The Influence of Anaerobic Pretreatment on the Treatability of a Milk Waste

Jerry Lee Siegel

Follow this and additional works at: https://openprairie.sdstate.edu/etd

Recommended Citation
https://openprairie.sdstate.edu/etd/3605
THE INFLUENCE OF ANAEROBIC PRETREATMENT ON
THE TREATABILITY OF A MILK WASTE

BY

JERRY LEE SIEGEL
THE INFLUENCE OF ANAEROBIC PRETREATMENT ON
THE TREATABILITY OF A MILK WASTE

BY
JERRY LEE SIEGEL

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Civil Engineering, South Dakota
State University

1969

SOUTH DAKOTA STATE UNIVERSITY LIBRARY
THE INFLUENCE OF ANAEROBIC PRETREATMENT ON

THE TREATABILITY OF A MILK WASTE

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for the degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

[Signature]
Thesis Adviser
Date

Head, Civil Engineering
Department
Date
ACKNOWLEDGMENTS

The author sincerely valued the guidance and encouragement provided by Professor Dwayne A. Rollag, Dr. James N. Dornbush, and Dr. John R. Andersen of the Civil Engineering Department.

Appreciation is extended to T. Alvin Biggar, Civil Engineering Technical Assistant, for his help in constructing the apparatus needed for the study.

This study was supported in part by Federal Water Pollution Control Administration Training Grant No. 5T1-WP-93.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>Use of Pilot Plants in Biological Treatment Studies.</td>
<td>3</td>
</tr>
<tr>
<td>Aerobic Treatment</td>
<td>5</td>
</tr>
<tr>
<td>Anaerobic Treatment</td>
<td>7</td>
</tr>
<tr>
<td>Anaerobic-Aerobic Treatment Systems</td>
<td>9</td>
</tr>
<tr>
<td>Use of the Warburg Respirometer for Determining Waste Treatability</td>
<td>14</td>
</tr>
<tr>
<td>EXPERIMENTAL METHODS AND TEST PROCEDURES</td>
<td>17</td>
</tr>
<tr>
<td>Design and Operation of the Pilot Plant System</td>
<td>17</td>
</tr>
<tr>
<td>Development of Testing Technique</td>
<td>20</td>
</tr>
<tr>
<td>Chemical Oxygen Demand Versus Time</td>
<td>20</td>
</tr>
<tr>
<td>Oxygen Uptake Using the Warburg Respirometer</td>
<td>22</td>
</tr>
<tr>
<td>Laboratory Test Procedures</td>
<td>24</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>26</td>
</tr>
<tr>
<td>Preliminary Studies</td>
<td>26</td>
</tr>
<tr>
<td>Rate of Substrate COD Removal</td>
<td>27</td>
</tr>
<tr>
<td>Oxygen Uptake for Acclimated Systems</td>
<td>30</td>
</tr>
<tr>
<td>Oxygen Uptake for Non-acclimated Systems</td>
<td>34</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>38</td>
</tr>
<tr>
<td>FUTURE STUDY</td>
<td>40</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>41</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (continued)

APPENDIX I. Amount of Substrate COD Remaining in Solution at Indicated Time after Feeding ................................................. 46

APPENDIX II. Oxygen Uptake Data. .................................................. 48
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Photograph of the pilot plant system.</td>
<td>18</td>
</tr>
<tr>
<td>2.</td>
<td>Amount of substrate COD remaining in the supernatant solution versus time after feeding activated sludge with non-treated milk and pretreated milk</td>
<td>28</td>
</tr>
<tr>
<td>3.</td>
<td>Oxygen uptake of the acclimated treatment systems</td>
<td>32</td>
</tr>
<tr>
<td>4.</td>
<td>Oxygen uptake of anaerobically pretreated milk using acclimated and non-acclimated activated sludges at two MLSS concentrations.</td>
<td>35</td>
</tr>
<tr>
<td>5.</td>
<td>Oxygen uptake of non-treated milk using acclimated and non-acclimated activated sludges at two MLSS concentrations.</td>
<td>36</td>
</tr>
</tbody>
</table>
INTRODUCTION

Both anaerobic and aerobic treatment of wastewater has been used for many years. Some of the common aerobic treatment systems include oxidation or stabilization ponds, activated sludge, trickling filters, and aerated lagoons. Anaerobic treatment has been most commonly used for digestion of sewage sludges. However, septic tanks, anaerobic lagoons, and anaerobic activated sludge (contact stabilization) units are used to treat wastewater anaerobically.

More recently, the two systems have been used in combination, with the anaerobic system preceding the aerobic one. This type of treatment has been used primarily for lagooning of industrial wastes, and most commonly for meat-packing wastes.

In all instances, the anaerobic unit of combination systems has been designed to provide some waste stabilization, that is, to remove a portion of the BOD, biochemical oxygen demand, of the waste. This requires the relatively long detention time which is characteristic of anaerobic treatment. The aerobic unit serves to reduce the remaining BOD of the waste to a level dictated by government regulation or by the capacity of the receiving stream to handle the organic load.

The combination anaerobic-aerobic system evaluated in this study differs from the previously mentioned system in one important aspect. The anaerobic unit was not designed to provide actual waste stabilization. Instead, the anaerobic unit was utilized only for
pretreatment of the waste, that is, conversion of the more complex organics present in the waste into simpler organic compounds without the formation of gaseous end products. This initial breakdown of the waste should make it more easily and rapidly treatable in the subsequent aerobic system.

A close examination of the fundamentals of anaerobic treatment indicates that eliminating the necessity of actual waste stabilization in the anaerobic unit might reduce two of the main disadvantages of anaerobic treatment: sensitive operation and long detention periods.

The advantages and uses of this type of combination treatment could be many. Rather than adding a more expensive aerobic unit to an overloaded aerobic waste treatment system, anaerobic pretreatment, such as that provided in a simple septic tank, might make the existing system perform satisfactorily. In the design of a new system, the initial construction cost could be cut if the size of the aerobic unit required to handle the wastewater could be substantially reduced by anaerobic pretreatment.
Use of Pilot Plants in Biological Treatment Studies

A review of the literature indicated that a pilot plant evaluation often precedes the design of new treatment plants and additions to existing plants. The pilot plant serves to indicate how stabilization of the particular waste will be affected by such variable factors as time, temperature, suspended solids, waste strength, and waste characteristics. By evaluating these variables in a pilot plant study, serious errors in design are often avoided.

There are two basic pilot plant designs: continuously fed systems and batch fed, fill-and-draw type systems. The continuously fed system has the advantage of being operated similar to an actual wastewater treatment plant. Because of this, it is easier to evaluate the pilot plant data and apply it to actual plant operation. Continuously fed pilot plants, however, create problems of sludge settling and sludge return which are difficult to solve on a small scale.

Batch fed pilot plant systems are simpler in design and operation. They need to be fed only once a day. Low capacity pumps, which are expensive and cumbersome, are not required. In the batch-fed units, the high concentration of organic matter at the start of the aeration period often stimulates necessary adaptive enzymes for metabolism which would not be stimulated under the low concentration conditions in a continuously fed, completely mixed
pilot plant. For an activated sludge pilot plant, a one-tank system suffices. In the aeration vessel, introduction of reactants, aeration, and product separation and removal are all carried out in sequence after the proper time interval (1)(2). Symons et al. (1) recommend using batch fed aeration units when evaluating the biological treatability of an industrial waste.

For several years, Roy F. Weston, Inc., Environmental Science and Engineering Consultants of Newton Square, Pa., has used batch pilot plant laboratory data in the design of activated sludge units. These consultants establish the fundamental reaction kinetics using the batch system and integrate this with mixing theory to predict the performance of a continuous process. They found from their experience that this method prevents design errors inherent in less fundamental approaches (3). Eckenfelder (4-179) describes a method of operating and evaluating a batch activated sludge pilot plant.

For studies evaluating activated sludge treatment systems, a soluble synthetic waste is often used. Eckhoff and Jenkens (5) and Washington et al. (6) used a synthetic sewage while Johnson and Schroepfer (7) and Milbury et al. (8) successfully used a dry skim milk solution. In skim milk there is a non-biodegradable fraction which remains in solution. In one study (9) there was 58 mg/l non-biodegradable COD per 1000 mg/l skim milk COD for the dry skim milk solids which were used. This portion will pass through the biological treatment system unoxidized.
Before activated sludge pilot plant units can be evaluated, it is necessary to acclimate the sludge. As waste is fed to the sludge seed, the biota undergoes a change, such that those organisms capable of growth in the particular waste increase in numbers while others die off. Eckenfelder (4-178) suggested using activated sludge from a sewage treatment plant and allowing a week for an active culture to develop. Milbury et al. (8) used activated sludge seed but waited one month for the culture to develop before conducting their studies.

In order to measure the organic concentration of the pilot plant effluent, Milbury et al. (8) centrifuged a 100 ml sample for 15 minutes at 700 times gravity and ran a COD test on the supernatant. They reported that the skim milk suspension they used as a substrate was not removed from solution using this centrifuging procedure.

**Aerobic Treatment**

In aerobic treatment, wastewater is mixed with large quantities of microorganisms and air. The microorganisms use the organic waste for food and use molecular oxygen to burn a portion of this food to \( \text{CO}_2 \) and \( \text{H}_2\text{O} \) for energy. Their growth is rapid; thus, a large portion of the organic matter is converted to new cells. This produces a large amount of biological sludge requiring subsequent disposal because this sludge is not stabilized but only changed in form (10).
Activated sludge is an aerobic system in which flocculated biological growths are mixed with wastewater and aerated. The biological solids are separated from the treated waste by settling in another tank. A portion of this settled biological sludge is returned to the aeration tank to be mixed with the incoming waste. The amount of sludge returned must be sufficient to utilize all the organic matter in the settled sewage. When too much food is present, some of this substrate will not be adsorbed and digested. This results in a poor effluent with bulking sludge which goes over the weirs, reducing the quality of the final effluent even further (11). Retaining excessive sludge concentrations, however, results in a poor nutritional condition of the sludge and brings a quick reduction in the activity of the sludge per unit weight (12).

With the activated sludge process, it is important to maintain a biological sludge of good physical quality. It must flocculate well and settle rapidly. The mixed liquor suspended solids (MLSS) represent all the suspended matter in the aeration chamber, inert material (inorganics) and organic biota, plus suspended organics which enter with the waste. The determination of volatile suspended solids (VSS) evaluates only the organic portion of the MLSS. For activated sludge treatment of normal domestic sewage, the VSS is generally about 80% of the total MLSS (2).

Activated sludge provides removal of the substrate in several steps. There is initial removal by adsorption of the food on the flocculated biological growths followed by oxidation of the adsorbed
substrate and then oxidation of the biological protoplasm itself (endogenous respiration). A long detention period is not needed for substrate adsorption, but appears to be necessary for assimilation and oxidation of the substrate to occur. Stabilization of the substrate has occurred when the bacteria have consumed most of the available food and have passed into the endogenous phase. When this occurs, synthesis of new bacteria is minimal. The remaining bacteria obtain energy through degradation of the sludge mass by metabolism of food stored within their cells or by metabolism of biota which has undergone lysis (13)(14)(15)(9).

**Anaerobic Treatment**

In an anaerobic system, the waste is mixed with large quantities of microorganisms without air. Under these conditions, the bacteria which grow are capable of using combined oxygen to convert organic matter to CO₂ and methane gas. This anaerobic conversion to methane yields very little energy to the microorganisms. Only a small portion of the waste is converted to new cells, resulting in low nutrient requirements and a low production of biological sludge (10).

For simplification, anaerobic treatment is considered to be a two-stage process. In the first stage, complex organics such as fats, carbohydrates, and proteins are hydrolyzed, fermented and biologically converted into simpler soluble organic compounds called organic or volatile acids, the most prevalent being acetic and
propionic acids. This breakdown is initiated by extra-cellular enzymes, which are secreted by a variety of different organisms. The function of these enzymes is to render the organic matter soluble so it can pass through the cell wall of the bacteria and be made available for biochemical reactions of metabolism. Although waste stabilization does not occur because methane is not produced during this step, the first stage is required so that the organic matter is in a form suitable for the second stage of treatment (10)(16).

During the second stage, the organic acids are converted into gaseous end products, carbon dioxide and methane, by a second group of bacteria, the methane formers. These bacteria are strictly anaerobic as they develop only in the absence of free oxygen and in the presence of a suitable reducing agent. There are several different groups of methane formers that have the capacity for methane fermentation. Each group is characterized by its ability to ferment only a relatively small number of organic compounds. The major methane formers, that is, those living on acetic and propionic acid, grow slowly (10)(16).

In anaerobic treatment, there are several factors which affect the performance of the unit, particularly that of the methane formers. Anaerobic conditions must be maintained. High temperatures (85-95°F) are needed for optimum operation. The pH in the anaerobic unit must be kept between 6.6 and 7.6. Below pH 6.2, acidic conditions
become toxic to the methane bacteria. High volatile acid concentrations resulting from unbalanced fermentation can depress the pH and interfere with normal anaerobic digestion. In addition, the slow rate of growth of methane formers creates two disadvantages for anaerobic treatment. A long time period is required to start the process and the process has a limited rate of adjustment to changing waste load, temperature, and other environmental conditions. The slow rate of growth and acid utilization by the methane formers represents the limiting step in anaerobic process design (10)(17)(16).

Thus, it appears that making use of the first stage of the anaerobic treatment process could result in an improvement in the treatability of the waste due to the breakdown of complex organics into simpler organic compounds during this stage. Eliminating the utilization of the second stage would eliminate two of the main disadvantages of anaerobic treatment: sensitive operation and long detention periods.

Anaerobic-Aerobic Treatment Systems

The most frequently used combination anaerobic-aerobic waste treatment system is a series operation in which an anaerobic lagoon is followed by an aerobic one. The Public Health Service made a survey of the use, design, and performance of industrial waste lagoons in the early 1960's (18). The results of this survey revealed that combination lagoon systems accounted for 30 out of 466 reported lagoon installations or 6.4%. These 30 installations
included 11 for meat and poultry wastes; 7 for canning wastes; 2 each for leather and paper wastes; 1 each for chemical, petroleum, dairy, sugar, machine operations, and corn wastes; and two for miscellaneous industrial wastes.

The Division of Public Health Engineering of the Louisiana Department of Health has been developing and promoting the use of anaerobic-aerobic pond systems for treatment of abattoir and packing-house wastes (19). From a study of these systems, the Health Department concluded that the combination pond systems are by far the least expensive units to build and operate; that they can be successfully used to treat packinghouse wastes, including blood and paunch manure; and that they are nuisance free except for slight odors during initial operation. Sollo (20), reporting on the operation of a waste treatment plant at Moultrie, Georgia, stated that the combination pond system for meat-packing wastes has the advantages of low investment, simplicity of operation, and production of an effluent that is stable without dilution.

At the Wilson and Company, Inc. meat packing plant at Albert Lea, Minnesota, an anaerobic contact system was used instead of an anaerobic pond in the anaerobic-aerobic system (21). Two conventional aerobic lagoons successfully handled an anaerobic process effluent containing an average BOD loading of 129 mg/l or 410#/day/acre. The aerobic effluent was less than 30 mg/l BOD. During one period when the stickwater evaporator in the plant was out of order,
BOD loadings in excess of 700#/day/acre were reported. The pond system removed 90% of the BOD during this period without nuisance conditions.

A treatment system designed for the MID Packing Plant at Luverne, Minnesota, consisted of two anaerobic lagoons operated in series followed by two aerobic lagoons (22). Even though the actual BOD loading on the first aerobic pond was 67.7#/day/acre versus the design loading of 25#/day/acre, no nuisance conditions developed. It was theorized that this success might be attributed to a change in the nature of the organic material in the anaerobic ponds making it more easily assimilated by the aerobic organisms.

Other adaptations of anaerobic-aerobic treatment have been used. Rand and Cooper (23) reported on the anaerobic-aerobic treatment of meat packing wastes using oxidation ponds preceded by anaerobic digestion rather than anaerobic lagoons. Wymore and White (24) reported on the treatment of slaughterhouse waste in Iowa Falls, Iowa, using two anaerobic lagoons in parallel followed by two aerated lagoons, operated in series, rather than conventional stabilization ponds.

Eye and Aldous (25) made a pilot plant study of the anaerobic-aerobic lagoon treatment of spent vegetable tan liquors. The detention time in the anaerobic lagoon was only 6.6 days. Although the overall BOD reduction in the system was about 80%, the BOD reduction in the anaerobic lagoon was slight. The authors commented,
"It appears, however, that the anaerobic degradation of the organics in the influent rendered them more readily available to the aerobic organisms."

A combination system for domestic sewage was designed by the Peoria Sanitary District of Peoria, Illinois (26). This system consisted of three units: an anaerobic contact process followed by a trickling filter and an oxidation pond. The anaerobic contact removed 34% of the BOD it received versus 29% for the trickling filter and 53% for the pond. Ordinarily an oxidation pond can be expected to treat 35 to 50# BOD/day/acre. This one, originally designed for secondary settling, accepted 235# BOD/day/acre without producing nuisance conditions. In a discussion of this system, Coulter and Ettinger (27) wrote, "It is hard to believe that aerobic conditions could be maintained winter and summer for two years at that loading of anaerobic sewage."

Oswald et al. (28) proposed the use of a landscaped anaerobic-aerobic pond system for waste treatment when developing new subdivisions where the lots are too small for satisfactory septic tank disposal and where sewerage is not yet provided to the area.

Harvey F. Ludwig (29) presented some pertinent information regarding anaerobic pretreatment. His consulting firm had investigated many oxidation pond installations producing satisfactory effluents but possessing odor problems. From their investigations, these consultants resolved a design principle for ponds receiving
any portion of industrial waste. That principle was: a preliminary anaerobic digestion chamber should be included in the design because, in effect, this guarantees that the following oxidation pond will function without the production of odors. In reference to these preliminary anaerobic units, Ludwig stated:

We found that the loading measured in terms of BOD is not too significant as far as the production of odor is concerned. Once this (waste) has been subjected to anaerobic treatment for 24 hours, something drastic has happened so that it has been conditioned to the point where that particular type of BOD is readily adapted to subsequent treatment in the pond. In our experience, it isn't the loading on the anaerobic chamber, it is the detention period involved.

These findings by Ludwig tend to confirm and explain observations by Steffen (21), Rollag and Dornbush (22), and Fall and Kraus (26) that BOD loadings for aerobic lagoons greatly in excess of the maximum commonly imposed did not produce nuisance conditions when anaerobic treatment preceded the aerobic lagoons.

The conclusions reached in a study made by King and Bann (30) were in disagreement with the findings mentioned above. This study was a laboratory pilot plant comparison of anaerobic-aerobic and aerobic lagoon treatment of a synthetic sewage. The separate aerobic unit was reported to be more efficient in the removal of COD than was the aerobic polishing unit of the anaerobic-aerobic system. The polishing unit treated a waste which was the effluent from an anaerobic cell. This anaerobically treated substrate being fed to the aerobic polishing unit was similar in strength to the untreated substrate being fed to the separate aerobic unit. It was
suggested that antibiotic substances produced with the anaerobic system may have retarded the aerobic organisms. The authors concluded:

It would appear then, that the organic material entering the polishing cell, although with similar characteristics to the organics entering the aerobic lagoon, was converted by anaerobic decomposition such that it was difficult to treat by aerobic means.

An examination of the data, however, revealed that the anaerobic cell had removed 62% of the BOD and COD of the waste received by the polishing cell. This would seem to indicate that the more easily treatable portion of the waste being fed to the aerobic polishing cell had already been removed in the anaerobic cell. Thus, the poor performance of the polishing cell, when compared to the aerobic cell, would not necessarily mean that anaerobic decomposition had made the waste more difficult to treat by aerobic means, as concluded by the authors.

Use of the Warburg Respirometer for Determining Waste Treatability

Hunter and Heukelekian (31) used the Warburg respirometer to determine the biodegradability of a waste; that is, the ability of a waste to be oxidized by microorganisms with the production of cell matter, energy and waste products. They reported that the "oxygen uptake" method of Warburg lends itself readily to biodegradability determinations. The authors were proposing the use of the Warburg to determine the biodegradability of new economically important
organic materials synthesized by man, many of which are partially or wholly resistant to microbial degradation.

The use of the Warburg respirometer is based on the principle that at a constant temperature and constant gas volume, any changes in the amount of gas can be measured by changes in its pressure. The apparatus consists basically of a flask of known volume connected to a manometer containing a liquid of known density. The flasks are shaken in a constant temperature environment. The Warburg respirometer is commonly used to measure the amount of oxygen utilized by the respiration of living cells. In most cells, the utilization of oxygen results in a release of CO₂. If these two gases (CO₂ and O₂) are the only ones involved, the respiration (oxygen uptake) can be measured by absorbing the liberated CO₂ in alkali and measuring the decrease in pressure via the manometer (32).

The organisms absorb oxygen that has been dissolved in the liquid. If the rate of oxygen uptake measured by the Warburg respirometer is to represent the uptake of oxygen by the cells, the controlling step in the reaction must not be the rate of oxygen diffusion into the liquid phase from the gas phase. The principle reason for shaking the flasks of the respirometer is to maintain a liquid phase saturated with oxygen (32-9)(33).

It is important also that the CO₂ is absorbed completely and very rapidly. Otherwise, the CO₂ pressure will not be zero and the reading on the manometer will not represent oxygen uptake (32-12).
The temperature of the flasks should be controlled within 0.05°C and corrections should be made for changes in the atmospheric pressure of the room (32-6).

It appears that many applications could be made of oxygen uptake data. The rate of oxygen uptake for a particular waste could be used to indicate the availability of the waste as food for the microorganisms, and the total oxygen uptake could be used to determine the amount of oxygen which would be required to stabilize the waste.
The purpose of this study was to compare the treatability of a non-treated dry skim milk to that of a similar milk which had been pretreated anaerobically. Throughout the study it was important to follow exacting testing procedures so that the results could be compared with a high degree of confidence. This was complicated by the variable nature of biological treatment. Because of this, duplicate samples were tested for all determinations made throughout the study. A definite methodology was developed for the operation of the pilot plant and for evaluating the treatability of the milk substrate.

Design and Operation of the Pilot Plant System

A batch-fed laboratory pilot plant was designed to evaluate the influence of anaerobic pretreatment on the treatability of a skim milk substrate. Figure 1 is a photograph showing the pilot plant apparatus.

A five-gallon glass carboy was used as the anaerobic pretreatment unit. The milk substrate was fed to this unit by connecting the tube from the bottom of the feed jar to the feed tube on the anaerobic unit. Air bubbles were removed from the tubing before feeding was begun. When the clamp on the sample tube was opened, milk flowed by gravity from the feed jar into the anaerobic unit. The milk was discharged from the feed tube about one inch above the bottom of the carboy forcing the pretreated milk out near
Figure 1. Photograph of the pilot plant system
the top of the unit via the variable depth sampling tube. A gasometer was initially utilized to measure the production of gaseous end products. Because the gas produced between daily feedings was not sufficient to be measured, the gasometer was disconnected. The anaerobic unit was operated at a theoretical detention time of five days.

Two 2.5 gallon narrow-mouth, plain glass reagent bottles served as the batch operated activated sludge units. One unit served as the aerobic treatment system while the second activated sludge unit served as the aerobic cell of the anaerobic-aerobic system. A five liter volume was maintained in these units. Each morning, after the mixed liquor was allowed to settle for one hour, two liters of supernatant were siphoned off and wasted. Then two liters of the appropriate milk substrate were added and aeration was resumed until the next daily feeding.

Development of an acclimated sludge culture in the pilot plant was begun in early March, 1968. Sludge from a sludge digester at the Brookings sewage treatment plant was used to seed the anaerobic unit. Trickling filter effluent was used to seed the two activated sludge units. During acclimation the strength of the untreated milk, that was being fed to the activated sludge unit of the aerobic system, was increased from 0.1 gram/liter to 1.0 gram/liter. The activated sludge unit for the anaerobic-aerobic system was acclimated by starting with a daily feeding of two liters of non-treated milk. An increasing proportion of anaerobically pretreated milk was fed
until at the end of three weeks the activated sludge unit was receiving pretreated milk only. Excess sludge was removed from both activated sludge units to maintain the suspended solids concentration near 2000 mg/l, thus keeping the sludge floc in an active condition.

It was desired that the COD of the milk substrate being fed to both the activated sludge units be approximately the same. A COD of approximately 1000 mg/l was suggested by Symons et al. (1). Mixing 1.00 grams of dry skim milk solids in one liter of hard tap water resulted in a COD of about 1050 mg/l. A slightly higher strength milk solution was fed to the anaerobic unit to allow for the expected small amount of COD reduction in this unit. Initially, 1.30 grams/liter was tried. Later testing indicated that 1.05 grams/liter was sufficient to produce an anaerobic effluent of approximately 1050 mg/l COD.

Development of Testing Technique

Chemical Oxygen Demand Versus Time. The main parameter selected to evaluate the effect of anaerobic pretreatment on the treatability of the skim milk substrate was COD, chemical oxygen demand. The evaluation of treatability was to be accomplished by measuring the COD remaining in the mixed liquor supernatant of the activated sludge units at selected time intervals after the milk substrate had been added. Two MLSS (mixed liquor suspended solids)
concentrations, 1000 mg/l and 2000 mg/l, were utilized in evaluating both systems. The procedure developed was as follows:

1. The milk substrate was obtained—either by reconstituting the dry milk solids at 1.00 gram/liter or by feeding the anaerobic unit with this non-treated milk and subsequently collecting a sample of the pretreated milk.

2. The suspended solids concentration in the activated sludge unit was measured after concentrating the activated sludge, by settling, to a two-liter volume.

3. The COD of the milk substrate, either non-treated or pretreated, and a centrifuged activated sludge sample was measured so that the initial COD of the mixed liquor could be calculated.

4. One liter of activated sludge was prepared, by dilution, at twice the desired MLSS concentration and placed in a one-gallon jug in a 20°C constant bath.

5. One liter of the milk substrate was added to the activated sludge and aeration was begun immediately.

6. Duplicate samples were collected for 24 hours, at increasing time intervals, after aeration was begun.

7. These samples were centrifuged immediately for three minutes. This removed the MLSS from solution so that the COD remaining in the supernatant could be determined.

8. The COD of this supernatant, which represented a settled activated sludge effluent, was measured.
9. The percent of COD which remained after the various aeration periods was computed and then plotted against elapsed time.

Data from preliminary COD testing indicated that the differences between the two systems were small. For both systems, the COD of the supernatant decreased within two hours from an initial value of over 500 mg/l to values between 20 and 40 mg/l. It became evident that this COD testing procedure was probably measuring primarily the rate of adsorption of the food by the activated sludge floc. An additional parameter to evaluate the rate and the amount of stabilization of the waste was deemed necessary.

**Oxygen Uptake Using the Warburg Respirometer.** The Warburg respirometer was selected as an additional method of evaluating treatability. A refrigerated Warburg apparatus made by Precision Scientific Company of Chicago was available. Large 130 ml respirometer flasks were used. The manometers were calibrated with mercury, while the flasks were calibrated with distilled water as described in *Manometric Techniques* (32-46,48). The flask constants were calculated to the 20.0 centimeter mark on the manometers using Brodie's manometer fluid (32-63).

The flasks were oscillated at 90 strokes per minute with an amplitude of 4 centimeters per stroke. This shake rate appeared to be satisfactory to maintain adequate oxygen diffusion into the liquid. Preliminary tests were run, however, at very high suspended solids
concentrations to determine if the rate of oxygen diffusion was limiting at the lower suspended solids concentrations used in the actual experimental tests. At the high MLSS concentrations, much higher uptakes were achieved, indicating that sufficient oxygen would be present for the tests.

Precautions suggested in Manometric Techniques (31-12,13) were taken to assure that the rate of CO₂ adsorption was not a limiting factor. Although 1% KOH (potassium hydroxide) is sufficient under most circumstances, two milliliters of 5% KOH were used. In addition, accordion folded pieces of analytical grade filter paper were placed in the alkali cup. These pieces projected about 5 mm above the side walls of the cup providing a large increase in the surface area of the alkali. The top of the cup was greased with silicone stopcock grease before inserting the papers to avoid "creeping over" of the toxic alkali.

The procedure described in Manometric Techniques (32-13) was used to set up the system for each set of tests. The volume of the three liquid constituents of the flask were as follows: substrate - 15 ml; seed - 10 ml; and KOH - 2 ml. The flasks were allowed to equilibrate with shaking for ten minutes to attain temperature equilibrium.

The oxygen uptake experiments were run at 20°C. The temperature was maintained by a thermo-regulator which was sensitive to within ± 0.005°F. Changes in the barometric pressure in the room and small changes in the temperature of the water bath were
corrected for by a thermobarometer consisting of a manometer attached to a flask containing 25 milliliters of water.

The COD of the substrate was approximately 1050 mg/l for both non-treated and pretreated milk. Oxygen uptake experiments were run at 1000 and 2000 mg/l MLSS concentrations for both systems. Duplicate samples were used for each set of oxygen uptake tests. Single flasks containing the seed only were used to evaluate the amount of oxygen that the microorganisms would have used without food, that is, endogenous respiration. Manometer readings were taken after 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 16.0, 24.0 hours elapsed time and were recorded to the nearest 0.1 centimeter.

**Laboratory Test Procedures**

The COD determinations were conducted according to procedure prescribed in Standard Methods for the Examination of Water and Wastewater (34-510). An alternate dilute method for COD utilizing 0.05 N potassium dichromate was used for low strength supernatant samples because of the inaccuracy involved using 0.25 N for these measurements.

Suspended solids determinations were based upon the use of a Millipore filter apparatus as described by Sawyer and McCarty (35-441). Glass fiber filters (Reeve's Angel-Grade No. 9340AH) of 4.2 centimeter diameter were used. Drying temperature for total suspended solids determinations was 103-105°. For volatile
suspended solids, the sample was ignited at 600°C. A 10 milliliter sample size was filtered.

Volatile acid determinations were made according to the direct titration procedure as described by DiLallo and Albertson (36). The titrants used were 0.1 N sulphuric acid and 0.05 N sodium hydroxide. Alkalinity was determined from this titration data.
RESULTS AND DISCUSSION

Because of early difficulty encountered in referring to the two treatment systems when discussing and comparing them, the following simplified terminology was developed. The abbreviation, AS, was used to refer to an activated sludge system. Likewise, A-AS referred to the activated sludge which was acclimated to non-treated milk while An-AS represented the activated sludge which was acclimated to the anaerobically pretreated milk.

Preliminary Studies

During and after acclimation of the pilot plant, considerable data were collected on the characteristics of the two substrates and activated sludges. The average pH of the various liquids was found to be as follows: non-treated milk, pH 8.0; pretreated milk, pH 6.2; A-AS, pH 8.3; and An-AS, pH 8.7. The low pH of the pretreated milk indicated that methane formers were not thriving in the anaerobic chamber. It would seem likely that a small shock loading effect would result from the pretreated milk with pH 6.2 being fed to the An-AS with pH 8.7, even though the sludge was acclimated to this substrate.

The volatile portion of the suspended solids of the A-AS and An-AS was 87 percent and 81 percent respectively. The values would indicate slightly more inert material in the An-AS. Both values were higher than the 80 percent common for activated sludge used to treat normal domestic sewage. The anaerobic pretreatment unit removed an average of 2.5 percent of the COD it received.
From the volatile acid testing, both alkalinity and volatile acids were calculated. The total alkalinity of the non-treated milk averaged 168 mg/l as calcium carbonate. The alkalinity of the hard tap water used to prepare the milk solution averaged 130 mg/l during this period. The pretreated milk had a much higher average alkalinity at 545 mg/l, indicating a much higher buffering capacity. The volatile acids of the non-treated milk averaged 35 mg/l as CaCO3 versus 447 mg/l for the pretreated milk. This more than 12-fold increase in volatile acids during pretreatment would suggest that the complex organics in the non-treated milk were indeed being broken down into organic (volatile) acids.

Rate of Substrate COD Removal

Figure 2 is a graphical representation of the percent of COD remaining in solution versus elapsed time after feeding for both treatment systems. Average data for both 1000 mg/l and 2000 mg/l suspended solids concentrations are plotted. The initial COD for these tests was about 550 mg/l. COD versus time data are found in Appendix I.

It can be seen from Figure 2 that the rate of removal of non-treated milk COD from the mixed liquor was more rapid, because the percent remaining was lower, than was the removal of pretreated milk COD. This was true at both suspended solids concentrations although the difference between the two treatment systems was more evident at 1000 mg/l. For 1000 mg/l MLSS concentration, only about 50 percent of the pretreated milk COD had been removed from solution 0.5 hours
Figure 2. Amount of substrate COD remaining in the supernatant solution versus time after feeding activated sludge with non-treated milk and pretreated milk.
after feeding, whereas about 80 percent of the non-treated milk COD had been removed. At 2000 mg/l MLSS the corresponding values were about 75 and 91 percent. It is important, however, that after two hours, the amount of substrate COD remaining in solution was essentially the same for both systems.

From these data, the possibility that a portion of the non-adsorbed milk substrate was also being removed during the three-minute centrifuging period was suspected even though this centrifuging was intended to remove only the flocculant suspended solids from the solution. To evaluate this possibility, both substrates were centrifuged for three minutes and the COD of the supernatant was measured. It was found that there was no change in COD of the non-treated milk while 11 percent of the COD of the pretreated milk was removed, suggesting that anaerobic bacteria were contributing to the initial COD of the pretreated milk. This information if applied to the COD data would increase the apparent difference in percent substrate remaining for the two treatment systems because the initial COD of the pretreated milk substrate would be lower than the COD used in the calculation of percent of initial COD remaining. Thus, correcting the data would make the values for percent pretreated milk COD remaining even higher in magnitude.

The more rapid rate of COD removal of non-treated milk substrate by the activated sludge floc might be explained on the basis of particle size. The non-treated milk was a fine colloidal suspension, whereas the organic matter in the pretreated milk was
partially volatile acids in solution. It would probably be easier for the AS to adsorb or flocculate the colloidal non-treated milk particles than the dissolved organics in the pretreated milk.

From the data presented for substrate COD remaining with time, it could be concluded that for aeration periods under two hours, the aerobic system could achieve higher COD removals than the anaerobic-aerobic system if the flocculant AS solids for both systems possessed good settling characteristics. If, however, the AS floc with the adsorbed substrate could not be settled after short aeration periods, the faster rate of adsorption of the non-treated milk in the activated sludge unit would probably not yield higher COD removals from the system.

Oxygen Uptake for Acclimated Systems

Three individual oxygen uptake tests, using duplicate samples for each test, were run on the non-treated milk substrate at both 1000 mg/l and 2000 mg/l suspended solids concentrations. Four individual runs were made on the pretreated milk. Because the initial COD of the substrate for both treatment systems varied slightly, the data were plotted in terms of oxygen uptake per milligram of initial COD. This put all the tests for both substrates on a common basis to allow logical comparison of the data. The pretreated milk was aerated for 10 minutes before it was added to the activated sludge for two of the oxygen uptake runs. For two other runs, the pretreated milk was not aerated. When these
results were calculated in terms of initial COD, the rate of oxygen uptake for the aerated and non-aerated pretreated milk was observed to be similar. Therefore, the data from the four runs were averaged together.

Figure 3 is a graphical representation of the average oxygen uptake data for both systems. The four curves representing the rates of endogenous respiration were plotted in terms of total µl of oxygen uptake. The oxygen uptake data calculated in terms of mg of initial COD are found in Appendix II.

From Figure 3 it can be shown that during the first four hours, the rate of oxygen uptake per mg of initial COD of the anaerobic-aerobic system utilizing pretreated milk was much greater than that of the aerobic system. At the end of two hours the total uptake per mg of initial COD at 1000 mg/1 MLSS concentration for the two systems was as follows: An-AS system = 16.1 µl O₂ and A-AS system = 6.9 µl O₂. Similar differences were observed at the end of four hours. This large initial difference in the rate of uptake would suggest that organic material in the pretreated milk was in the form that was readily available as food for the activated sludge bacteria, that is, it was more easily assimilated and oxidized.

After four hours, the rate of oxygen uptake was similar for the two treatment systems; therefore, at the end of 24 hours the total uptake per mg of initial COD of the anaerobic-aerobic system using pretreated milk was still much higher than that of the aerobic system. At 1000 mg/1 MLSS the 24-hour average oxygen
Figure 3. Oxygen uptake of the acclimated treatment systems
uptake for the pretreated milk was 44.0 µl while for the non-treated milk, the uptake was 29.3 µl. At 2000 mg/l MLSS the corresponding values were 47.9 and 35.7 µl.

The higher 24-hour oxygen uptake for the pretreated milk is very interesting because this oxygen uptake was based on an equal amount of chemically oxidizable organic (COD) material. Thus, after a skim milk substrate has been partially broken down by anaerobic pretreatment, it appears that a greater amount of oxygen is required for stabilization of the milk.

Because both the initial rate of oxygen utilization and the total oxygen required for waste stabilization were greater for pretreated milk, it would appear that the application of the pretreatment process may be limited. For waste treatment systems, such as activated sludge and aerated lagoons, which rely on mechanical oxygen transfer, the limiting criteria in economical design is frequently oxygen transfer capabilities. For stabilization ponds, however, oxygen is supplied by the photosynthetic activity of algal populations. The literature has revealed that stabilization ponds receiving anaerobically treated wastes have functioned properly at organic loading rates much higher than those used in standard practice. Anaerobic pretreatment might prove beneficial if it altered the waste through the breakdown of solids to release the nutrients for the support of algal populations which supply the oxygen in stabilization ponds.
Oxygen Uptake for Non-acclimated Systems

To gain further insight into the characteristics of the substrate and activated sludges, the non-treated milk was fed to the An-AS and the pretreated milk was fed to the A-AS. Oxygen uptake tests were run on these two non-acclimated systems. The uptake data for these tests are found in Appendix II.

Figure 4 contains four curves which represent the oxygen uptake of pretreated milk when fed to both An-AS and A-AS. This graph shows that there was an initial lag in oxygen uptake in the A-AS which was a non-acclimated sludge, indicating either a shock loading effect or an insufficient number of organisms in this environment. The low pH of the pretreated milk (pH 6.2) may have inhibited the non-acclimated bacteria which were accustomed to food with pH 8.0. After a period of adjustment, however, the rate of oxygen uptake with the non-acclimated sludge increased so that after 24 hours, the total uptake was nearly the same with both acclimated and non-acclimated sludges. The results were similar at both MLSS concentrations, although the period of adjustment was longer at the lower concentration (1000 mg/l).

Figure 5 shows the oxygen uptake for non-treated milk using both An-AS and A-AS. Again there was an initial lag in oxygen uptake for the non-acclimated system (An-AS) but the lag was not as pronounced as for the previous system. In this case, the non-acclimated sludge did not fully adjust quickly to the new substrate. At the end of 24 hours the total uptake for the acclimated system was still 22
Figure 4. Oxygen uptake of anaerobically pretreated milk using acclimated and non-acclimated activated sludges at two MLSS concentrations.
Figure 5. Oxygen uptake of non-treated milk using acclimated and non-acclimated activated sludges at two MLSS concentrations.
percent and 25 percent higher than for the non-acclimated system at 1000 mg/l and 2000 mg/l MLSS, respectively.

The initial lag in oxygen uptake for both non-acclimated systems may be explained in terms of basic principles of aerobic treatment. In the biological treatment of wastes, the ingestion of food by bacteria is accomplished by passage of the compounds through the cell walls. Bacterial cells require food in true solution, that is, organic wastes which are finely divided and available immediately to the bacteria as food. This would be the situation with the soluble organic acids present in the anaerobically pretreated milk. Larger bacteria are capable of ingesting more complex molecules. Larger particles of organic matter, such as the colloidal skim milk particles, must be chemically acted upon with the aid of enzymes outside the cell to bring the organic material into solution prior to being available to the bacteria as food (37-375). Likely these enzymes were not present in the An-AS because these aerobic bacteria had never had to bring about the initial breakdown of the milk particles. Instead, the initial breakdown of the milk particles was accomplished by the acid forming bacteria along with their enzymes in the anaerobic unit. The continued disparity between the total uptake of the non-acclimated and acclimated system shown in Figure 5 would indicate that these enzymes were not developed in the An-AS within the 24-hour period.
CONCLUSIONS

Based on the batch-fed laboratory pilot plant study, the following conclusions have been drawn.

1. The removal of the COD of the non-treated milk substrate, that is, the adsorption of the non-treated milk by the activated sludge, was more rapid than the adsorption of the COD of the anaerobically pretreated milk during the first two hours. If both activated sludges possessed good settling characteristics after short (less than two hours) aeration periods, the removal of substrate COD in the aerobic system would probably be greater than for the anaerobic-aerobic system.

2. During the first four hours, the rate of oxygen uptake per milligram of initial substrate COD was much higher for the pretreated milk than for the non-treated milk. During this period, the accumulated amount of oxygen uptake was nearly twice as much for the pretreated milk which would indicate that the pretreated milk was more treatable, that is, more readily available to the activated sludge bacteria as food, than was the non-treated milk.

3. The 24-hour oxygen uptake per milligram of initial substrate COD was higher for the pretreated milk than for the non-treated milk. Thus, the amount of oxygen required
for stabilization was probably greater for the pre­
treated milk.

4. The two preceding conclusions would indicate that anaerobic
pretreatment had rendered the milk more treatable, i.e.,
biologically oxidizable. Whether or not this improvement
in treatability would be considered economically advan­
tageous would depend on the method by which the oxygen
was supplied in the subsequent aerobic system. For
mechanical aeration systems where oxygen transfer is a
costly item, pretreatment may be of negative value. If,
however, pretreatment stimulates algal populations which
supply oxygen for the aerobic bacteria, as occurs in
stabilization ponds, pretreatment may prove to be
advantageous.
FUTURE STUDY

Additional studies are required to more completely evaluate the influence of anaerobic pretreatment on subsequent aerobic treatment. The effect of shorter detention periods in the anaerobic chamber should be evaluated. Additional pilot plant study is needed using stabilization ponds or the newly developed activated algae for the aerobic system rather than activated sludge. In addition, continuously fed, anaerobic-aerobic pilot plant systems should be used to evaluate anaerobic pretreatment. With this type of operation, the effect of high organic loadings on the production of odor could be more easily evaluated.


APPENDIXES
APPENDIX I

Amount of Substrate COD Remaining in Solution at Indicated Time after Feeding

1000 mg/l MLSS

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% COD mg/l Remaining</td>
<td>% COD mg/l Remaining</td>
<td>% COD mg/l Remaining</td>
<td>% COD mg/l Remaining</td>
<td>% COD mg/l Remaining</td>
</tr>
<tr>
<td>0 min</td>
<td>538</td>
<td>518</td>
<td>556</td>
<td>631</td>
<td>580</td>
</tr>
<tr>
<td>15 min</td>
<td>235</td>
<td>233</td>
<td>216</td>
<td>354</td>
<td>394</td>
</tr>
<tr>
<td>30 min</td>
<td>116</td>
<td>97</td>
<td>94</td>
<td>267</td>
<td>300</td>
</tr>
<tr>
<td>45 min</td>
<td>58</td>
<td>49</td>
<td>58</td>
<td>175</td>
<td>192</td>
</tr>
<tr>
<td>1.0 hr</td>
<td>44</td>
<td>33</td>
<td>37</td>
<td>112</td>
<td>184</td>
</tr>
<tr>
<td>1.5 hr</td>
<td>27</td>
<td>29</td>
<td>27</td>
<td>52</td>
<td>86</td>
</tr>
<tr>
<td>2.0 hr</td>
<td>25</td>
<td>27</td>
<td>20</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>4.0 hr</td>
<td>22</td>
<td>23</td>
<td>18</td>
<td>41</td>
<td>47</td>
</tr>
<tr>
<td>8.0 hr</td>
<td>20</td>
<td>21</td>
<td>16</td>
<td>32</td>
<td>39</td>
</tr>
<tr>
<td>24.0 hr</td>
<td>17</td>
<td>8</td>
<td>16</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>
# APPENDIX I

Amount of Substrate COD Remaining in Solution at Indicated Time after Feeding

2000 mg/l MLSS

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/l Remaining</td>
<td>% COD</td>
<td>mg/l Remaining</td>
<td>% COD</td>
</tr>
<tr>
<td>0 min</td>
<td>527</td>
<td>100.0</td>
<td>533</td>
<td>100.0</td>
</tr>
<tr>
<td>15 min</td>
<td>107</td>
<td>20.3</td>
<td>136</td>
<td>25.5</td>
</tr>
<tr>
<td>30 min</td>
<td>43</td>
<td>8.2</td>
<td>50</td>
<td>9.4</td>
</tr>
<tr>
<td>45 min</td>
<td>30</td>
<td>5.7</td>
<td>40</td>
<td>7.5</td>
</tr>
<tr>
<td>1.0 hr</td>
<td>27</td>
<td>5.1</td>
<td>39</td>
<td>7.3</td>
</tr>
<tr>
<td>1.5 hr</td>
<td>23</td>
<td>4.4</td>
<td>34</td>
<td>6.4</td>
</tr>
<tr>
<td>2.0 hr</td>
<td>23</td>
<td>4.4</td>
<td>33</td>
<td>6.1</td>
</tr>
<tr>
<td>4.0 hr</td>
<td>20</td>
<td>3.8</td>
<td>31</td>
<td>5.8</td>
</tr>
<tr>
<td>8.0 hr</td>
<td>19</td>
<td>3.7</td>
<td>24</td>
<td>4.5</td>
</tr>
</tbody>
</table>
APPENDIX II

Oxygen Uptake Data

Acclimated Treatment Systems

<table>
<thead>
<tr>
<th>Date</th>
<th>MLSS (mg/l)</th>
<th>Type System</th>
<th>Accumulative Oxygen Uptake (µl O₂/mg initial COD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Elapsed Time after Feeding (hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>6-18</td>
<td>1000</td>
<td>A*</td>
<td>4.8</td>
</tr>
<tr>
<td>6-20</td>
<td>1000</td>
<td>A</td>
<td>4.8</td>
</tr>
<tr>
<td>7-4</td>
<td>1000</td>
<td>A</td>
<td>4.5</td>
</tr>
<tr>
<td>6-18</td>
<td>1000</td>
<td>An**</td>
<td>6.0</td>
</tr>
<tr>
<td>7-1</td>
<td>1000</td>
<td>An</td>
<td>10.2</td>
</tr>
<tr>
<td>7-1</td>
<td>1000</td>
<td>An</td>
<td>10.0</td>
</tr>
<tr>
<td>7-1</td>
<td>1000</td>
<td>An</td>
<td>10.8</td>
</tr>
<tr>
<td>6-18</td>
<td>2000</td>
<td>An</td>
<td>5.8</td>
</tr>
<tr>
<td>6-20</td>
<td>2000</td>
<td>An</td>
<td>6.1</td>
</tr>
<tr>
<td>7-4</td>
<td>2000</td>
<td>An</td>
<td>6.7</td>
</tr>
<tr>
<td>6-18</td>
<td>2000</td>
<td>An</td>
<td>10.5</td>
</tr>
<tr>
<td>7-1</td>
<td>2000</td>
<td>An</td>
<td>9.8</td>
</tr>
</tbody>
</table>
APPENDIX II

Oxygen Uptake Data

Acclimated Treatment Systems

<table>
<thead>
<tr>
<th>Date</th>
<th>MLSS (mg/l)</th>
<th>Type System</th>
<th>Accumulative Oxygen Uptake (µl O₂/mg initial COD)</th>
<th>Elapsed Time after Feeding (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>7-1</td>
<td>2000</td>
<td>An</td>
<td></td>
<td>9.5</td>
</tr>
<tr>
<td>7-4</td>
<td>2000</td>
<td>An</td>
<td></td>
<td>10.8</td>
</tr>
</tbody>
</table>

*Aerobic Treatment System (Non-treated Milk)

**Anaerobic-Aerobic Treatment System (Pretreated Milk)*
## APPENDIX II

### Oxygen Uptake Data

#### Acclimated and Non-Acclimated Systems

<table>
<thead>
<tr>
<th>Date</th>
<th>MLSS (mg/1)</th>
<th>Milk Substrate</th>
<th>AS Seed</th>
<th>Accumulative Oxygen Uptake (µl O₂/mg initial COD)</th>
<th>Elapsed Time after Feeding (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7-20</td>
<td>1000</td>
<td>A*</td>
<td>A-AS</td>
<td>4.6</td>
<td>6.6</td>
</tr>
<tr>
<td>7-22</td>
<td>1000</td>
<td>A</td>
<td>A-AS</td>
<td>3.5</td>
<td>6.7</td>
</tr>
<tr>
<td>7-20</td>
<td>1000</td>
<td>An**</td>
<td>An-AS</td>
<td>8.3</td>
<td>16.1</td>
</tr>
<tr>
<td>7-22</td>
<td>1000</td>
<td>An</td>
<td>An-AS</td>
<td>8.4</td>
<td>15.7</td>
</tr>
<tr>
<td>7-20</td>
<td>2000</td>
<td>A</td>
<td>A-AS</td>
<td>6.9</td>
<td>9.6</td>
</tr>
<tr>
<td>7-22</td>
<td>2000</td>
<td>A</td>
<td>A-AS</td>
<td>5.8</td>
<td>9.7</td>
</tr>
<tr>
<td>7-20</td>
<td>2000</td>
<td>An</td>
<td>An-AS</td>
<td>11.2</td>
<td>18.3</td>
</tr>
<tr>
<td>7-22</td>
<td>2000</td>
<td>An</td>
<td>An-AS</td>
<td>11.2</td>
<td>17.6</td>
</tr>
<tr>
<td>7-20</td>
<td>1000</td>
<td>An</td>
<td>A-AS</td>
<td>3.0</td>
<td>4.3</td>
</tr>
<tr>
<td>7-22</td>
<td>1000</td>
<td>An</td>
<td>A-AS</td>
<td>1.9</td>
<td>2.1</td>
</tr>
</tbody>
</table>
### APPENDIX II

**Oxygen Uptake Data**

*Acclimated and Non-Acclimated Systems*

<table>
<thead>
<tr>
<th>Date</th>
<th>MLSS (mg/l)</th>
<th>Milk Substrate</th>
<th>AS Seed</th>
<th>Accumulative Oxygen Uptake (µl O₂/mg initial COD)</th>
<th>Elapsed Time after Feeding (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>7-20</td>
<td>1000</td>
<td>A</td>
<td>An-AS</td>
<td></td>
<td>1.6</td>
</tr>
<tr>
<td>7-22</td>
<td>1000</td>
<td>A</td>
<td>An-AS</td>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td>7-20</td>
<td>2000</td>
<td>An</td>
<td>A-AS</td>
<td></td>
<td>3.7</td>
</tr>
<tr>
<td>7-22</td>
<td>2000</td>
<td>An</td>
<td>A-AS</td>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>7-20</td>
<td>2000</td>
<td>A</td>
<td>An-AS</td>
<td></td>
<td>2.7</td>
</tr>
<tr>
<td>7-22</td>
<td>2000</td>
<td>A</td>
<td>An-AS</td>
<td></td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Non-treated Milk Substrate  **Pretreated Milk Substrate*