a synthetic wastewater and various mixtures of municipal sewage and slaughterhouse wastewater.

The most obvious result obtained was that no beneficial effect can be attributed to the addition of biocatalysts on the COD reduction in a properly operated activated sludge unit containing slaughterhouse wastewater. This result is similar to the results of McKinney (12), who investigated the use of biocatalysts on BOD removal from raw sewage and a synthetic wastewater about 20 years previous to this investigation. Figures 16 and 17 show the results McKinney obtained from his studies.

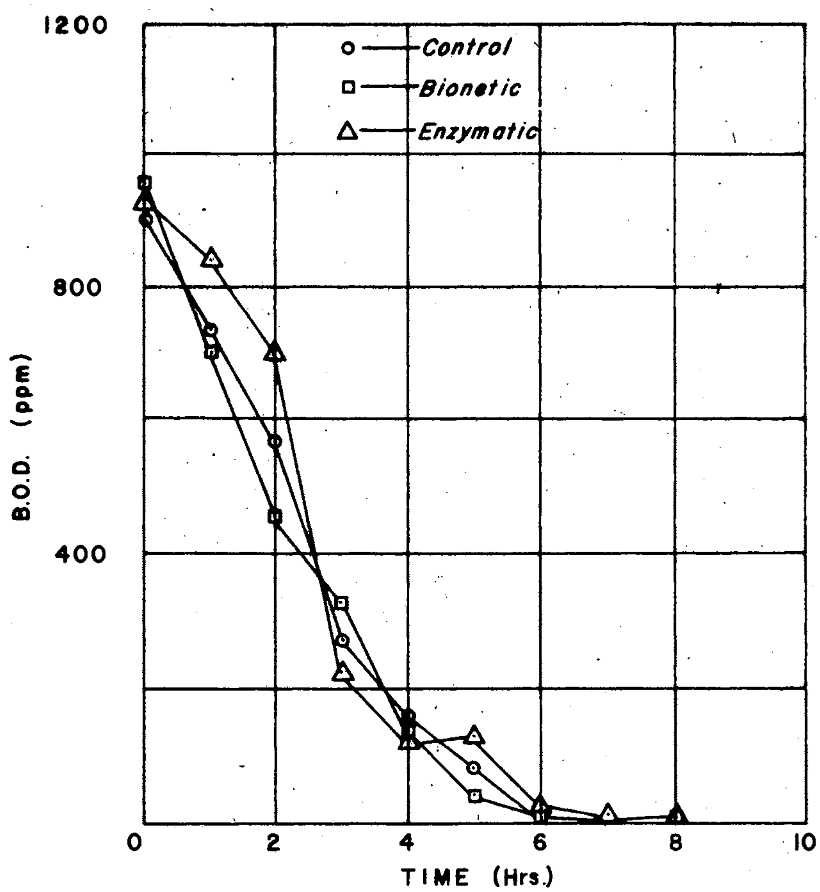
From the study of synthetic wastewater, it was learned that the dried bacteria in the biocatalyst preparations were viable by preparing culture plates and incubating the inoculated plates at 30^{o}C. It was seen that the factor in COD removal from glucose wastewater which was seeded with 10 mg/l of raw domestic sewage, a detention time of 45 hours for 80 percent removal was obtained by doubling the biocatalyst from 40 mg/l to 80 mg/l. The detention time for 80 percent

Figure 16. BOD of Effluent From Activated Sludge Units Fed Raw Sewage and 100 mg/l Catalysts Twice Daily After 24-hr Aeration



Control
Bionetic
Enzymatic
400
300
200
100
B.O.D. (ppm)
0
2
4
6
8
10
12
TIME (Days)

Figure 17. BOD of Effluent From Activated Sludge Units Fed Synthetic Industrial Waste and 500 mg/l Catalysts; Eighth Day of Aeration



Control
Bionetic
Enzymatic
1200
800
400
B.O.D. (ppm)
0
2
4
6
8
10
TIME (Hrs.)

removal using 40 mg/l biocatalyst for 76 hours. It was seen that the COD increased for various periods of time until an adequate microorganism population was produced. After this population was adequate, the rate of removal was the same for all units containing biocatalysts; therefore, the bacterial population was the major factor involved in reducing the detention time. This also supports McKinney's statement that biocatalysts could be used in units which have insufficient biological populations.

The acclimated study showed that the five biocatalysts used had no significant beneficial effect on decreasing the detention time or increasing the rate of COD removal from the domestic and slaughterhouse wastewaters, using present day bacterial enzyme preparations.

Figure 3 shows that biocatalyst additions from 0 to 80 mg/l had no effect on COD removal. The manufacturers of four of the biocatalysts recommended the use of one pound of catalyst for each million gallons of slaughterhouse wastewater. The concentration used in the study resulting in the data in Figure 3 far exceeded the recommended dosage.

Studies conducted to find the effect of the use of biocatalysts on inline waste treatment indicated that oxygen was the limiting factor of biological action in sewage lines. One manufacturer recommended the use of biocatalysts to reduce the BOD or COD of the wastewater by enhancing biological action, but the low dissolved oxygen conditions of sewage lines inhibits the process of aerobic processes. Two of the biocatalysts recommended for biological waste reduction seemed to inhibit COD reduction.

Five wastewaters containing 80 mg/l of one of the five biocatalysts were compared to a control unit containing no biocatalyst. None of the wastewaters containing biocatalysts gave better filtered COD reduction rates than the control unit. One of the biocatalysts did have an effect in increasing the unfiltered COD removal rate.

Studies of acclimated wastewater were conducted to find what effect the biocatalysts had on activated sludge units containing sludge which had been acclimated for seven to 14 days. Both studies showed that the biocatalysts had no effect on the removal rate of filtered COD in activated sludge units.

It is interesting to note that Figure 9 shows that slaughterhouse wastewater added to 283 mg of acclimated sewage seed solids had a slightly higher rate of COD removal than the same wastewater added to 212 mg of acclimated slaughterhouse wastewater seed solids. This similarity in removal rate obtained from both sewage seed and slaughterhouse seed indicates that slaughterhouse waste characteristics are similar to domestic waste, and that they can be treated efficiently either separately or combined.

Figures 9, 11, 12, and 13 show that biological solids concentration is the major factor involved in the treatment of slaughterhouse wastewater in activated sludge units.

This and other studies show that biocatalysts have no significant effect in removing COD from wastewater similar to domestic wastewater when sufficient microorganism population is present. Biocatalysts can be effective in starting biological treatment of wastewaters which contained a small or no microbial population.

It is concluded from the results obtained in this study that biocatalysts have not been improved in the last 20 years for application to well operated activated sludge processes, and that these biocatalysts have limited use in the treatment of wastewaters. Various performance claims have been made by manufacturers, but reports indicate that many of the biological units in which biocatalysts were added had not been operated properly. Although there may be an application of biocatalysts to improperly operated biological units, the high cost of the biocatalysts encourages the maintenance of good operation and biological testing programs.

CHAPTER VI

CONCLUSIONS

Conclusions based on the results of this study are:

(1) Biocatalysts can be of use on wastewaters having low microbial populations.

(2) Some of the biocatalysts acted as inhibitors during low dissolved oxygen conditions.

(3) Sewage collection systems cannot be used as treatment units without the addition of air into the sewage line throughout the system.

(4) Biocatalysts are not beneficially effective on domestic sewage and slaughterhouse wastewaters when the bacterial population is adequate and activated sludge units are operated properly.

CHAPTER VII

SUGGESTIONS FOR FUTURE STUDY

Based on the findings of this study, the following suggestions are made for future study of the application of biocatalysts on wastewater:

(1) Investigation of the effect of biocatalysts on grease and petroleum products.

(2) Investigation of biocatalysts on solids reduction and sludge drying.

(3) Investigation of the use of biocatalysts and domestic sewage on wastewaters containing inadequate microbial populations.

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