# CHEMICAL AND MICROBIOLOGICAL CONSEQUENCES OF ANAEROBIC DIGESTION OF LIVESTOCK MANURE, A LITERATURE REVIEW

Elizabeth A. Casman, Ph.D.

ICPRB Report # 96-6
October, 1996

This publication has been prepared by the staff of the Interstate Commission on the Potomac River Basin. Funds for this project were provided by the U.S.E.P.A. Office of Air and Radiation and the Interstate Commission on the Potomac River Basin. The opinions expressed are those of the author and should not be construed as representing the opinions or policy of the United States government or any of its agencies, the several states, or the Commissioners of the Interstate Commission on the Potomac River Basin

# EXECUTIVE SUMMARY

This report summarizes the literature on the effects of anaerobic digesters and lagoons on components of animal manure slurry that are potential water pollutants: plant nutrients, oxygen demanding substances, trace metals, and pathogenic microorganisms. The fate of these constituents in pre-digestion separated solids, liquid effluent, and settled sludge is discussed.

It has long been known that anaerobic digestion reduces oxygen demanding substances in animal manure slurry by up to 90%. This has an immediate water quality impact in cases where appreciable amounts of slurry reach water bodies. Other slurry components of particular water quality significance are nitrogen and phosphorus compounds, because of their role in promoting eutrophication. The dominant species of nitrogen in anaerobically digested slurry is ammonium. Through pH manipulation and temperature control, anaerobic digesters can be operated to either conserve ammonium-nitrogen in the effluent or to gas it off. (Elevated pH and/or temperature favor the existence of the gaseous ammonia over the non-volatile ammonium ion.) Phosphorus compounds and potassium are partitioned between sludge and supernatant, but are totally conserved. Both ammonium-ammonia transformation and the partition of nutrients are accelerated with increasing temperatures. This is evident even within the range of ambient temperatures at which lagoons operate.

Pathogenic organisms demonstrate a wide variety of sensitivities to anaerobic digestion in animal waste. Temperature, retention time, and organism type are key factors in predicting the ability of a digester to inactivate pathogens.

At ambient temperatures, retention times on the order of months or years would be required to inactivate many species of bacteria, viruses, and parasites. Anaerobic lagoons with residence times in excess of 300 days will demonstrate substantial reductions in many (but not all) pathogenic species. The extended retention times typical of lagoons designed for methane recovery are likely to produce effluents of significantly improved microbiological quality.

Mesophilic or thermophilic anaerobic digestion of slurry causes an accelerated inactivation of vegetative, non-spore-forming bacteria when compared with inactivation during anaerobic storage at lower temperatures. Decimal reduction times (the time required to reduce the concentration of organisms by 90%) of these bacteria are of the order of hours or minutes at thermophilic temperatures (46° to 66°C) , days at mesophilic temperatures (27° to 46°C) and weeks or months at psychrophilic temperatures (2° to 27°C).

Spore forming bacterial species, whether anaerobic like *Clostridium* or aerobic like *Bacillus,* and certain life stages of some helminth parasites seem to be quite resistant to anaerobic digestion at ambient temperatures or even at elevated temperatures. Some viruses can withstand 50 days digestion at 50°C. However, thermophilic as well as mesophilic digestion coupled with thermophilic pre-treatment will result in a sufficient reduction of both vegetative pathogenic

bacteria and intestinal parasites occurring in concentrations usually found in animal waste to allow for unrestricted use of the effluent in agriculture. This cannot be said for brief mesophilic digestion used alone.

This report contains an exhaustive tabulation of the decimal reduction times for a variety of pathogens under anaerobic digestion in animal slurry at various temperatures. These statistics can be used to size a lagoon or a digester -- to determine the retention time necessary to clear or reduce initial pathogen populations to acceptable levels. For example, to reduce an initial population of $10^7$ total coliforms per milliliter to $10^2$ per milliliter an anaerobic lagoon must be large enough to provide a retention time of 390 days, which is 6 times the decimal reduction time of 65 days at 6° to 15°C (see Table 22). A digester operating at 35°C should provide a retention time of 33 days ( 6 times 5.5 days, same table). To reduce the concentration of $10^4$ *Giardia* cysts per milliliter to less than one per milliliter, it would take about 150 days at 15°C, that is, 5 times 30 days (Table 39).

If anaerobic digesters and lagoons are designed with pathogen reduction requirements in mind, their ability to eliminate certain pathogens from animal slurry has potential public health benefits where concentrated livestock production facilities are located near drinking water sources. Similarly, if sized for pathogen reduction, anaerobic digesters have the potential to reduce the cycle of re-infection of livestock via land disposal of contaminated waste.

# Table of Contents

# List of Tables

# Chemical and Microbiological Consequences of Anaerobic Digestion of Livestock Manure, a Literature Review

## INTRODUCTORY REMARKS

### PURPOSE OF REPORT

This report is intended to serve as a reference summarizing the effects of anaerobic digesters and lagoons on constituents of livestock manures considered to be potential sources of water pollution, specifically human and animal pathogens, chemical and biochemical oxygen demanding carbon compounds, and the plant nutrients, nitrogen, phosphorus, and potassium. Since the literature is sparse on the subject of water quality effects of methane recovery systems, information primarily describing effluent and sludge quality from anaerobic digesters and lagoons is assembled. The benefits are inferred from the difference between the raw waste and the stabilized end products. Issues affecting the end product quality are discussed as well.

A second purpose of this report is to provide detailed information useful in modeling studies that could support claims of possible "collateral benefits" of installing methane recovery systems (MRS) on livestock producing farms.

Collateral benefits for MRS are those benefits that will accrue in addition to the generation of methane and its use as energy. Primarily they fall into four categories, fertilizer savings, water pollution abatement, odor control, and disease control.

The amount of fertilizer savings realized depends on how manure was disposed prior to the installation of the methane recovery system. There may be in fact no fertilizer savings, though it is conceivable that a MRS which provided an enhanced storage capacity could give the farmer more flexibility in the timing of the effluent and sludge applications, which could translate into fertilizer savings.

Likewise, the degree of water pollution abatement achieved is also relative to the degree of water pollution experienced prior to installation of a methane recovery system. A well designed MRS can be used to clean up a messy barnyard, and may significantly improve the storm water runoff quality. Furthermore, the timing of waste application to fields can be improved to minimize contaminated runoff from cropland and pasture. A Methane Recovery System can be used to conserve nutrients or to get rid of them. Farms which generate significantly more nitrogen than should be applied to the available fields can operate a MRS to strip out ammonia-nitrogen from the waste, or to blow it off into the atmosphere.

A methane recovery system can be managed to reduce odor problems, though some unpleasant odors are inevitable when parts of the system go aerobic, such as when a lagoon needs to be emptied for the removal of accumulated sludge. Fly problems are also mitigated by the installation

of MRS.

Disease can be transmitted to humans and other animals via livestock manure when the manure is released into the environment by spreading on fields and pastures and becomes available for transport by surface runoff. Table 1 lists a sampling of major pathogens found in animal manures.

Table 1. Some diseases and parasites transmittable to humans from animal manure #50

| **DISEASE** | **RESPONSIBLE ORGANISM** |
|---|---|
| **Bacteria** (0.5 - 2.0 μm diameter) | |
| Salmonellosis | *Salmonella* sp. |
| Leptospirosis | *Leptospira pomona* |
| Anthrax | *Bacillus anthracis* |
| Tuberculosis | *Mycobacterium tuberculosis, Mycobacterium avium* |
| Johnes disease | *Mycobacterium paratuberculosis* |
| Brucellosis | *Brucella abortus, Brucella melitensis, Brucella suis* |
| Listeriosis | *Listeria monocytogenes* |
| Tetanus | *Clostridium tetani* |
| Tularemia | *Pasteurella tularensis* |
| Erysipelas | *Erysipelothrix rhusiopathiae* |
| Colibaciliosis | *Escherichia coli* (some serotypes) |
| Coliform mastitis-metritis | *Escherichia coli* (some serotypes) |
| **Rickettsia** | |
| Q fever | *Coxiella burneti* |
| **Viruses** (0.025 - 0.1 μm diameter) | |
| New Castle | virus |
| Hog Cholera | virus |
| Foot and Mouth | virus |
| Psittacosis | virus |
| **Fungi** | |
| Coccidioidomycosis | *Coccidioides immitus* |
| Histoplasmosis | *Histoplasma capsulatum* |
| Ringworm | Various *microsporum* and *trichophyton* |
| **Protozoa** (7.0 - 50 μm diameter) | |
| Coccidiosis | *Eimeria* sp. |
| Balantidiasis | *Balatidium coli* |
| Giardiasis | *Giardia lamblia* |
| Cryptospridiasis | *Cryptosporidium* sp. |
| Toxoplasmosis | *Toxoplasma* sp. |
| **Parasites/Metazoa** (>50μm diameter) | |
| Ascariasis | *Ascaris lumbricoides* |
| Sarcocystiasis | *Sarcosystis* sp. |

Many of the microorganisms pathogenic to both animals and man are transmitted via the fecal-oral route. According to Stelma, *et al.*, #13, most of these pathogens could conceivably be transmitted through a shellfish vector. Bacteria potentially transmitted from animal to man via shellfish include most of the salmonellae, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Escherichia coli* O157:H7, *Campylobacter jejuni* and *Listeria monocytogenes*. The protozoa most likely to be transmitted in this way are *Giardia lamblia* and *Cryptosporidium* spp. Because the enteric viruses are highly species specific, they are not likely to be transmitted from animals to humans. A possible exception are the rotaviruses, which have been isolated from a variety of animals including cattle, sheep, and goat. Cross-species transmissions of rotaviruses is recognized: human to non-human and *vice versa*. Rotaviruses are also environmentally stable in fresh and marine waters.

Reservoirs for *Yersinia enterocolitica* include most domestic mammals, particularly swine. Reservoirs for *Yersinia pseudotuberculosis* include a wide variety of domestic mammals and fowl. The recently discovered hemorrhagic colitis strains of *Escherichia coli* belonging to the O157:h7 serotype are usually acquired after ingestion of either rare ground beef or raw milk. They have also been shown to have been transmitted via water. These verotoxin producing strains have been isolated from calves and pigs with enteric diseases and from retail pork and lamb. Reservoirs of *Campylobacter jejuni* include cattle, sheep, swine, dogs, and domestic poultry.

Table 2 lists the number of waterborne disease outbreaks reported to the Center for Disease Control from 1966 through 1984. The shaded entries are those that probably had no agricultural link.

Table 2. Etiology of Waterborne Disease Outbreaks in the U. S. 1966 - 1984 #107

| | **1966-1970** | **1971-1975** | **1976-1980** | **1981-1984** |
|---|---|---|---|---|
| Amoebiasis | 3 | | | 1 |
| Campylobacterosis | | | 3 | 6 |
| Chemical poisoning | 4 | 13 | 25 | 11 |
| Cholera | | | | 1 |
| Cryptosporidium | | | | 1 |
| Gastroenteritis, unknown origin | 21 | 63 | 114 | 59 |
| Gastroenteritis, viral | | | 10 | 8 |
| Giardiasis | 2 | 13 | 26 | 48 |
| Hepatitis A | 19 | 14 | 2 | 7 |
| Salmonellosis | 4 | 2 | 6 | 2 |
| Shigellosis | 14 | 14 | 10 | 7 |
| Toxigenic *Escherichia coli* AGI | | 1 | | |
| Typhoid fever | 4 | 4 | | |
| Yersiniosis | | | | 1 |

Many of the diseases endemic to livestock operations are transmitted to the herds by the fecal oral

route. For example, Paratuberculosis in ruminants, caused by infection with *Mycobacterium paratuberculosis*, is mainly acquired by oral ingestion of contaminated water or feed. Application of contaminated slurry or manure to pasture may therefore contribute to the spread of the disease. #66

Anaerobic digestion is known to be able to deactivate many pathogens present in animal waste. The lethality is temperature and pH sensitive, with differing levels of deactivation achieved under different conditions. Also the mixing characteristics and detention time of the reactor influence the survival of pathogens in the effluent.

It is conceivable that anaerobic digesters operated at high temperatures could be used to decontaminate large amounts of animal wastes. The tables found later in this text contain the deactivation rates of a wide variety of pathogenic organisms under anaerobic digestion in manure. One can find the organism of interest and determine the temperature and duration of digestion necessary to reduce the population to acceptable levels.

Before the microbiological aspects of anaerobic digestion are considered, the effects of anaerobic digestion of manure in lagoons, digesters, and laboratory glassware on nitrogen, phosphorus, potassium, oxygen demanding carbon compounds, and trace metals are presented.

## ANAEROBIC DIGESTION: INFLUENT AND EFFLUENT CHEMISTRY

### *Lagoons and Ponds*

Anaerobic lagoons provide biological treatment and long-term storage. They usually are smaller than aerobic lagoons and decompose more organic matter per unit volume, but aerobic lagoons provide a higher degree of treatment with less odor production. #69

Lagoons can be covered, partially covered, or uncovered; earthen, clay lined, or concrete. They operate at ambient temperature, are usually not intentionally mixed, and have longer detention times than digesters. They have long been used as to treat concentrated organic wastes. Their end products are in the forms of liquid supernate, semi-solid sludge, and waste gases.

Large reductions in $BOD_5$ and COD can be expected in effluents from anaerobic lagoons, especially when the detention time is long. #67 Organic constituents of swine waste are removed in anaerobic lagoon systems primarily by microbial stabilization and subsequent release of methane and carbon dioxide. Net sludge settling also accounts for about 30% losses of both total organic carbon (TOC) and chemical oxygen demand (COD). #57 As with organic nitrogen compounds, some surface losses due to volatilization of short chain molecules occurs. BOD reductions in anaerobic lagoons can be a respectable 40-90% (Table 3); however, due to the high

Table 3. Percent Reduction of Nutrients, Solids, Oxygen Demanding Substances Achieved in Anaerobic Lagoons

| | | percent reduction | animal type | reference |
|---|---|---|---|---|
| BOD | anaerobic lagoon | 40 - 90% | -- | #67 |
| BOD | anaerobic lagoon | 80 - 90% | swine | #57 |
| BOD | anaerobic lagoon | 88% | dairy | #93 |
| | | | | |
| COD | anaerobic lagoon | 89 - 90% | swine | #70 |
| COD | anaerobic lagoon | 75 - 90% | swine | #57 |
| COD | covered lagoon | 70% | dairy | #74 |
| | | | | |
| TS | anaerobic lagoon | 50% | dairy | #93 |
| | | | | |
| VS | anaerobic lagoon | 83% | dairy | #92 |
| VS | 2-cell anaerobic lagoon with recycle | 76 - 84% | dairy | #97 |
| VS | average of 5 anaerobic lagoons | 55% | dairy | #101 |
| VS | average of 8 anaerobic lagoons | 81% | swine | #101 |
| VS | average of 7 anaerobic lagoons | 80% | poultry | #101 |
| | | | | |
| TN | bottom loading pit | 0 - 20% | swine | #76 |
| TN | ponds and pits | 8 - 50% | dairy | #52 |
| TN | covered lagoon | 30% | dairy | #74 |
| TN | covered lagoon | 20 - 30% | swine | #76 |
| TN | uncovered lagoon | 30 - 40% | swine | #76 |
| TN | anaerobic lagoon | 65 - 80% | beef and dairy | #50 |
| TN | anaerobic lagoon | 70 - 80% | swine and poultry | #50 |
| TN | anaerobic lagoon | 45 - 65% | swine | #52 |
| TN | anaerobic slurry | 60% | poultry | #52 |
| TN | anaerobic lagoon plus surface spreading | 55 - 80% | swine | #76 |
| TN | deep pit plus liquid spreading | 35 - 65% | -- | #52 |
| TN | anaerobic lagoon plus liquid spreading | 60 - 80% | -- | #52 |
| TN | anaerobic lagoon | 70 - 90% | swine | #69 |
| TN | 2-cell anaerobic lagoon with recycle | 73 - 85% | dairy | #97 |
| | | | | |
| org.N | 2-cell anaerobic lagoon with recycle | 58 - 63% | dairy | #97 |
| | | | | |
| TP | covered lagoon | 30% | dairy | #74 |
| TP | anaerobic lagoon | 50 - 65% | beef and dairy | #50 |
| TP | anaerobic lagoon | 50 - 65% | swine and poultry | #50 |
| TP | anaerobic lagoon plus liquid spreading | 30 - 50% | -- | #52 |
| | | | | |
| o-$PO_4$ | anaerobic lagoon | 32% | dairy | #93 |
| | | | | |
| K | anaerobic lagoon | 25% | swine | #57 |
| K | anaerobic lagoon | 35 - 50% | beef and dairy | #50 |
| K | anaerobic lagoon | 40 - 50% | poultry and swine | #50 |

loading rates, the effluent from anaerobic lagoons is unlikely to be suitable for discharge to surface waters, even with high BOD removals (#67). The effluent will contain significant concentrations of oxygen demanding material, solids, and nutrients. The quality of the effluent is decreased during start-up operations and when low temperatures exist in the lagoon.

The settling of particulate phosphorus and potassium forms accounts for the observed losses of 25% to 65% of influent concentrations. The salts in swine waste are removed in an anaerobic lagoon by chelation-settling or precipitation. #57 Losses of various cations can range from less than 10% to over 90%. #63 Settling solids prior to discharging swine wastes to a lagoon significantly decreases phosphorus levels in the lagoon.

The TN story is more complex. A wide range of nitrogen losses are recorded, from zero to 80%. These studies are really not contradictory, but require a bit of interpretation. The study reporting 0% reduction concerned a system where effluent entered in the bottom, and settled solids were not considered "lost". In the other studies there is an immediate loss of 25 to 40% of the N to the settled solids. Detention time has a non-intuitive influence on nitrogen loss. One might expect that nitrogen loss would increase monotonically with detention time due to settling and volatilization, however, Overcash and Humenik #57 reported that for swine waste, initial nitrogen loss due to settling is approximately 40% of the input, but after sludge biological activity and transfer to the supernatant, the net amount of nitrogen loss to the sludge is about 25%.

Actual nitrogen loss from anaerobic lagoons (assuming that sludge bound nitrogen is not lost but stored) is attributed primarily to surface volatilization of ammonia (#57), which can be as little as 5% or 10% of the total N or could be in excess of 50%. Lorimer *et al.* #63 observed that nitrogen concentrations decreased by as much as 50% during the summer period in a deep pit holding beef manure (from a spring high of 10000 mg/l TN to a summer low of 5,000 mg/l), and attributed the loss to ammonia volatilization. The magnitude of ammonia losses through volatilization is temperature and pH dependent, with the largest losses occurring at summer temperatures and pHs above 7.0.

Nitrogen loss from anaerobic lagoons occurs primarily as surface volatilization of ammonia. #57 In the case of swine waste, because of the high fraction of ammonia in the raw waste (50% initially) and lagoon supernatant, the conversion of organic nitrogen to ammonia does not appear to be a limiting step in nitrogen loss. Secondary nitrogen losses can be attributed to the net solid material remaining in the lagoon sludge (around 25%). A tertiary nitrogen loss mechanism is the volatilization of short chain organic compounds such as amines. While this pathway probably is small as a nitrogen loss, the impact as an odor or nuisance problem is well documented.

Overcash and Humenik #57 claimed that the anaerobic lagoon is a flexible treatment process which can be designed or operated to yield a variety of effluent concentrations. With respect to nitrogen, a lagoon can be used for nitrogen conservation or for dissipating the majority of the input nitrogen. These authors generalized 12 data sets and derived regression equations for

Table 4. Beef and Dairy Anaerobic Lagoon Effluent, Nutrient Concentrations

| | Operation type | Concentration | Reference |
|---|---|---|---|
| TS | Beef deep pit | 11.7% | #63 |
| TS | Dairy anaerobic lagoon | 0.25% | #50 |
| TS | Dairy covered lagoon | 0.38% | #74 |
| TS | Beef anaerobic lagoon | 0.478% - 0.59% | #56 |
| TS | Beef anaerobic lagoon | 0.412% | #100 |
| | | | |
| COD | Dairy anaerobic lagoon | 1506 mg/l | #50 |
| COD | Dairy covered lagoon | 3440 mg/l | #74 |
| COD | Beef anaerobic lagoon | 4700 - 5500 mg/l | #56 |
| COD | Beef anaerobic lagoon | 2450 mg/l | #100 |
| | | | |
| VS | Dairy anaerobic lagoon | 1104 mg/l | #50 |
| VS | Dairy covered lagoon | 1976 mg/l | #74 |
| VS | Beef anaerobic lagoon | 2870 - 3710 mg/l | #56 |
| | | | |
| BOD | Dairy anaerobic lagoon | 200 - 1200 mg/l | #50 |
| BOD | Beef anaerobic lagoon | 200 - 2500 mg/l | #50 |
| BOD | Dairy anaerobic lagoon | 352 mg/l | #50 |
| BOD | Beef anaerobic lagoon | 1340 - 1420 mg/l | #56 |
| | | | |
| TN | Beef deep pit | 6992 mg/l | #63 |
| TN | Dairy anaerobic lagoon | 201 mg/l | #50 |
| TN | Dairy covered lagoon | 511 mg/l | #74 |
| TN | Beef anaerobic lagoon | 360-500 mg/l | #56 |
| TN | Beef anaerobic lagoon | 274 mg/l | #100 |
| | | | |
| $NH_4$-N | Beef anaerobic lagoon | 162 mg/l | #100 |
| | | | |
| P | Beef deep pit | 1230 mg/l | #63 |
| P | Dairy covered lagoon | 125 mg/l | #74 |
| P | Dairy anaerobic lagoon | 58 mg/l | #50 |
| P | Beef anaerobic lagoon | 73 mg/l | #100 |
| | | | |
| o-$PO_4$ | Beef anaerobic lagoon | 61 mg/l | #100 |
| | | | |
| K | Beef anaerobic lagoon | 562 mg/l | #100 |
| K | Beef deep pit | 1902 mg/l | #63 |
| K | Dairy anaerobic lagoon | 502 mg/l | #50 |

Table 5. Swine and Poultry Anaerobic Lagoon Effluent, Nutrient Concentrations

| | **Operation type** | **Concentration** | **Reference** |
|---|---|---|---|
| TS | Swine anaerobic lagoon | 0.5% | #63 |
| TS | Swine anaerobic lagoon | 0.13% | #70 |
| TS | Swine anaerobic lagoon | 0.25% | #50 |
| TS | Swine anaerobic lagoon | 3091 mg/l | #98 |
| TS | 5 Swine anaerobic lagoons with recycle | 0.173 - 0.521% | #100 |
| TS | 2 Poultry anaerobic lagoons | 0.437 - 0.534% | #100 |
| | | | |
| COD | Swine anaerobic lagoon | 880 mg/l | #70 |
| COD | Swine anaerobic lagoon | 1205 mg/l | #50 |
| COD | Swine anaerobic lagoon | 940 - 3850 mg/l | #56 |
| COD | Poultry anaerobic lagoon | 590 - 2550 mg/l | #56 |
| COD | 5 Swine anaerobic lagoons with recycle | 970 - 2371 mg/l | #100 |
| COD | 2 Poultry anaerobic lagoons | 1882 - 1972 mg/l | #100 |
| | | | |
| VS | Swine anaerobic lagoon | 580 mg/l | #70 |
| VS | Swine anaerobic lagoon | 850 - 2330 mg/l | #56 |
| VS | Swine anaerobic lagoon | 1329 mg/l | #98 |
| | | | |
| BOD | Swine anaerobic lagoon | 300 - 3600 mg/l | #50 |
| BOD | Poultry anaerobic *lagoon* | 600 - 3800 mg/l | #50 |
| BOD | Poultry anaerobic lagoon | 320 - 1350 mg/l | #56 |
| BOD | Swine anaerobic lagoon | 401 mg/l | #50 |
| | | | |
| TN | Swine anaerobic lagoon | 804 mg/l | #63 |
| TN | Swine anaerobic lagoon | 300 mg/l | #70 |
| TN | Swine anaerobic lagoon | 351 mg/l | #50 |
| TN | Poultry anaerobic lagoon | 113 - 290 mg/l | #56 |
| TN | Swine anaerobic lagoon | 439 mg/l | #98 |
| TN | 5 Swine anaerobic lagoons with recycle | 391 - 827 mg/l | #100 |
| TN | 2 Poultry anaerobic lagoons | 489 - 800 mg/l | #100 |
| | | | |
| $NH_4$-N | 5 Swine anaerobic lagoons with recycle | 336 - 778 mg/l | #100 |
| $NH_4$-N | 2 Poultry anaerobic lagoons | 403 - 737 mg/l | #100 |
| | | | |
| P | Swine anaerobic lagoon | 73 mg/l | #63 |
| P | Swine anaerobic lagoon | 76 mg/l | #50 |
| P | Swine anaerobic lagoon | 70 mg/l | #98 |
| P | 5 Swine anaerobic lagoons with recycle | 69 -175 mg/l | #100 |
| P | 2 Poultry anaerobic lagoons | 53 - 112 mg/l | #100 |
| | | | |
| o -$PO_4$ | 5 Swine anaerobic lagoons with recycle | 56 - 164 mg/l | #100 |
| o -$PO_4$ | 2 Poultry anaerobic lagoons | 42 - 102 mg/l | #100 |
| | | | |
| K | Swine anaerobic lagoon | 416 mg/l | #63 |
| K | Swine anaerobic lagoon | 381 mg/l | #50 |
| K | Swine anaerobic lagoon | 366 mg/l | #98 |
| K | 5 Swine anaerobic lagoons with recycle | 285 - 1129 mg/l | #100 |
| K | 2 Poultry anaerobic lagoons | 936 - 1338 mg/l | #100 |

effluent quality as a function of loading rate of volatile solids. The data spread was enormous and the regressions were pretty weak. Regardless, their results are summarized in Table 6.

Table 6. Effluent TKN, COD and $BOD_5$ as a percent of influent for different lagoon loading rates, anaerobic swine lagoons, literature synthesis #57 and #97

| **Loading rate, kg VS/week·m³** | **COD remaining in effluent** | **$BOD_5$ remaining in effluent** | **TKN remaining in effluent** |
|---|---|---|---|
| 1.08 | 40 - 60% | 25 - 40% | 70% |
| 0.55 | 10 - 25% | 10 - 20% | 40% |
| 0.13 | 2 - 10% | 3 - 6% | 15 % |

Nitrogen in fresh manure is mostly in the organic form (60 to 80% of the total N). In an anaerobic lagoon, the organic fraction is typically 20 to 30 percent of total N. #50

The $NH_4$-N shows a cyclic trend over time, probably due to temperature effects on lagoon biological activity. #100 Highest $NH_4$-N concentrations usually occur during the spring and summer, similar to data reported in #102.

Table 4 contains a few samples of the concentrations of certain compounds of interest found in the effluents from different beef and dairy lagoons. Table 5 contains reports of these compounds in effluents from swine and poultry lagoons. The effluent quality obviously depends heavily on the influent chemistry and especially on the amount of dilution. For instance, one can see the influence of dilution by contrasting the results of study #63 a beef operation with study #50 of a highly diluted effluent from a dairy lagoon.

Three years of monitoring of the influent and effluent of a methane generating covered lagoon allowed the following analysis (Figure 1 and Tables 5 and 6) of the effect of operating temperature on nutrient removal, # 74. The lagoon held dairy waste from a 200 cow operation for a retention time of 60 days. Solids were separated prior to digestion. This study demonstrates that even within the operating range of 7°C to 30°C the nutrient removal rate can differ by as much as a factor of two. The worst performance occurred at the lowest temperatures.

**Figure 1**. Temperature Effects on the Nutrient Removal Efficiency of an Anaerobic Lagoon: Ratio of Effluent Concentration to Influent Concentration

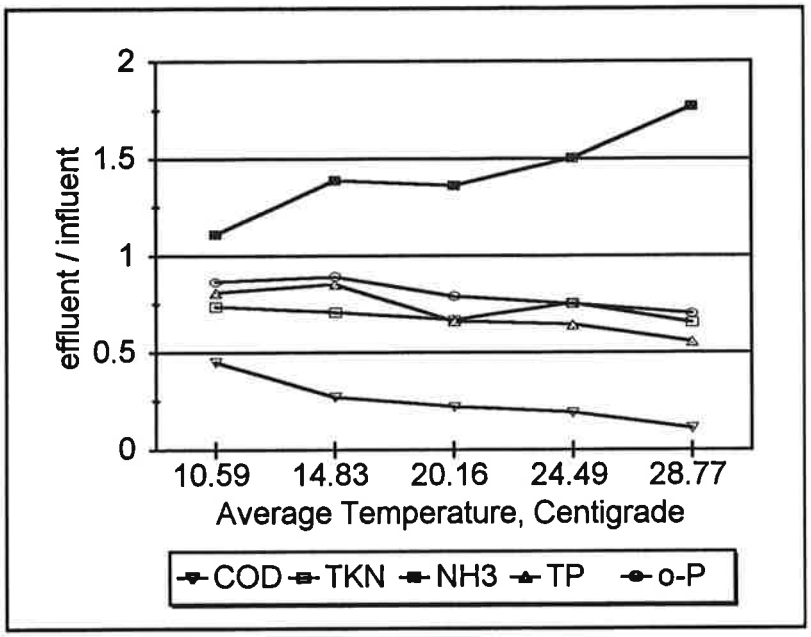


Table 7. Temperature Effects on the Operation of a Covered Lagoon, Percent reduction #74

| **Average Temperature, °C** | **TKN % reduction** | **NH3 % increase** | **TP % reduction** | **o-P % reduction** | **COD % reduction** |
|---|---|---|---|---|---|
| 10.6 | 26% | 11% | 19% | 14% | 55% |
| 14.8 | 29% | 39% | 15% | 11% | 73% |
| 20.2 | 33% | 36% | 34% | 21% | 78% |
| 24.5 | 25% | 50% | 36% | 25% | 81% |
| 28.8 | 34% | 77% | 44% | 30% | 89% |

Table 8. Temperature Effects on the Operation of a Covered Lagoon, Influent and Effluent Quality #74

| Average Temperature, °C | Influent TKN, mg/l | Influent NH3, mg/l | Influent TP, mg/l | Influent o-P, mg/l | Influent COD, mg/l |
|---|---|---|---|---|---|
| 10.6 | 842 | 276 | 212 | 138 | 15012 |
| 14.8 | 706 | 223 | 165 | 114 | 10709 |
| 20.2 | 693 | 232 | 166 | 117 | 11214 |
| 24.5 | 569 | 205 | 144 | 112 | 8538 |
| 28.8 | 625 | 185 | 162 | 115 | 12016 |
| **Average Temperature, °C** | **Effluent TKN, mg/l** | **Effluent NH3, mg/l** | **Effluent TP, mg/l** | **Effluent o-P, mg/l** | **Effluent COD, mg/l** |
| 10.6 | 620 | 307 | 171 | 119 | 6831 |
| 14.8 | 499 | 310 | 140 | 102 | 2909 |
| 20.2 | 461 | 316 | 110 | 92 | 2486 |
| 24.5 | 428 | 308 | 93 | 84 | 1653 |
| 28.8 | 409 | 327 | 90 | 80 | 1320 |

Overcash and Humenik #57 generalized 12 studies of anaerobic lagoons and reported attenuation of a variety of cations and anions (Table 9). The effluent calcium, potassium sodium, and chlorine concentrations were about 75% of the influent while magnesium, copper, manganese, and zinc are present at about 10% of the influent levels.

Table 9. Cations and Anions in Effluent from Anaerobic Swine Lagoons, Literature Review #57

| | % remaining in effluent | median effluent concentration, mg/l |
|---|---|---|
| K | 75% | 340 |
| Na | 75% | 220 |
| Ca | 20% | 89 |
| Mg | 60% | 53 |
| Cu | 3% | 0.4 |
| Zn | 10% | 0.9 |
| Mn | 15% | 1.0 |
| Cl | 75% | 335 |
| Al | | 5.6 |
| Ni | | 0.4 |
| B | | 0.65 |
| Cr | | 3.1 |
| Fe | | 2.8 |
| Pb | | 1.5 |
| Mb | | 0.02 |

Tables 10 and 11 report coupled lagoon effluent and sludge characteristics. Table 11 makes the distinction between the sludge "bed" and a sludge "blanket", a whimsical nomenclature that describes the denser semisolid sludge and the wispy semi-liquid solids layer that covers it, respectively.

Table 10. Average Composition of Swine Lagoon Supernate and Sludge #98

| **Parameter** | **Supernate** | **Sludge** |
|---|---|---|
| TS | 3,091 mg/l | 203,843 mg/l |
| VS/TS | 0.43 | 0.31 |
| TKN | 439 mg/l | 4,531 mg/l |
| $NH_3$-N/TKN | 0.78 | 0.25 |
| $NH_4$-N | 342 mg/l | 1134 mg/l |
| P | 70 mg/l | 3,723 mg/l |
| K | 366 mg/l | 1,773 mg/l |
| Na | 470 mg/l | 4627 mg/l |
| Ca | 257 mg/l | 6,176 mg/l |
| Mg | 64 mg/l | 1,514 mg/l |
| Cu | 0.99 mg/l | 40 mg/l |
| Zn | 1.84 mg/l | 181 mg/l |

Table 11. Average TKN, $NH_4$-N and P in Supernatant and Sludge of Livestock Lagoons #101

| **Animal** | **Depth** | **TKN mg/l** | **$NH_4$-N mg/l** | **P mg/l** |
|---|---|---|---|---|
| Poultry: Layer | supernatant | 491 | 402 | 88 |
| | sludge blanket | 1875 | 620 | 1369 |
| | sludge bed | 3884 | 898 | 5525 |
| Poultry: Pullet | supernatant | 334 | 259 | 81 |
| | sludge blanket | 1436 | 427 | 879 |
| | sludge bed | 2912 | 589 | 3323 |
| Swine | supernatant | 285 | 180 | 105 |
| | sludge blanket | 871 | 286 | 524 |
| | sludge bed | 2830 | 496 | 2493 |
| Dairy | supernatant | 179 | 119 | 48 |
| | sludge blanket | 704 | 171 | 162 |
| | sludge bed | 2546 | 328 | 1143 |

*Anaerobic Digesters*

The data on changes in plant nutrients during the operation of livestock manure digesters are even more sparse than data for lagoons. Ideally one would like to present data for the range of temperatures at which digesters are operated[1] and for a representative range of hydraulic retention times. Unfortunately, such an analysis could not be assembled from the peer-reviewed literature. Most of the studies which specified operating temperature and which reported both influent and effluent concentrations or which reported percent reduction of N, P, and K compounds were for digesters operating at 35°C, a mesophilic temperature (Table 12).

Digester effluent has half the COD and 20% of the BOD of untreated waste. A subjective assessment of odor indicated a large reduction by digestion. Most of the sulfur compounds which would be responsible for waste odors appear to be released as gasses during digestion, which then dissolve in the water in the condensate traps of the gas holder. #3

The concentration of N, P, and K are the same in the effluent from an anaerobic digester as in the influent manure. #8 The nitrogen in the effluent is approximately 70% ammonia nitrogen whereas the nitrogen in the influent is approximately 30% ammonia. Anaerobic treatment of livestock manure converts a large proportion of the organic nitrogen content of fresh manure into ammonia, converting on the average of two thirds of the organic nitrogen to ammonia. #30

Comparing Tables 3 and 12, one observes that less nitrogen is lost from digesters than from lagoons. This is because digesters are more tightly "closed" than lagoons, allowing less ammonia to escape, and because in digesters the nitrogen entering the solid phase is not considered lost. For the latter reason one would also expect negligible phosphorus and potassium loss. This has led to the claim that the effluent from a digester still contains most of the original nutrients. #2

*Laboratory Studies*

Laboratory studies of the anaerobic digestion of animal wastes (Table 13) give results loosely comparable to the performance of digesters operated under similar mixing and feeding schedules. Of course, laboratory studies have no spillage, leakage, incomplete mixing, variable feed composition, non-uniform heating, or other problems that commonly beset actual digesters. Furthermore, lab studies often utilize small test volumes and long detention times. Therefore, laboratory results should be considered separately from field study results, though they may produce very similar trends. The laboratory studies cited employed continuous mixing, which means that no sludge layer was allowed to form. Therefore, these lab studies are not analogous to anaerobic lagoon studies, where significant losses may occur to in the settled solids. There are

---

[1]Three temperature regimes are commonly referenced, psychrophilic temperatures, from 1°C to 27°C; mesophilic temperatures, from 27°C to 45°C; and thermophilic temperatures, from 45°C to 66°C. These terms refer to the temperatures preferences of the digester bacteria.

really too few studies here to form generalizations about the curious loss of phosphorus in the continuous-feed experiment #10.

Table 12. Performance of Anaerobic Digesters at Mesophilic and Thermophilic Temperatures

| | **Temperature** | **Manure type** | **Percent Reduction** | **Reference** |
|---|---|---|---|---|
| BOD | 35°C | swine | 84% - 90% | #73 |
| BOD | 35°C | swine | 80% | #3 |
| | | | | |
| COD | 35°C | swine | 30% -53% | #73 |
| COD | 35°C | swine | 51% | #3 |
| COD | 45°C | beef | 41% | #75 |
| COD | 50°C | beef | 42% | #75 |
| COD | 55°C | beef | 44% | #75 |
| | | | | |
| TS | 35°C | swine | 28% - 45% | #73 |
| TS | 35°C | swine | 40% | #3 |
| TS | 35°C | swine | 40% | #1 |
| TS | 45°C | beef | 48% | #75 |
| TS | 50°C | beef | 44% | #75 |
| TS | 55°C | beef | 50% | #75 |
| | | | | |
| TN | 35°C | swine | 12% | #2 |
| TN | 35°C | swine | 0% | #1 |
| TN | 45°C | beef | 0% | #75 |
| TN | 50°C | beef | 0% | #75 |
| TN | 55°C | beef | 0% | #75 |
| | | | | |
| $NH_4$ | 35°C | swine | 95% increase | #2 |
| $NH_4$ | 35°C | swine | 10% reduction | #3 |
| | | | | |
| organic N | 35°C | swine | 58% | #3 |
| organic N | 35°C | swine | 56% | #2 |
| | | | | |
| TP | 35°C | swine | 0% | #1 |
| | | | | |
| K | 35°C | swine | 0% | #1 |

Table 13. Laboratory studies of Anaerobic Digestion of Animal Wastes (vessels continuously mixed)

| | Feeding | Mean Residence Time | Manure Type | % Change | Temperature | Reference |
|---|---|---|---|---|---|---|
| TKN | batch | | dairy | 1% loss | not reported | #44 |
| TKN | batch | 56 days | swine | 0% | 15°C to 36°C | #10 |
| TKN | continuous | 64 days | swine | 0% | 15°C to 36°C | #10 |
| | | | | | | |
| TP | batch | 56 days | swine | 0% | 15°C to 36°C | #10 |
| TP | continuous | 64 days | swine | 17% loss | 15°C to 36°C | #10 |
| | | | | | | |
| o-$PO_4$ | batch | 56 days | swine | 30% gain | 15°C to 36°C | #10 |
| o-$PO_4$ | continuous | 64 days | swine | 50% loss | 15°C to 36°C | #10 |
| | | | | | | |
| COD | continuous | 65 days | swine | 95% loss | 15°C to 36°C | #10 |
| | | | | | | |
| K | batch | 56 days | swine | 0% | 15°C to 36°C | #10 |
| K | continuous | 64 days | swine | 0% | 15°C to 36°C | #10 |
| | | | | | | |
| $NH_4$ | batch | 56 days | swine | 30% gain | 15°C to 36°C | #10 |
| $NH_4$ | continuous | 64 days | swine | 30% gain | 15°C to 36°C | #10 |
| $NH_4$ | batch | | dairy | 200% gain | | #44 |
| | | | | | | |
| organic N | batch | | dairy | 14% loss | not reported | #44 |

## AMMONIA/AMMONIUM CHEMISTRY UNDER ANAEROBIC CONDITIONS

All of the studies, whether for lagoons, digesters, or laboratory glassware, document the conversion of organic nitrogen forms to ammonium during anaerobic digestion. Ammonia/ammonium chemistry is one of the most important features in anaerobic digestion both from the aspect of the toxicity of ammonia (but not ammonium) to bacteria and viruses contained in the influent manures and to anaerobic digester flora, and from the aspect of ammonia (but not ammonium) as a gaseous nitrogen compound that could be removed from the effluent, if desired.

Ammonia is quite toxic to anaerobic organisms when the concentration is in the range of 2000 mg/l and can be detrimental to digester function. Waste disposal systems that capture the entire waste discharge including urine, are especially susceptible in that undiluted ammonia concentrations often exceed 3000 mg/l. #62

Basically, the amount of ammonium, $NH_4^+$, present in the ionized form is controlled by pH and

temperature.

The following calculation was presented by Schmid *et al.* #62. Ammonia exists in equilibrium with ammonium ion in water

$$NH_4^+ + H_2O \rightleftharpoons NH_3 + H_2O + H^+ \quad (1)$$

Undissociated ammonia ($NH_3$) is the only form that can come off as a gas, and the amount available is described by the equation

$$NH_3 = NH_4^+ \cdot \frac{10^{pH}}{\frac{k_b}{k_w} + 10^{pH}} \quad (2)$$

where $k_b$ and $k_w$ are the ionization constants of aqueous ammonium and water, respectively. The temperature effect can be calculated from the equation

$$k_b/k_w = [-3.398 \ \ln(0.0241 \cdot {}^\circ C)] \cdot 10^9 \quad (3)$$

Solving the these two equations at $NH_4$ -N concentration of 2000 mg/l results in a free $NH_3$-N concentration at pH 7 and 10°C of 4.1 mg/l; at 35°C the concentration would be 34 mg/l. The NH3-N concentration would be 292 mg/l at pH 8 and 35°C, 66 times more than at pH 7 and 10°C (Table 14).

Table 14. Undissociated Ammonia ($NH_3$) Concentrations in an Aqueous Solution of 2000 mg/l Ammonium Ion ($NH_4^+$) at Various Temperatures and Acidities

| | **10°C** | **35°C** |
|---|---|---|
| **pH 7** | 4.1 mg/l $NH_3$ | 34 mg/l $NH_3$ |
| **pH 8** | 38 mg/l $NH_3$ | 272 mg/l $NH_3$ |

Thus one would not expect ammonia to be volatilized to any great extent in the pH range near 7 or less, where most anaerobic digesters operate. A heated digester can operate at near 35°C, at which temperature the fraction of dissociated ammonia available for volatilization is nearly 8.5 times greater than it would be at 10°C. So, it is possible to remove ammonia at near neutral pH.

#62 discusses how ammonia could be captured by stripping it from the gas by an acid solution, ideally phosphoric acid, in that it would be desirable to keep the nitrogen to use later as a fertilizer. The ammonia would neutralize the acid, resulting in liquid ammonium phosphate, which would be stable and storable for use as a fertilizer.

#62 estimated that the total ammonia available for stripping was 0.04 lb $NH_3$-N per 150 lb animal per day (18g/67.5 kg). If 50% of the ammonia could be stripped and captured, the system could

collect 14,600 lb (6570 kg) of nitrogen from a 200-head swine operation per year.

A relatively small amount of N is lost to biogas. #68 reports that biogas contains more than 1 percent ammonia and more than 4% $N_2$.

## SLUDGE CHARACTERISTICS

Table 15 presents several measurements of sludge accumulation rates in lagoons. Fulhage #98 estimates that sludge accumulates at 0.0022 $m^3$ per kg porcine animal per year, which is significantly less than the other estimates. This may be due to the fact that Fulhage investigated older lagoons, which had more completely digested sludge layers.

Table 15. Average Sludge Accumulation Rates in Lagoons

| **Animal** | **$m^3$/animal·year** | **$m^3$/kg TS** | **Reference** |
|---|---|---|---|
| Poultry: Layer | 0.016 | 0.00184 | #101 |
| Poultry: Pullet | 0.009 | 0.00284 | #101 |
| 45.4 kg Hog | 0.356 | 0.00303 | #101 |
| 454 kg lactating Cow | 7.08* | 0.00455 | #101 |
| Dairy Cow | | 0.0033 | |
| 454 kg Cow | 7.53 | 0.00456 | #50 |
| Grower/Finisher Swine | 0.340 | 0.00303 | #50 |

*includes some mud from washing cows

Nordstedt and Baldwin #58 found no apparent difference in the volume of sludge buildup in dairy lagoons at 25°C and 10°C. However, since the solids reduction was two to three times greater at the higher temperature, the solids accumulated at a lower temperature had to be more concentrated.

The rate at which solids decompose in a lagoon will depend upon such environmental factors as the temperature of the lagoon, the degree of mixing that takes place, and the pH and alkalinity in the lagoon. At low temperatures, the quality of the settled solids will be quite similar to those that entered the lagoon. Little decomposition will take place. #56

Settling solids prior to discharging swine wastes to a lagoon significantly decreases phosphorus levels in the lagoon. #63 Phosphorus may be lost by the formation of phosphorus containing precipitates in anaerobic treatment or storage facilities which then settle to the bottom sludges. The precipitation of magnesium ammonium phosphate in anaerobic swine lagoons is an example of this. Losses of potassium in the supernatant of lagoons and holding ponds and high potassium contents in the bottom sludges have been observed. #52

The composition of the sludge produced by anaerobic digestion is determined by the composition and biodegradability of the raw material fed to the digester. #44 and #56 Examples of chemical analyses of lagoon sludges are found in Table 16.

Table 16. Composition of Sludges from Anaerobic Lagoons

| parameter | amount | type of sludge | reference |
|---|---|---|---|
| % moisture | 90% | dairy lagoon sludge | #50 |
| % moisture | 92% | swine lagoon sludge | #50 |
| | | | |
| TS | 10% | dairy lagoon sludge | #50 |
| TS | 6% | dairy lagoon sludge after 57 months | #58 |
| TS | 8% | swine lagoon sludge | #50 |
| TS | 20% | swine lagoon sludge | #98 |
| | | | |
| COD | 52188 mg/l | dairy lagoon sludge | #50 |
| COD | 64841 mg/l | swine lagoon sludge | #50 |
| | | | |
| N | 2510 mg/l | dairy lagoon sludge | #50 |
| N | 3012 mg/l | swine lagoon sludge | #50 |
| N | 1720-2440 mg/l | cattle lagoon sludge | #56 |
| N | 8% dry weight | sludge, laboratory batch swine waste digestion | #10 |
| N | 1.3% dry weight | sludge, laboratory continuous digestion, swine | #10 |
| N | 6.8 % dry weight | swine lagoon sludge | #78 |
| N | 4531 mg/l | swine lagoon sludge | #98 |
| N | 1436 - 3884 mg/l | poultry lagoon sludge | #101 |
| N | 871 - 2830 mg/l | swine lagoon sludge | #101 |
| N | 704 - 2546 mg/l | dairy lagoon sludge | #101 |
| N | >3000 mg/l | dairy lagoon sludge after 57 months | #58 |
| | | | |
| $NH_4^+$-N | 2510 mg/l | dairy lagoon sludge | #50 |
| $NH_4^+$-N | 763 mg/l | swine lagoon sludge | #50 |
| $NH_4^+$-N | 1133 mg/l | swine lagoon sludge | #98 |
| $NH_4^+$-N | 427 - 898 mg/l | poultry lagoon sludge | #101 |
| $NH_4^+$-N | 286 - 496 mg/l | swine lagoon sludge | #101 |
| $NH_4^+$-N | 171 - 328 mg/l | dairy lagoon sludge | #101 |
| | | | |
| P | 1104 mg/l | dairy lagoon sludge | #50 |
| P | 2711 mg/l | swine lagoon sludge | #50 |
| P | 4% dry weight | sludge, laboratory batch swine waste digestion | #10 |
| P | 1% dry weight | sludge, laboratory continuous digestion, swine | #10 |
| P | 2.5% dry weight | swine lagoon sludge | #78 |
| P | 3723 mg/l | swine lagoon sludge | #98 |
| P | 879 - 5525 mg/l | poultry lagoon sludge | #101 |
| P | 524 - 2493 mg/l | swine lagoon sludge | #101 |
| P | 162 - 1143 mg/l | dairy lagoon sludge | #101 |
| | | | |
| K | 1506 mg/l | dairy lagoon sludge | #50 |
| K | 7628 mg/l | swine lagoon sludge | #50 |
| K | <1% dry weight | sludge, laboratory batch swine waste digestion | #10 |
| K | <1% dry weight | sludge, laboratory continuous digestion, swine | #10 |
| K | 1.2% dry weight | swine lagoon sludge | #78 |
| K | 1773 mg/l | swine lagoon sludge | #98 |

The salts in swine waste are removed in an anaerobic lagoon by chelation-settling or precipitation. Losses of various cations can range from less than 10% to over 90%. A substantial fraction of influent heavy metals remain in the sludge. #57 (Table 17)

Table 17. Approximate Sludge Content as a Percentage of Total Lagoon Contents, Various Chemical Species from Anaerobic Swine Lagoons.

| | **Reference #57** | **Reference #98** |
|---|---|---|
| K | 25% | 25% |
| Na | 25% | |
| Ca | 80% | 63% |
| Mg | 40% | 64% |
| Cu | 97% | 75% |
| Zn | 90% | 86% |
| Mn | 85% | |
| Cl | 25% | |
| P | 65% | 80% |
| TKN | 25% | 41% |
| VS | | 77% |
| TS | | 82% |

The sludge layer in an anaerobic lagoon has two components, a layer of dense refractory material covered by a wispy watery biologically active layer. #99 and #58 Smith #99 describes these as the sludge bed and the sludge blanket, respectively. This phenomenon is documented in Table 18.

Table 18. Total Solids Concentration Variation With Depth From Swine Lagoon Bottom #99

| **Depth from Bottom, meters** | **TS, mg/l** | **VS, %TS** |
|---|---|---|
| 3.3 (surface of lagoon) | 1294 | 36 |
| 3.0 | 1309 | 36 |
| 2.7 | 1281 | 36 |
| 2.4 | 1228 | 36 |
| 2.1 | 1393 | 36 |
| 1.8 | 1275 | 36 |
| 1.5 | 21900 | 51 |
| 1.2 | 42300 | 65 |
| 0.9 | 47390 | 66 |
| 0.6 | 50800 | 66 |
| 0.3 | 55600 | 66 |
| 0 | 250000 | |

(After 5 years operation average sludge depth: 1.5 m; average TS concentration: 150,000 mg/l)

## ANAEROBIC DIGESTION: EFFECT ON PATHOGENIC ORGANISMS

### BACTERIA

The pathogenic bacteria found in animal excreta can be a health hazard to livestock exposed via unclean living conditions or by contamination of feed or pasture. A few of the most important livestock bacterial pathogens and the diseases they cause are listed below.

Table 19. Some Important Bacterial Agents of Disease in Animals

| **Bacterium** | **Diseases** |
|---|---|
| *Staphylococcus aureus* | mastitis in dairy cows and joint disease in poultry |
| *Mycobacterium avium* | tuberculosis in swine and poultry |
| *Brucella suis* | brucellosis or Traum's disease resulting in abortion in swine |
| *Salmonella* species | abortion in mares and sheep and other disease in poultry and swine |
| *Erysipelothrix rhusiopathia* | erysipelas in swine |
| *Escherichia coli* | a major cause of death in newborn animals |
| *Mycobacterium bovis* | tuberculosis in cattle and other animals |
| *Brucella abortis* | brucellosis or Bang's disease resulting in abortion in cows |
| *Bacillus anthracis* | anthrax in cattle, sheep and other animals |
| *Clostridium* species | blackleg in cattle and other animals, overeating disease in lambs |
| *Salmonella gallinarum* | bacillary white diarrhea of chickens |

Any disposal of human or animal wastes to land entails some potential risk of introducing disease agents (viruses, bacteria, and parasites) into the food chain via plants or animals, or the water supply. #5 Animals are the chief reservoir of most enteric bacteria that are pathogenic to humans.

The inactivation of pathogenic microorganisms in animal manures subjected to anaerobic digestion may be a valuable collateral benefits to the methane production, since anaerobic digestion of animal manures has been shown to cause a temperature/time related reduction of vegetative pathogenic bacteria (#6 and #43).

The section compiles results of studies of the effects of anaerobic digestion on bacterial die-off in animal slurries.

When the elimination of an organism from a medium under defined condition follows an exponential curve over time, it is appropriate to describe the die-off rate as either k, the decay constant or as $D_{90}$, the decimal reduction time or decimation constant. $D_{90}$ represents the time necessary for a 90% reduction of the original population. Its use assumes the exponential death rate that does not vary over an extended period of time. The decimal reduction time is not to be confused with the time needed to clear the inoculum of organisms, which could be considerably longer. For example, in the case of an inoculum containing $10^8$ organisms per milliliter, treatment lasting for a single $D_{90}$ would decrease the population only to $10^7$ organisms per milliliter. It

would take ten times the decimation time to totally clear the culture.

Because it is so commonly reported in studies involving anaerobic digestion of animal slurries (#38) and because it allows quick mental calculations of the effectiveness of treatment[2], this report will focus on the $D_{90}$ statistic to describe microbial death rates.

Since initial densities of individual bacterial species in livestock slurries can be in the $10^8$ organisms per milliliter range or higher, one sees that it would take more than one $D_{90}$ (in this case 10 $D_{90}$'s) to theoretically eliminate the organism entirely. Complete elimination of pathogens is not usually the goal of treatment. The amount of treatment required depends on the type of pathogen present, the expected exposure of the animals to the effluent, and the susceptibility of the animals to the pathogen[3].

Not all authors have observed the typical exponential die-off curve in their long term studies of the effects of anaerobic digestion of animal waste slurries on bacteria. Some authors (#15, #34) have disputed the assumption of an exponential die-off distribution after the first or second $\log_{10}$ reduction, citing the selection for and emergence of resistant organisms, which die at slower rates. Populations of microorganisms can contain a mixture of susceptible and resistant members. The susceptible members succumb first, leaving a residual population with a new death rate. Therefore, in cases of extremely pathogenic organisms, calculations based on $D_{90}$'s should incorporate a safety factor.

Another caveat of which to be aware is the difference between batch and continuous digester operation. In batch processes the influent is treated as a unit. In mixed or continuous-feed digesters, the influent is mixed with the partially digested material currently in the digester. The effluent is removed simultaneously with the addition of influent, and therefore, in well mixed digesters, the treated effluent contains a fraction of undigested influent. For example, a completely mixed operation with a mean residence time of 20 days means that one-twentieth of the volume in the reactor is removed each day, that one-twentieth of this newly added volume leaves the reactor by the following day. Unless conditions in the digester are extremely lethal, this inherent one-in-four-hundred short circuiting may pass a significant amount of infectious pathogen when compared to what would have survived 20 days' residence. Prolonged subsequent holding (secondary digestion or lagooning) is one solution to this problem. #45 Another solution is to run a thermophilic biogas plant semi-continuously, with an appropriate interval between input and withdrawing of slurry, to ensure the destruction of serious pathogens. #66

---

[2]To calculate the time necessary for a 99% reduction, simply multiply the $D_{90}$ by two. For a 99.9% reduction, multiply the $D_{90}$ by three, etc.

[3]For instance, Calves are susceptible to salmonellosis when exposed to an inoculum of $10^5$ to $10^{11}$ cfu $ml^{-1}$, but adult cattle are infected when exposed to inocula of $10^{11}$ cfu $ml^{-1}$ or greater. #34

One further caution, the degrees and rates of inactivation of bacteria in lab experiments tend to be greater than in full scale digesters, because the latter seldom are operated under conditions ideal for inactivation or because indigenous salmonellas are more resistant #60.

*Compilation of Bacterial Die-Off Study Results*

Total and fecal coliforms, though not necessarily pathogenic, were studied because of their widespread use as indicators of water pollution. It is interesting to note that the mixed populations of coliforms appear to be somewhat more resistant to inactivation by anaerobic digestion than their representative species, *Escherichia coli*. (Tables 20, 21, 22)

Table 20. Total Coliforms Decimal Reduction Times under Anaerobic Digestion with Livestock Manures

| **Temperature** | **$D_{90}$** | **Comments** | **Reference** |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 6 - 9°C | 31.5 - 126 days | mean 65 days, cattle and pig slurry | #38 |
| 6 - 9°C | 56.7 days | non-aerated dairy and swine slurry | #20 |
| 6 - 15°C | 65 days | slurry | #108 |
| 18 - 20°C | 10.5 - 38.5 days | mean 15 days, cattle and pig slurry | #38 |
| 18 - 21°C | 15 days | slurry | #108 |
| 20°C | 14.7 days | non-aerated dairy and swine slurry | #20 |
| MESOPHILIC | | | |
| 23 - 28°C | 5.1 days | anaerobic swine lagoon | #71 |
| 35°C | 3.1 days | indigenous total coliforms | #6 |

Table 21. Fecal Coliforms Decimal Reduction Times under Anaerobic Digestion of Livestock Manures

| **Temperature** | **$D_{90}$** | **Comments** | **Reference** |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 6 - 9°C | 24.5 - 120 days | mean of 62 days, cattle and pig slurry | #38 |
| 18 - 20°C | 10.5 - 31.5 days | mean of 14 days, cattle and pig slurry | #38 |

Table 22. *Escherichia coli* Decimal Reduction Times under Anaerobic Digestion of Livestock Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 4°C | >29 days* | *E. coli* K12 strA, in cattle slurry | #34 |
| 6 - 9°C | 11.9 days | *E. coli* serovar 078, cattle and pig slurry | #28 |
| 6 - 9°C | 12 days | *E. coli* serovar 08 in cattle and pig slurry | #38 |
| 6 - 9°C | 61.6 - 65.1 days | | #28 |
| 6 - 9°C | 33.6 days | *E. coli* serovar 08 | #28 |
| 6 - 15°C | 56 days | | #108 |
| 10°C | 5 days | rabbit waste slurry | #23 |
| 17°C | >29 days* | *E. coli* K12 strA, in cattle slurry | #34 |
| 18 - 20°C | 11.2 days | *E. coli* serovar 078, cattle and pig slurry | #28 |
| 18 - 20°C | 6.3 days | *E. coli* serovar 08 in cattle and pig slurry | #38 |
| 18 - 20°C | 14 - 14.7 days | | #28 |
| 20°C | 3.3 days | rabbit waste slurry | #23 |
| MESOPHILIC | | | |
| 33°C | 1 day | | #41 |
| 33 - 35°C | 2.2 - 5.5 days | total *E. coli* in cattle and pig slurry | #43 |
| 33 - 35°C | 1 - 2 days | pathogenic *E. coli*, cattle and pig slurry | #43 |
| 35°C | 1.8 days | | #79 |
| 35°C | 1.2 days | *E. coli* K-12 10083 strA in beef slurry | #5 |
| 35°C | 0.8 day | batch incubation E. coli K12 strA | #34 |
| 35°C | 1.5 days | semicontinuous feed, *E. coli* K12 strA | #34 |
| 37°C | 1.4 days | rabbit waste slurry | #23 |
| THERMOPHILIC | | | |
| 50°C | 5 - 6 hours | | #41 |
| 53°C | 0.4 hour | | #79 |
| 53°C | 0.5 - 1 hour | | #41 |
| 50 - 59°C | 0.2 - 1.5 hours | pathogenic *E. coli*, cattle and pig slurry | #43 |

*longer than the duration of the experiment

Another widely used indicator species is fecal *Streptococcus*. (Table 23)

Table 23. Fecal Streptococci Decimal Reduction Times under Anaerobic Digestion in Livestock Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 6 - 9°C | 84 - 280 days | mean 150 days, cattle and pig slurry | #38 |
| 6 - 9°C | 149.8 days | dairy and swine slurry, anaerobic storage | #20 |
| 18 - 20°C | 28 - 49 days | mean 40 days, cattle and pig slurry | #38 |
| 20°C | 39.9 days | dairy and swine slurry, anaerobic storage | #20 |
| MESOPHILIC | | | |
| 33 - 35°C | 7 - 8 days | cattle and pig slurry | #43 |
| 35°C | 2 days | | #79 |
| THERMOPHILIC | | | |
| 53°C | 1 hour | | #79 |
| 50 -59°C | 2 - 3.5 hours | cattle and pig slurry | #43 |

Salmonellosis is regarded as the most important disease spread by slurry. Carriers can contaminate pastures and water supplies, and consequently cause infection in other animals.#34

Although salmonellas may survive for up to 150 days in stored anaerobic cattle slurry, 90% die during the first two to four weeks of storage. Survival is greatest at temperatures below 10°C and in slurries containing more than 5% solids.#21

Tables 24 and 25 present $D_{90}$ times for this genus.

Table 24. *Salmonella typhimurium* Decimation Times under Anaerobic Digestion of Livestock Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 4°C | 21.3 days | strain 0035, lab test, beef slurry | #34 |
| 7°C | 39.8 days | | #28 |
| 5 - 10°C | 21 days | | #81 |
| 5 - 10°C | 13 - 25 days | | #80 |
| 6 - 9°C | 41.3 days | non-aerated dairy and swine slurry | #20 |
| 6 - 9°C | 21 - 56 days | mean of 41 days | #38 |
| 6 - 9°C | 41.3 days | | #28 |
| 17°C | 17.5 days | strain 0035, lab test, beef slurry | #34 |
| 18 - 20°C | 14 days | | #28 |
| 18 - 20°C | 7 - 21 days | mean of 14 days | #38 |
| 20°C | 14 days | non-aerated dairy and swine slurry | #20 |
| 20°C | 14.4 days | | #28 |
| 23 - 28°C | 5.3 days | swine slurry | #71 |
| MESOPHILIC | | | |
| 33°C | 2 - 3 days | | #41 |
| 33 - 35°C | 2 - 4 days | | #43 |
| 35°C | 0.9 days | strain 0035, batch lab experiment | #34 |
| 35°C | 1.1 days | strain 0035 semi-continuous lab test | #34 |
| 35°C | 2.4 days | | #6 |
| 35°C | 1.1 days | | #15 |
| THERMOPHILIC | | | |
| 50°C | 0.7 - 0.8 hour | | #41 |
| 53°C | 0.7 hour | | #79 |
| 53°C | 0.5 - 1 hour | | #41 |
| 50 - 59°C | 0.2 - 1.5 hour | | #43 |

Table 25. Other Salmonella species Decimal Reduction Times under Anaerobic Digestion in Livestock Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 5 - 10°C | 10 - 72 days | *S. dublin* | #80 |
| 7°C | 33.6 days | *S. dublin* | #28 |
| 7°C | 30.8 days | *S. derby* | #28 |
| 10°C | 2.5 days | *S. typhi*, rabbit waste slurry | #23 |
| 20°C | 2.1 days | *S. typhi*, rabbit waste slurry | #23 |
| 23 - 28°C | 5.1 - 6.6 days | *S. choleraesuis* | #71 |
| MESOPHILIC | | | |
| 35°C | 0.7 days | *S. duesseldorf* | #60 |
| 35°C | 2.1 days | *S. dublin* | #79 |
| 37°C | 1.5 days | *S. typhi*, rabbit waste slurry | #23 |
| THERMOPHILIC | | | |
| 48°C | 20 minutes | *S. duesseldorf* | #60 |
| 53°C | 36 minutes | *S. dublin* | #79 |
| 55°C | 5 minutes | *S. typhi* | #42 |
| 55°C | 5 minutes | *S. paratyphi* | #42 |
| 55°C | 5.5 minutes | *S. enteritidis* | #42 |
| 57°C | 31 minutes | *S. senftenberg* 775W* | #42 |

*the most heat tolerant salmonella strain known

*Mycobacterium paratuberculosis* exhibits an extraordinarily high resistance to various environmental conditions. In anaerobically-stored cattle slurry it survives 98 days at 15°C and 252 days at 5°C. #66 Table 26 concerns this species.

Table 26. *Mycobacterium paratuberculosis* Decimation Times under Anaerobic Digestion of Livestock Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 5°C | | complete die off after 252 days | #66 |
| 6 - 9°C | 63 days | | #28 |
| 6 - 9°C | | > 4 months in dairy and swine slurry | #20 |
| 15°C | | complete die off after 98 days | #66 |
| 18 - 20°C | 42 days | | #28 |
| MESOPHILIC | | | |
| 35°C | 6 days | | #38 |
| 35°C | 5 - 6.5 days | | #66 |
| THERMOPHILIC | | | |
| 53°C | 0.7 hour | | #38 |
| 53°C | 0.5 hour | | #66 |

Two other vegetative bacterial species are covered in Tables 27 and 28.

Table 27. *Yersinia enterocolitica* Decimal Reduction Times under Anaerobic Digestion of Livestock Manures

| Temperature | D90 | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 4°C | 20.8 days | beef slurry, lab study | #34 |
| 7°C | 10.1 days | | #28 |
| 6 - 9°C | 11.2 days | dairy and swine slurry, anaerobic storage | #20 |
| 6 - 9°C | 7 -17.5 days | mean 11 days, cattle and pig slurry | #38 |
| 17°C | 12.8 days | beef slurry, lab study | #34 |
| 18 - 20°C | 4.2 days | | #28 |
| 18 - 20°C | 3.5 - 7 days | mean 4 days, cattle and pig slurry | #38 |
| 20°C | 4.2 days | dairy and swine slurry, anaerobic storage | #20 |
| MESOPHILIC | | | |
| 35°C | 0.9 days | semicontinuous lab digestion, beef slurry | #15 |
| 35°C | 0.7 days | batch incubation, beef slurry | #34 |
| 35°C | 2.5 days | lab semicontinuous incubation, beef slurry | #34 |

Table 28. *Staphylococcus aureus* Decimal Reduction Times under Anaerobic Digestion with Livestock Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 5 - 10°C | 6 - 18 days | | #80 |
| 6 - 9°C | 10.5 - 120 days | mean of 50 days, cattle and pig slurry | #38 |
| 6 - 9°C | 49.7 days | non-aerated dairy and swine slurry | #28 |
| 10°C | 6.4 days | rabbit waste slurry | #23 |
| 18 - 20°C | 3.5 - 21 days | mean of 6 days, cattle and pig slurry | #38 |
| 18 - 21°C | 6.3 days | | #108 |
| 20°C | 5.3 days | rabbit waste slurry | #23 |
| 20°C | 6.3 days | non-aerated dairy and swine slurry | #20 |
| MESOPHILIC | | | |
| 33 - 35°C | 0.5 - 1 day | | #80 |
| 35°C | 0.9 day | | #79 |
| 37°C | 4.7 day | rabbit waste slurry | #23 |
| THERMOPHILIC | | | |
| 53°C | 0.5 hour | | #79 |
| 50 - 59°C | 0.4 - 1.5 hour | cattle and pig slurry | #43 |

Clostridia are anaerobic spore forming bacteria. (Table 29). They are unaffected by anaerobic digestion even at 53°C.

Table 29. *Clostridium perfringens* Decimation Times under Anaerobic Digestion of Animal Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 6 - 9°C | no reduction | 7 month experiment | #20 |
| 20°C | no reduction | 7 month experiment | #20 |
| MESOPHILIC | | | |
| 35°C | no reduction | type C | #79 |
| THERMOPHILIC | | | |
| 53°C | no reduction | type C | #79 |

Table 30. Miscellaneous Bacterial Decimal Reduction Times under Anaerobic Digestion of Animal Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 4°C | >84 days* | *Listeria monocytogenes* LM1 | #34 |
| 4°C | >112 days* | *Campylobacter jejuni* 4451 | #34 |
| 4°C | 70 days | *Treponema hyodysenteria* | #20 |
| 5 - 10°C | 6 - 18 days | *Brucella abortus* | #80 |
| 6 - 15°C | 150 days | Group D Streptococci | #108 |
| 17°C | 29.4 days | *Listeria monocytogenes* LM1 | #34 |
| 17°C | >112 days* | *Campylobacter jejuni* 4451 | #34 |
| 18 - 21°C | 40 days | Group D Streptococci | #108 |
| 20°C | < 9 days | *Treponema hyodysenteria* | #20 |
| MESOPHILIC | | | |
| 33 - 35°C | 1 - 2 days | *Erysipelothrix rhusiopathia* | #43 |
| 35°C | 1.8 days | *Erysipelothrix rhusiopathia* | #79 |
| 35°C | 7.1 days | Group D Streptococci | #79 |
| 35°C | 0.8 days | *Listeria monocytogenes* LM1 | #15 |
| 35°C | 12.3 - 35.7 days | *Listeria monocytogenes* | #34 |
| 35°C | 3.6 days | *Campylobacter jejuni* 4451 | #15 |
| 35°C | >71 days* | *Campylobacter jejuni* | #34 |
| 35°C | no reduction | *Bacillus cereus* | #79 |
| THERMOPHILIC | | | |
| 53°C | 1.2 hours | *Erysipelothrix rhusiopathia* | #79 |
| 53°C | no reduction | *Bacillus cereus* | #79 |

* longer than the duration of the experiment

*Discussion and Conclusions*

Mesophilic or thermophilic anaerobic digestion of slurry causes a significantly accelerated inactivation of vegetative, non-spore-forming bacteria when compared with inactivation during anaerobic storage at lower temperatures (6 to 20°C). Decimal reduction times of these bacteria are of the order of hours or minutes at thermophilic temperatures, days at mesophilic temperatures and weeks or months at psychrophilic temperatures. #66, #43, #38

Spore forming bacterial species, whether anaerobic like *Clostridium*, or aerobic like *Bacillus* seem to be quite resistant to anaerobic digestion at commonly encountered digestion temperatures.

Gadre *et al.* #59 reported that there is no conclusive evidence that intestinal pathogens are totally inactivated during anaerobic digestion although the number of these pathogens is reduced considerably. Dudley *et al.* #82 showed that klebsiellas, shigellas, salmonellas, mycobacteria, staphylococci and pseudomonads survived anaerobic digestion.

Thermophilic as well as mesophilic digestion coupled with thermophilic pretreatment will result in a sufficient reduction of both vegetative pathogenic bacteria and intestinal parasites occurring in concentrations usually found in animal waste to allow for unrestricted use of the effluent in agriculture. This cannot be said for mesophilic digestion used alone. #33

Anaerobic swine lagoons with residence times of greater than 300 days would be expected to give substantial pathogen die-off and maintain strong, mixed populations of microorganisms for organic decomposition.#57

VIRUSES

Viruses shed in large amounts in feces and urine fall for the most part in the categories of the adenoviruses, the reoviruses, and the enteroviruses[4].

The enteroviruses are of particular importance to managers of animal wastes, although the majority of cases of animals infected by enteric viruses will remain without clinical manifestations #36. Some of the diseases associated with human enteroviruses are listed below.

---

[4]The enteroviruses include polio virus, coxsackievirus, ECHO virus, rhinovirus and others which are unclassified. They are also sometimes called picornaviruses, *i.e.*, small RNA viruses.

Table 31. Clinical Syndromes Associated with Infections by Human Enteroviruses

| | |
|---|---|
| Poliovirus | Muscular paralysis<br>Aseptic meningitis<br>Febrile episodes |
| Coxsackievirus A | Herpangina<br>Acute lymphatic pharyngitis<br>Aseptic meningitis<br>Paralysis<br>Exanthem (hand-foot-mouth disease)<br>Pneumonitis of infants<br>Common cold<br>Hepatitis<br>Infantile Diarrhea |
| Coxsackievirus B | Pleurodynia<br>Aseptic meningitis<br>Paralysis<br>Meningoencephalitis and myocarditis<br>Pericarditis and myocarditis<br>Upper respiratory illness and pneumonia<br>Rash or hepatitis |
| ECHO virus | Aseptic meningitis<br>Paralysis<br>Gullain-Barre's syndrome<br>Exanthem<br>Respiratory disease<br>Diarrhea<br>Epidemic myalgia<br>Pericarditis and myocarditis<br>Hepatitis |

The majority of studies of viral inactivation by anaerobic digestion employed human enteroviruses. The results are presented alongside of studies of animal viruses with the understanding that related animal viruses may or may not have similar abilities to withstand the conditions of digestion. (Table 32)

*Compilation of Viral Die-Off Studies*

Table 32. Enterovirus Decimal Reduction Times Under Anaerobic Digestion of Animal Manures

| Temperature | $D_{90}$ | Comments | Reference |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 5°C | 19 days | poliovirus type 1 | #26 |
| 5°C | 200 - 300 days | porcine enterovirus | #36 |
| 5°C | 35 days | coxsackievirus B3 | #36 |
| 14.4°C | 29.9 days | poliovirus type 1 | #47 |
| 15°C | 20 days | poliovirus type 1 | #26 |
| 20°C | 9 days | coxsackievirus B3 at pH 6.9 to 7.8 | #36 |
| 20°C | 13 days | porcine enterovirus (Talfan) pH 6.9 - 7.8 | #36 |
| 20.8°C | 18.7 days | poliovirus type 1 | #47 |
| 25°C | 6.8 days | poliovirus type 1 | #47 |
| 25°C | 17 days | poliovirus type 1 | #26 |
| MESOPHILIC | | | |
| 32°C | 2.06 days | coxsackievirus B3 | #85 |
| 33°C | 2 days | coxsackievirus | #41 |
| 35°C | 0.64 days | coxsackievirus A9 | #16 |
| 35°C | 0.99 days | coxsackievirus B4 | #16 |
| 35°C | 0.46 days | coxsackievirus B3 | #85 |
| 35°C | 1.67 days | ECHO virus II | #16 |
| 35°C | 1.0 day | poliovirus type 1 | #86 |
| 35°C | 4.12 days | poliovirus type 1 | #86 |
| 37°C | 2.60 days | poliovirus type 1 | #86 |
| 37°C | 1.3 days | poliovirus type 1 | #47 |
| THERMOPHILIC | | | |
| 50°C | 3 hours | poliovirus type 1 | #86 |
| 50°C | 0.8 - 1.2 hours | coxsackievirus | #41 |
| 53°C | 1 - 7 hours | coxsackievirus | #41 |
| 53°C | 0.5 hour | ECHO virus | #41 |
| 55°C | 1 hour | poliovirus type 1 | #42 |
| 56°C | 3 hours | coxsackie virus | #41 |
| 80°C | 2 minutes | enterovirus | #36 |

Table 33. Decimal Reduction Times of Viruses Other Than the Enteroviruses Under Anaerobic Digestion in Animal Manures

| Temperature | $D_{90}$ | pH | Virus | Reference |
|---|---|---|---|---|
| PSYCHROPHILIC | | | | |
| 5°C | 34.6 days | 7.04 | hepatitis A virus | #14 |
| 5°C | 48.5 days | 7.58 | hepatitis A virus | #14 |
| 5°C | 188 days | | bovine parvovirus | #41 |
| 5°C | 200 - 300 days | | bovine parvovirus | #36 |
| 10.1°C | 22.2 days | | f2 coliphage | #7 |
| 10 - 20°C | 34.7 days | 6.9 | f2 coliphage | #7 |
| 10 - 20°C | 78.1 days | 6.9 | bovine encephalomyocarditis virus | #7 |
| 10 - 20°C | 197 days | 6.9 | bovine rotavirus | #7 |
| 10 - 20°C | 38 days | 6.9 | bovine parvovirus | #7 |
| 10 - 20°C | 22.9 days | 6.9 | bovine adenovirus | #7 |
| 10 - 20°C | < 2.5 days | 6.9 | bovine herpes virus | #7 |
| 10 - 20°C | 23 days | 7.4 | f2 coliphage | #7 |
| 10 - 20°C | 28.9 | 7.4 | bovine encephalomyocarditis virus | #7 |
| 10 - 20°C | 104 days | 7.4 | bovine rotavirus | #7 |
| 10 - 20°C | 29 days | 7.4 | bovine parvovirus | #7 |
| 10 - 20°C | 37.3 days | 7.4 | bovine adenovirus | #7 |
| 10 - 20°C | < 2.5 days | 7.4 | bovine herpes virus | #7 |
| 10 - 20°C | 27.3 days | 7.9 | f2 coliphage | #7 |
| 10 - 20°C | 44.6 days | 7.9 | bovine encephalomyocarditis virus | #7 |
| 10 - 20°C | 61.8 days | 7.9 | bovine rotavirus | #7 |
| 10 - 20°C | 24.5 days | 7.9 | bovine parvovirus | #7 |
| 10 - 20°C | 34.9 days | 7.9 | bovine adenovirus | #7 |
| 10 - 20°C | < 2.5 days | 7.9 | bovine herpes virus | #7 |
| 10 - 20°C | 9.4 days | 8.7 | f2 coliphage | #7 |
| 10 - 20°C | 8.2 days | 8.7 | bovine encephalomyocarditis virus | #7 |
| 10 - 20°C | 27.5 days | 8.7 | bovine rotavirus | #7 |
| 10 - 20°C | 20.3 days | 8.7 | bovine parvovirus | #7 |
| 10 - 20°C | 8.4 days | 8.7 | bovine adenovirus | #7 |
| 10 - 20°C | < 2.5 days | 8.7 | bovine herpes virus | #7 |
| 19.2°C | 7.1 days | | f2 coliphage | #7 |
| 20°C | 20 days | | bovine parvovirus | #41 |
| 22°C | 17.1 days | 7.0 | hepatitis A virus | #14 |
| 22°C | 23 days | 7.6 | hepatitis A virus | #14 |
| MESOPHILIC | | | | |
| 35°C | 0.86 day | | coliphage MS2 | #16 |
| THERMOPHILIC | | | | |
| 80°C | 16 minutes | | parvovirus | #36 |

At the pH value of raw pig manure most of the ammonia is in the charged state, which is non-toxic. #4 Table 34 shows the different end pH values of effluents from the anaerobic storage of various mixtures of livestock urine, feces and bedding, demonstrating the dependence of the stored waste acidity on the composition of the waste stream feeding it. The results of this study underline the importance of both virus type and pH as determinants of viral survival during anaerobic storage of livestock waste at ambient temperatures. #7

Table 34. $D_{90}$ in days of anaerobic storage at 10°C to 20°C #7

| **Waste Type** | **pH** | **f2 coli-phage** | **bovine enceph-alomyo-carditis virus** | **bovine rotavirus** | **bovine parvo-virus** | **bovine adeno-virus** | **bovine herpes virus** |
|---|---|---|---|---|---|---|---|
| cattle feces and urine, cleaning water, and sub-stantial amounts of bedding material | 6.9 | 34.7 | 78.1 | 197 | 38 | 22.9 | <2.5 |
| swine feces and urine, cleaning water, and sub-stantial amounts of bedding material | 7.4 | 23 | 28.9 | 104 | 29.3 | 37.3 | <2.5 |
| mixture of all three types of cattle and swine wastes | 7.9 | 27.3 | 44.6 | 61.8 | 24.5 | 34.9 | <2.5 |
| cattle urine, cleaning water, and minor amounts of bedding material | 8.7 | 9.4 | 8.2 | 27.5 | 20.3 | 8.4 | <2.5 |

Though not as precise as decimal reduction times, times for total inactivation were reported by #109 for a range of temperatures. (Table 35). These results illustrate the wide variability in temperature sensitivity of livestock viruses and serve as a caution against over-confidence in the abilities of mesophilic and thermophilic digestion to produce a pathogen free effluent.

Table 35. Inactivation Times* for Animal Viruses in Slurry at Various Storage and Digestion Temperatures #109

| Disease and Storage Medium | 5°C | 20°C | 35°C | 40°C | 45°C | 50°C | 55°C |
|---|---|---|---|---|---|---|---|
| Swine influenza, pig slurry | 9 wks | 2 wks | | | | | 1 hr |
| Porcine parvovirus, pig slurry | >10 mo | >10 mo | 21 wks | 9 wks | | 5 da | 8 da |
| Bovine virus diarrhoea, cattle sl. | 3 wks | 3 da | 3 hr | 50 min | 20 min | 5 min | 5 min |
| Bovine rhinotracheitis, cattle sl. | > 1 mo | 2 da | 1 da | 3 hr | 90 min | 40 min | 10 min |
| Aujeszky's disease, pig slurry | 15 wks | 14 da | 5 hr | 2 hr | 45 min | 20 min | 10 min |
| Foot & mouth disease, cattle sl | >14 wks | 2 wks | 1 da | 10 hr | 5 hr | 1 hr | 1 hr |
| Foot & mouth disease, pig slurry | | 5 wks | | | | | |
| Swine fever, pig slurry | > 6 wks | 2 wks | 4 hr | | | | |
| TGE in pigs, pig slurry | > 2 mo | 2 wks | 1 da | | 2.5 hr | 1 hr | 30 min |

* > indicates that time of complete inactivation was not reached at conclusion of test

*Viral Recovery on Separated Solids*

Derbyshire *et al.* #25 examined viral survival in the various liquids and solids associated with a mesophilic MRS digester. The study unit was a 375 sow farrow to finish operation with slatted floors from which manure was collected into a holding pit with a submersible mixer. The raw liquid manure was pumped daily into a 6.6 m diameter by 6.2 m high cylindrical, insulated, digestion tank. The contents of the tank were continuously mixed and maintained at 35°C. Digester effluent was withdrawn continuously for 8 to 16 hours each day. The effluent was pumped through a vibrating screen separator, and the screened digester solids were used as soil conditioner. Anaerobic digestion residue was removed from the screened digester effluent by a 30 centimeter long solid bowl centrifuge, stored as a high-moisture cake and blended with other ingredients for refeeding to fattening pigs. The watery centrifuge centrate remaining after recovery of the digestion residue was pumped into a storage tank and subsequently distributed onto farm land.

Viral recovery measurements taken during various steps in this process are found in Table 36.

Table 36. Isolation of Porcine Enterovirus from an Anaerobic Digestion System for Liquid Pig Manure #25

| | June | July | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Raw manure | | | + | + | + | + | + | + | + | 7 |
| Digester effluent | + | + | + | + | + | | | | | 5 |
| Screened digester solids | + | | | | | | | | + | 2 |
| Screened digester effluent | | + | + | + | + | | | | | 4 |
| Anaerobic digestion residue | | | + | | | | | | | 1 |
| Centrifuge centrate | | + | | | | + | + | | | 3 |
| Storage tank liquid | | + | | | | | | | | 1 |

Table 36 shows that enteroviruses were isolated more often from the raw liquid manure than any of the processed materials. These findings suggest significant reductions in viral infectivity, as a result of the anaerobic digestion and subsequent processing, for those materials which are utilized for distribution on the land or for refeeding to livestock, especially the solids.

*Discussion and Conclusions*

Virus inactivation in animal wastes stored under nonaerated conditions is an complex event. It is ultimately controlled by the inherent stability of the different viruses, by their association with suspended solids, by ambient temperature, and pH, by volatile and dissolved microbial metabolites, and possibly by added detergents[5] and disinfectants. #7

High temperature (above 33°C) #24, #86 , ammonia concentration, and virus type are the three major factors responsible for microbial inactivation in anaerobic digesters. #17, #18

[5]While ionic detergents accelerate inactivation of certain enteric viruses (Reoviridae), they protect others. Poliovirus and other enteroviruses were shown to be inactivated much more slowly in the presence of ionic detergents.#17

PsychrophilicTemperature

Temperature has been well established as a primary influence in determining rates of virus inactivation. In general viruses survive longer at low temperatures and are inactivated by heat. #14

Anaerobic storage of slurries at ambient temperatures result in $D_{90}$ values in the range of weeks or months (#38). Temperatures of 32°C or below do not destroy viruses rapidly. #72

EC guidelines recommend that manures to be spread on grazing land should be stored 60 days during the summer and 90 days during the winter before application. This review shows that these times are not sufficient for some viruses, especially the non-enveloped viruses such as rotaviruses, enteroviruses, and adenoviruses, as well as the thermoresistant parvoviruses.#7

Mesophilic and Thermophilic Temperature

As with bacteria, viral decimal reduction times for mesophilic anaerobic digestion fall in the range of days and for thermophilic temperatures in the range of hours or minutes.

Mesophilic digestion does not usually produce complete viral inactivation. #18 Enteroviruses and reoviruses are often detected in sludge from mesophilic (30 to 35°C) anaerobic digesters with retention times from 20 to 40 days. #72 Some viruses can even withstand 50 days at thermophilic 50°C in sludge. #89 #72 It has been concluded that, despite the best efforts at sludge digestion, total elimination of viruses from the sludges is probably not possible and low levels of viruses will probably be introduced into soil systems via land application. #88

The rates of virus inactivation at 30 to 35°C range between 50 and 99% per day #24, #16, #85 The rates of viral inactivation during anaerobic digestion can increase to 99.99999% per day when the digestion is performed at 50°C #86. The inactivation rate of enteroviruses can be expected to be 2 minutes per log unit at 80°C, and for many strains, considerably less, whereas, for parvovirus it may be 16 minutes per log unit of virus at 80°C, a very slow rate of inactivation.#36

Virus Type

Inactivation times depend strongly on the type of virus. #7, #14, #36 The influence of temperature is greater on enteroviruses than on parvovirus. Different kinds of viruses, even different strains of the same virus, have different temperature-dependence, the parvoviruses being the most resistant at higher temperatures. Adenoviruses are more sensitive than enteroviruses. #29

## Ammonia and pH

Enteric viruses are generally most stable near pH 7 but are more stable at low pHs (3 to 5) than at alkaline pHs (9 to 12).#14 #17 The sensitivity to high pHs is due almost exclusively to the presence of uncharged ammonia. (Increases in pH causes ammonium ions to be partially converted into aqueous ammonia, which inactivates poliovirus and other enterovirus.)

The microbe-inactivating capabilities of ammonia are greater at pH values >8, when the ammonia is in the uncharged state.#24 #65 For example, at pH 7, more than 99% of ammonia in $NH_4Cl$ is in the charged state; this value decreases to 95% at pH 8, 59% at pH 9, and 13% at pH 10. The ammonium ion has no detectable virucidal activity against poliovirus, but the uncharged form of ammonia is extremely active. The rate of enterovirus inactivation in digester supernatant has been shown to increase with increasing concentrations of ammonia. This relationship is linear and holds true for ammonia concentrations of up to 1500 mg/l. #87

Ward and Ashley #65 tested three strains of poliovirus, two strains of coxsackievirus, and one strain each of ECHO virus 11 and reovirus 3. Of these only one, the reovirus was relatively somewhat more resistant to inactivation by $NH_3$. All have single stranded RNA, except for the reovirus, which has double-stranded RNA.

## Field Scale vs Lab Scale Studies

Laboratory results predicting the fate of viruses during experimental sludge treatments have not always been consistent with observations of full scale digesters. The mixing patterns of sludge in large digesters seem not to be completely predictable and probably contribute to the variable results. #18

Furthermore, there seems to be a difference in the sensitivity of indigenous viruses and experimentally seeded laboratory strains. Berg and Berman #32 observed that mesophilic digestion reduced the numbers of viruses indigenous to raw sludges by about 90% in 20 days, but laboratory strains of viruses seeded into sludges usually have been reduced in numbers by about 90% in 1 to 4 days. Thus data gathered with laboratory strains of seeded viruses appear to overestimate considerably the rate at which viruses indigenous to raw sludges are inactivated by mesophilic digestion.

PROTOZOA AND METAZOA ("PARASITES")

In medical usage, the term"parasite" encompasses eukaryotic single and multicellular pathogens, primarily protozoa and helminths (worms). Some pathogenic parasites have stages in their life cycle which are adapted to survival outside of the host and are resistant to extreme environmental conditions. This adds to the concern of transmitting parasites through the disposal of wastes to land. Numerous species of protozoan and metazoan parasites are transmitted through feces (#84).

Table 37. Common Pathogenic Protozoa and Helminths Found in Animal Wastes #90

| **Cattle Slurry** | **Pig slurry** | **Poultry waste** |
|---|---|---|
| PROTOZOA | PROTOZOA | *PROTOZOA* |
| *Eimeria* oocysts | *Eimeria* | *Eimeria* |
| *Cryptosporidium* sp. | *Balantidium* | *Histomonas* |
| *Giardia* sp. | | |
| HELMINTHS | HELMINTHS | HELMINTHS |
| *Fasciola hepatica* | *Ascaris* | *Ascaridia* |
| *Stronglyoides papillosus* | *Oesophagostomum* | *Heterakis* |
| *Oesophagostomum* sp. | *Strongyloides* | *Capillaria* |
| *Dictylocaulus viviparus* | *Hyostrongylus* | |
| *Trichuris* sp. | *Trichuris* | |
| *Dictyocaulus viviparus* | *Fasciola* | |
| *Dicrocoelium dendriticum* | | |
| *Moniezia* sp. | | |
| *Toxocara vitulorum* | | |
| *Trichostrongyle* sp. | | |

Parasites are an important cause of morbidity and mortality in domestic animals and of considerable economic importance to the farming industry. Helminths are generally the most significant in terms of pathogenicity, but there are a number of protozoa which are of both veterinary and public health importance. #106

Of the parasitic protozoa, coccidian parasites can be a significant cause of disease and death in young cattle, sheep and poultry raised under intensive conditions. These animals may be infected with many different species of coccidia of varying pathogenicity. The majority belong to the genus *Eimeria*, which are characteristically highly host-specific. For example, sheep eimerian species do not infect goats. Protozoan parasites also exist which show little host specificity and which may be capable of producing widespread environmental contamination because of the potentially large host population. Contamination may possibly be increased by the spread of sewage, slurry or manure on land. #106

*Protozoa*

In the United States, *Giardia* is the most commonly recognized cause of waterborne disease in humans although *Cryptosporidium* is the cause of the largest outbreaks.#104[6]

Factors such as heavy rainfall leading to agricultural washoff, high turbidity in raw water, and problems with water treatment and distribution, are typical risk factors leading to waterborne epidemics of protozoan diseases. #104 Due to episodic surges in protozoan water contamination and the limitations of standard water treatment processes, water producers are currently not able to guarantee freedom from risk of parasites in all supplies at all times.

Apart from the potential zoonotic reservoir of *Giardia* and *Cryptosporidia* in livestock, high levels of infection in young animals can be clinically significant and any resultant effect on weight gain will inflict an important economic loss to agriculture. #12

Reduction in the level of environmental contamination by these protozoans through waste treatment would lessen the likelihood of both animal and human exposure.

*Cryptosporidium*

Parasites of the coccidial genus *Cryptosporidium* are small intracellular parasites which have been reported in more than 40 species of mammals, birds, reptiles and fish. Infection in man and domestic livestock, particularly calves, is usually associated with *Cryptosporidium parvum*. Cryptosporidiosis is predominantly found in young calves, less than 3 weeks old. Disease associated with *Cryptosporidium parvum* has also been reported in neonates of sheep, goats, and deer, and in non-domestic ruminants including antelope and oryx. Pigs, goats, and horses can also be infected. Most porcine cryptosporidial infections are asymptomatic with the majority of infections occurring in 6 to 12 week old pigs. #106 Other hosts include chickens, turkeys, raccoons, foxes, coyotes, beavers, muskrats, and squirrels. #94, #9 There are a few reports of

---

[6]In the spring of 1993, the largest documented waterborne disease outbreak in U. S. history occurred in Milwaukee, Wisconsin. An estimated 403,000 people developed watery diarrhoea after drinking municipal water contaminated with *Cryptosporidium parvum*. The water intakes of the treatment plants involved were on Lake Michigan. The source of oocysts in the lake remain speculative. The outbreak appears to have been an unfortunate confluence of several events. Heavy rainfall during early spring likely resulted in higher than usual levels of organic material, including cow manure that had been spread on fields within the watershed, being washed into streams that flow into Milwaukee's rivers. Other possible sources of oocysts include a slaughterhouse and meat packing plant in central Milwaukee and the sewage treatment plant, which is located at the confluence of Milwaukee's three rivers as they flow into Lake Michigan. #105

*Cryptosporidium* infections in companion animals. Dogs, cats and other pets are occasionally infected but they do not seem to be an important source of infection to other hosts. The avian cryptosporidia appear to have a high host specificity for birds. The *Cryptosporidium* infection rates of some important groups of domestic livestock are found in Table 38.

In man, person-to-person transmission is now recognized to be common, indicating that cryptosporidiosis is not always a zoonosis. Zoonotic transmission has been reported from calves and lambs to humans. #106

Table 38. Rates of Infection of Domestic Livestock with *Cryptosporidium* and *Giardia*

| | ***Cryptosporidium*** | ***Giardia*** | **Reference** |
|---|---|---|---|
| US calves without diarrhea* | 11% | 46% | #9 |
| US calves (with and without diarrhea) | 31% | 73% | #9 |
| Canadian sheep | | 18% | #12 |
| Canadian lambs | | 36% | #12 |
| Canadian cattle | | 10% | #12 |
| Canadian calves | | 28% | #12 |

*The epidemiological significance of this group is that a proportion of healthy as well as sick calves shed these pathogens.

#11 *Cryptosporidium* was first conclusively recognized as an agent of human waterborne disease in 1987. Like *Giardia*, cryptosporidia form environmentally resistant oocysts. Though reduced by chlorination and ozonation, current treatment practices do little to inactivate waterborne *Cryptosporidium*.

Cryptosporidium oocysts are resistant to most disinfectants used in hospitals and laboratories and can survive for months if kept cool and moist. #95

No effective specific treatment for cryptosporidiosis has been found. Prevention in animals is primarily aimed at limiting exposure. In animals the primary route of infection is likely to be the direct animal-to-animal fecal-oral route. Heavy infections can lead to high levels of environmental contamination which influences the rate of infection in susceptible hosts. #106

## *Giardia*

*Giardia lamblia* are flagellated protozoans which cause diarrhea among humans, dogs, cats, calves, and horses. #9 *Giardia* was first documented in 1966 as causative agents of human waterborne intestinal disease in the United States. Since that time, numerous outbreaks of giardiasis have occurred throughout the United States. During the period of 1971-1985, ninety two waterborne outbreaks were attributed to *Giardia* affecting over 24,000 individuals #11 and Table 2. *Giardia* is currently the predominant cause of waterborne illness, accounting for more than 50% of the identified cases.

*Giardia* have been isolated from a variety of mammalian, avian, reptilian, amphibian, and fish hosts. *Giardia* has been reported in cattle, sheep, goats, horses, dogs, cats, rodents, and psittacines (parrot family). *#106*

#106 There are three structural types of *Giardia*. The species affecting man and the majority of domestic animals is *Giardia lamblia* (also called *G. intstinalis* or *G. duodenalis*). The other morphologically different species are *Giardia muris* identified in rodents, birds, and reptiles and *Giardia agilis* from amphibians.

*Giardia lamblia* exists in two morphologically distinct forms, the trophozoite and the cyst. The infective cyst form is transmitted via the fecal-oral route. #5

*Giardia* infections in many species of animals are often asymptomatic. The host specificity of *Giardia* is still undecided, and as such the zoonotic potential of animal infection is the subject of much research. #106

Limited epidemiological studies suggest that direct animal-to-animal transmission is the most likely method of transmission, although water contamination can also be considered as a possible route. Human infection in the USA has been reported from drinking water contaminated with *Giardia* thought to have been shed by beavers #91 (or, as #104 observed, the giardiasis cases could have resulted from beavers transmitting infection which the beavers previously had acquired from water polluted by man.) Other animals may act as reservoirs of infection. The role of farm animals in the overall epidemiology of human giardiosis has yet to be investigated, though there is circumstantial evidence implicating them. #106

Studies in Canada indicate infections in sheep and cattle of 18% and 10% respectively (Table 38). Animal young are infected at somewhat higher rates. A more recent study in the UK indicated that nearly 70% of a flock of lambs to be infected with *Giardia* although the presence of the organism was not necessarily associated with clinical signs of disease. #106

*Helminths*

The two parasitic helminth phyla of most veterinary concern are Platyhelminthes (flat worms), especially classes Trematoda (flukes) and Cestoidea (tapeworms) and phylum Nemathelminthes (round worms or thread worms), especially of the class Nematoda.

Highly specific parasites such as the nodular worms of pig (*Oesophagostomum* sp.) will cause trouble in only the host species. Parasites with a wide range of hosts like the liver fluke (*Fasciola hepatica*) are a hazard to a variety of hosts. #90

While aquatic systems can be the vehicle for transmitting helminthal pathogens to humans,

modern water treatment methods are very effective in destroying these organisms. Thus, helminths pose hazards primarily to those persons who come into direct contact with untreated water. Sewage plant operators, swimmers, and farm laborers are at particular risk. #91

Helminth survival in cattle slurry varies between different species. Long survival under certain conditions enables some parasitic agents to accumulate in the environment of the excreters.#90

*Compilation of Parasite Die-Off Studies*

Table 39. Decimal Reduction Times for Protozoans and Helminths Stored Anaerobically in Animal Waste

| **Temperature** | **$D_{90}$** | **Organism** | **Reference** |
|---|---|---|---|
| PSYCHROPHILIC | | | |
| 4°C | >40 days | *Cooperia oncophora* eggs | #22 |
| 4°C | 28 days | *Dictyocaulus viviparus* larvae | #22 |
| 5°C | 130 days | *Giardia* cysts | #5 |
| 8°C | 4 days | *Strongyloides papillosus* 1st to 3rd stage larvae | #90 |
| 8°C | 64 days | *Trichostrongylus colubriformis* egg | #90 |
| 8°C | 76 days | *Trichostrongylus colubriformis* 3rd stage larvae | #90 |
| 8°C | >76 days | *Taenia pisiformis* eggs | #90 |
| 8°C | >76 days | *Fasciola hepatica* | #90 |
| 8°C | >85 days | *Ascaris suum* eggs | #90 |
| 15°C | <30 days | *Giardia* cysts | #5 |
| 18°C | 4 days | *Strongyloides papillosus* 1st to 3rd stage larvae | #90 |
| 18°C | 26 days | *Trichostrongylus colubriformis* eggs | #90 |
| 18°C | 37 days | *Trichostrongylus colubriformis* 3rd stage larvae | #90 |
| 18°C | 37 days | *Taenia pisiformis* eggs | #90 |
| 18°C | >55 days | *Fasciola hepatica* eggs | #90 |
| 18°C | >55 days | *Ascaris suum* eggs | #90 |
| 20°C | 17 days | *Dictyocaulus viviparus* larvae | #22 |
| 20°C | 20 days | *Cooperia oncophora* eggs | #22 |
| 22 - 27°C | >57 days | *Ascaris suum* eggs | #42 |
| MESOPHILIC | | | |
| 35°C | 2.5 days | *Cooperia oncophora* eggs | #22 |
| 35°C | <7 days | *Dictyocaulus viviparus* larvae | #22 |
| THERMOPHILIC | | | |
| 55°C | 7 - 10 min. | *Ascaris* eggs | #42 |
| 55°C | <10 min. | *Eimeria* sp. | #22 |
| 55°C | <10 min. | *Trichostrongyle* larvae | #42 |

*Discussion and Conclusions*

It appears that short term digestion at psychrophilic temperatures is of little value in the elimination of parasites from animal slurry. Detention times on the order of months to years would be required to achieve a 3 $\log_{10}$ unit reduction in these organisms (Table 39).

Downey and Moore #51 reported reduction of viable trichostrongylid eggs occurs in slurry during storage. Such eggs disappeared after two months storage in summer and after three to four months storage in winter. They concluded that for this reason, ideally, spring application of slurry from trichostrongylid contaminated herds to pastures or grassland was not advisable, it being preferable to delay application until July at the earliest.[7]

#22 Anaerobic digestion at thermophilic temperatures accelerates the inactivation of cattle nematodes. At temperatures of 50-60°C, semi-continuously run reactors will inactivate most bovine nematodes. However, temperatures of 30-37°C are too low to ensure that nematodes are killed.

Unfortunately, there is a dearth of published die-off rates during anaerobic digestion of manure for the two protozoan genera of highest water quality concern, *Giardia* and *Cryptosporidium*, especially in the mesophilic and thermophilic temperature ranges. Considering the potential benefit to be derived from reducing the spread of these parasites via animal manures, this research deserves support.

## PLANT PATHOGENS

The literature is sparse on the subject of the destruction of plant pathogens by anaerobic digestion. That is why they are singled out in this section, instead of being included in the animal pathogen sections.

It appears that plant pathogens may be somewhat more sensitive to anaerobic digestion at 35°C than animal pathogens, though there is not enough information presented here for accurate generalization.

---

[7]The transmission of helminth larvae to grass and subsequently to grazing stock has been demonstrated for bovine intestinal trichostrongylids #51, #22. Application of cattle slurry to pastures caused calves grazing such pastures to acquire higher worm burdens and to gain less weight than calves on naturally contaminated control pastures.

Turner *et al.* #61 determined the sensitivity of three plant pathogens to mesophilic anaerobic digestion in animal slurry. The pathogens were a fungus (*Fusarium oxysporum*: a dikaryotic fungus known to cause wilt and root rot of tomatoes, carnations and rice), a bacterium (*Corynebacterium michiganense*: a bacterial pathogen known to cause vascular wilt, canker and leaf and fruit spot of tomatoes, potatoes and tobacco), and a nematode (*Globodera pallida*: The potato root eelworm: a nematode worm which destroys the root tissues of its host plant), Table 40.

The destruction of plant pathogens by anaerobic digestion is important if diseased plant residues are digested with slurry and the effluent is subsequently applied to crop land.

Table 40. Approximate $D_{90}$ Times of Plant Pathogens at 35°C ± 2°C Anaerobic Digestion, Lab Study #61

| **Organism** | **$D_{90}$** |
|---|---|
| *Fusarium oxysporum* | 1 day |
| *Corynebacterium michiganense* | 17 hours |
| *Globodera pallida* | 6 hours |

**REFERENCES**

#1 Boersma, L., E. Gasper, J. E. Oldfield and P. R. Cheeke (1981) Methods for the recovery of nutrients and energy from swine manure. 1. Biogas. *Neth. J. agric. Sci.* 29, p 3-14.

#2 Pos, J., R. H. teBoekhorst, B. Walczak and V. Pavlicik (1981) Design and operation of a 1400 litre anaerobic digester utilizing swine manure. *Notes-Agric. Guelph: Ontario Agricultural College* v. 17(4) p.8-17.

#3 Mills, P. J. (1977). A comparison of an anaerobic digester and an aeration system treating piggery waste from the same source. IN *Food, Fertilizer and Agricultural Residues; Proceedings of the Cornell Agricultural Waste Management Conference*. Raymond C. Loehr, *ed.* Ann Arbor Science Pub. Ann Arbor, MI, p.415-422.

#4 Mateu, A., J. Mata-Alvarez, and R. Parés (1992) Enterobacterial and Viral Decay Experimental Models for Anaerobic Digestion of Piggery Waste. *Applied Microbiology and Biotechnology* , v.38, p.291-296.

#5 Deng, Ming Yi and D. O. Cliver (1992) Degradation of *Giardia lamblia* Cysts in Mixed Human and Swine Wastes, *Applied and Environmental Microbiology,* v. 58, p. 2368-2374.

#6 Olsen, J. E. and H. E. Larsen (1987) Bacterial Decimation Times in Anaerobic Digestion of Animal Slurries, Biological Wastes, v. 21, p.153-168.

#7 Pesaro, F., I. Sorg, and A. Metzler (1995) *In Situ* Inactivation of Animal Viruses and a Coliphage in Nonaerated Liquid and Semiliquid Animal Wastes, *Applied and Environmental Microbiology* v.61(1), p. 92-97.

#8 Fischer, J. R., M. R. Gebhardt, and C. D. Fulhage (1984) Effluent from a Swine Manure Anaerobic Digester as a Fertilizer for Corn. *Papers of the American Society of Agricultural Engineering*, St. Joseph, MI.

#9 USDA Animal and Plant Health Inspection Service, Veterinary Services (1994) *Cryptosporidium* and *Giardia* in Beef Calves. Ft. Collins CO.

#10 Wrigley, T. J., K. M. Webb and H. Venkitachalm (1992) A Laboratory Study of Struvite Precipitation after Anaerobic Digestion of Piggery Wastes. *Bioresource Technology*, v.41, p.117-121.

#11 LeChevallier, M. W., W. D. Norton, R. G. Lee, and J. B. Rose (1991) *Giardia and Cryptosporidium in Water Supplies*. AWWA Research Foundation, Denver, CO.

#12 Thompson, R. C. A. and J. A Reynoldson (1993) *Giardia* and Giardiasis, IN *Advances in*

*Parasitology* , Academic Press, v.32, p.71-160.

#13 Stelma, Jr., G. N. and L. J. McCabe (1992) Nonpoint Pollution From Animal Sources and Shellfish Sanitation, *J. Food Protection,* v.55(8) p.649-656.

#14 Deng. M. Y. and D. O. Cliver (1995) Persistence of Inoculated *Hepatitis* A Virus in Mixed Human and Animal Wastes. *Applied and Environmental Microbiology*, v. 61(1), p. 87-91.

#15 Kearney, T. E, M. J. Larkin, and P. N. Levett (1994) Metabolic activity of Pathogenic Bacteria during Semicontinuous Anaerobic Digestion, *Applied and Environmental Microbiology*, v. 60(10), p. 3647-3652.

#16 Bertucci, J. J., C. Lue-Hing, D. Zenz, S. J. Sedita (1977) Inactivation of Viruses during Anaerobic Sludge Digestion, *J. Water Pollution Control Federation,* v. 49(7) p. 1642-1651.

#17 Ward, R.L. (1987) Mechanism of Virus Inactivation, Chapter 8, IN *Wastewater Sludges in Human Viruses in Sediments, Sludges, and Soils*, V. C. Rao and J. L. Melnick, eds., CRC Press, Inc., Boca Raton.

#18 Cliver, D.O. (1987) Fate of Viruses during Sludge Processing, Chapter 9, IN *Wastewater Sludges in Human Viruses in Sediments, Sludges, and Soils*, V. C. Rao and J. L. Melnick, eds., CRC Press, Inc., Boca Raton.

#19 Bertucci, J. J., S. J. Sedita, and C. Lue-Hing (1987) Viral Aspects of Applying Sludges to Land, Chapter 10, IN *Wastewater Sludges in Human Viruses in Sediments, Sludges, and Soils*, V. C. Rao and J. L. Melnick, eds., CRC Press, Inc., Boca Raton.

#20 Larsen, H. E. and B. Munch (1981) Occurrence and Survival of Pathogenic Bacteria in Cattle and Pig Slurry, IN *Communicable Diseases Resulting from Storage, Handling, Transport and Landspreading of Manures*, J. R. Walton and E. G. White, *eds.* Commission of the European Communities, Hanover, p. 161-174.

#21 Jones, P. W. (1981) A Short Survey of the Most Important Pathogens Found in Farm Animal Wastes, IN *Communicable Diseases Resulting from Storage, Handling, Transport and Landspreading of Manures*, J. R. Walton and E. G. White, *eds.* Commission of the European Communities, Hanover, p. 139-147.

#22 Olsen, J. E. and P. Nansen (1987) Inactivation of Some Parasites by Anaerobic Digestion of Cattle Slurry, *Biological Wastes*, v.22, p.108-114.

#23 Singh, L., M. Sai Ram, S. I. Alam, M. S. Maurya (1995) Inactivation of Pathogens During Aerobic and Anaerobic Treatments at Low Temperatures, *Bulletin of Environmental Contamination and Toxicology*, v. 54, P. 472-478.

#24 Hurst, C. J. (1989) Fate of Viruses During Wastewater Sludge Treatment Processes, CRC Critical Reviews in Environmental Control, v.18(4), p. 317-343.

#25 Derbyshire, J. B. , H. D. Monteith, and E. E. Shannon (1986) Virological Studies on an Anaerobic Digestion System for Liquid Pig Manure, *Agricultural Wastes*, V.19 p.309-312.

#26 Snowdon, J. A., D. O. Cliver and J. C. Converse (1989) Inactivation of Poliovirus 1 as a Function of Temperature in Mixed Human and Dairy Animal Wastes, *Waste Management & Research*, v. 7, p. 135-142.

#27 Snowdon, J. A., D. O. Cliver, and J. C. converse (1989) Land Disposal of Mixed Human and Animal Wastes: A Review, *Waste Management & Research*, v. 7, p.121-134.

#28 Munch, B., H. E. Larsen and B. Aalbaæk (1987) Experimental Studies on the Survival of Pathogenic and Indicator Bacteria in Aerated and Non-aerated Cattle and Pig Slurry, *Biological Wastes* v. 22, p. 49-65.

#29 Lund, E. (1981) The Survival of Enteric Viruses in Aerated and Non-aerated Cattle and Pig Slurry, IN *Communicable Diseases Resulting from Storage, Handling, Transport and Landspreading of Manures*, J. R. Walton and E. G. White, *eds*. Commission of the European Communities, Hanover, p. 149-155.

#30 Roos, K. F. (1994) The AgSTAR Program: Energy for Pollution Prevention, Proceedings of the 2nd Conference on Environmentally Sound Agriculture, ASAE, Orlando.

#31 Reddy, K. R., R. Khaleel, and M. R. Overcash (1981) Behavior and Transport of Microbial Pathogens and Indicator Organisms in Soils Treated with Organic Wastes, *J. Environ. Quality*, v. 10(3) p. 255-264.

#32 Berg, G. and D. Berman (1980) Destruction by Anaerobic Mesophilic and Thermophilic Digestion of Viruses and Indicator Bacteria Indigenous to Domestic Sludges, Applied and Environmental Microbiology, v. 39(2) p. 361-368.

#33 Larsen, H. E., B. Munch and J. Schlundt (1994) Use of Indicators for Monitoring the Reduction of Pathogens in Animal Waste Treated in Biogas Plants, *Zentralblatt fur Hygiene und Umveltmedizin*, v. 195(5), p. 544-555.

#34 Kearney, T. E., M. J. Larkin and P. N. Levett (1993) The Effect of Slurry Storage and Anaerobic Digestion on Survival of Pathogenic Bacteria, Journal of Applied Bacteriology, v. 74, p. 86-93.

#35 Farrah, S. R. and G. Bitton (1983) Bacterial Survival and Association with Sludge Flocs During Aerobic and Anaerobic Digestion of Wastewater Sludge Under Laboratory Conditions,

*Applied and Environmental Microbiology*, v. 45(1), p. 174-181.

#36 Lund, E. and B. Nissen (1983) The Survival of Enteroviruses in Aerated and Unaerated Cattle and Pig Slurry, *Agricultural Wastes*, V. 7, p. 221-233.

#37 Derbyshire, J. B. and E. G. Brown (1978) Isolation of Animal Viruses from Farm Livestock Waste, *J. Hygiene, Cambridge*, v. 81, p. 295-302.

#38 Larsen, H. E. and B. Munch (1986) Pathogenic Bacteria in Extra-animal Environments: Occurrence, Survival, and Hygienic Significance with Special Reference to Farm Animal Waste Management, A Review, *Ugeskrift for Jordbrug, Selected Research Reviews*, p. 57-66.

#39 Farrah, S. R., G. Bitton, E. M. Hoffmann, O. Lanni, O. C. Pancorbo, M. C. Lutrick, and J. E. Bertrand (1981) Survival of Enteroviruses and Coliform Bacteria in a Sludge Lagoon, *Applied and Environmental Microbiology*, v. 41(2), p. 459-465.

#40 Burge, W. D. and J. F. Parr (1980) Movement of Pathogenic Organisms from Waste Applied to Agricultural Lands, IN *Environmental Impact of Nonpoint Source Pollution*, M.R. Overcash and J. M. Davidson, eds., Ann Arbor Science Publishers, Ann Arbor, p.107-127.

#41 Lund, E. (1983) Inactivation of Viruses under Anaerobic or Aerobic Stabilization in Liquid Manure and in Sludge from Sewage Treatment Plants, IN *Hygienic Problems of Animal Manures: Proceedings of a Joint Workshop of Expert Groups of the Commission of the European Communities, German Veterinary Medical Society, and Food and Agricultural Organization*, S. Strauch, ed., Stuttgart, p. 199-209.

#42 Plym-Forshell, L. (1983) Survival of Salmonella Bacteria and *Ascaris suum* Eggs in a Thermophilic Biogas Plant, IN *Hygienic Problems of Animal Manures: Proceedings of a Joint Workshop of Expert Groups of the Commission of the European Communities, German Veterinary Medical Society, and Food and Agricultural Organization*, S. Strauch, ed., Stuttgart, p. 217-222.

#43 Munch, B. and J. Schlundt (1983) On the Reduction of Pathogenic and Indicator Bacteria in Animal Slurry and Sewage Sludge Subjected to Anaerobic Digestion or Chemical Disinfection, IN *Hygienic Problems of Animal Manures: Proceedings of a Joint Workshop of Expert Groups of the Commission of the European Communities, German Veterinary Medical Society, and Food and Agricultural Organization*, S. Strauch, ed., Stuttgart, p. 130-149.

#44 National Academy of Sciences (1977) *Methane Generation from Human, Animal, and Agricultural Wastes*, Chapter 4, Washington, D. C.

#45 Cliver, D. O. (1980) Agricultural crops: Pathogens, in *Sludge -- Health Risks of Land Application*, G. Bitton, B. L. Damron, G. T. Edds and J. M. Davidson, eds., Ann Arbor Science,

Chapter 6, p. 141-152.

#46 Chandler, D. S. and J. A. Craven ( 1981) A note on the Persistence of *Salmonella havana* and Faecal Coliforms on a Naturally Contaminated Piggery Effluent Disposal Site, *Journal of Applied Bacteriology*, v. 51, p. 45-49.

#47 Deng, M. Y. and D. O. Cliver (1992) Inactivation of Poliovirus Type 1 in Mixed Human and Swine Wastes and by Bacteria from Swine Manure, *Applied and Environmental Microbiology*, v. 58(6), p. 2016-2021.

#48 Chandler, D. S., I. Farran, and J. A. Craven (1981) Persistence and Distribution of Pollution Indicator Bacteria on Land Used for Disposal of Piggery Effluent, *Applied and Environmental Microbiology*, v.42(3), p. 453-460.

#49 Hurst, C. J., C. P. Gerba, and I. Cech (1980) Effects of Environmental Variables and Soil Characteristics on Virus Survival in Soil, *Applied and Environmental Microbiology*, v. 40(6), p. 1067-1079.

#50 USDA Soil Conservation Service (1992) National Engineering Handbook: Agricultural Waste Management Field Handbook, Part 651, (210-AWMFH, 4/92) chapters 4 and 11.

#51 Downey, N. E. and J. F. Moore (1978) Trichostrongylid Infection on Pasture and in Calves as a Result of Slurry Application, IN *Animal and Human Health Hazards Associated with the Utilization of Animal Effluents*, W. R. Kelly, ED., p. 250-303. C. E. C. EUR 6009 EN.

#52 Vanderholm, D. H. (1975) Nutrient Losses from Livestock Waste During Storage, Treatment, and Handling, IN *Managing Livestock Wastes, Proceedings of the 3rd International Symposium on Livestock Wastes*, ASAE Publication PROC-275, p. 282-285, St. Joseph, MI.

#53 Muck, R. E. and B. K. Richards (1983) Losses of Manurial Nitrogen in Free-Stall Barns, *Agricultural Wastes*, v.7, p. 65-79.

#54 Moore, J. A., M. E. Grismer, S. R. Crane, and J. R. Miner (1983) Modeling Dairy Waste Management Systems' Influence on Coliform Concentration in Runoff, *TRANSACTIONS of the ASAE*, vol. 26(4), p. 1194-1200.

#55 Hess, E. and C. Breer (1975) Epidemiology of Salmonellae and Fertilizing Grassland with Sewage Sludge, Zbl. Bakt. Hyg., I. Abt. Orig. B 161, p.54-60.

#56 Loehr, R. C. (1973) *Pollution Implications of Animal Wastes -- A Forward Oriented Review*, U. S. E. P. A. 13040-07/68, Washington, D. C.

#57 Overcash, M. R. and F. J. Humenik (1976) *State-of-the-Art: Swine Waste Production and*

*Pretreatment Processes*, U. S. E. P. A. Environmental Protection Technology Series, EPA-600/2-76-290, Ada, OK.

#58 Nordstedt, R. A. and L. B. Baldwin (1975) Sludge Management for Anaerobic Dairy Waste Lagoons, IN *Managing Livestock Wastes, Proceedings of the 3rd International Symposium on Livestock Wastes*, ASAE Publication PROC-275, p. 535-536, St. Joseph, MI.

#59 Gadre, R. V., D. R. Ranade and s. H. Godbole (1986) A Note on Survival of Salmonellas during Anaerobic Digestion of Cattle Dung, *Journal of Applied Bacteriology*, v. 60, p. 93-96.

#60 Carrington, E. G., S. A. Harman and E. B. Pike (1982) Inactivation of *Salmonella* during Anaerobic Digestion of Sewage Sludge, *Journal of Applied Bacteriology*, v. 53, p. 331-334.

#61 Turner, J. D. A. Stafford, D. E. Hughes, and J. Clarkson (1983) The Reduction of Three Plant Pathogens (*Fusarium, Corynebacterium* and *Globodera*) in Anaerobic Digesters, *Agricultural Wastes*, v. 6, p. 1-11.

#62 Schmid, L. A., R. I Lipper, J. K. Koelliker, C. A. Cate, and J. W. Daber (1975) Separating Nutrients to Enhance Swine-Waste Digestion, IN *Managing Livestock Wastes, Proceedings of the 3rd International Symposium on Livestock Wastes*, ASAE Publication PROC-275, p. 248-251, St. Joseph, MI.

#63 Lorimor, J. C., S. W. Melvin, and B. M Leu (1975) Nutrient Characteristics of Wastes from Deep Pits and Anaerobic Lagoons, IN *Managing Livestock Wastes, Proceedings of the 3rd International Symposium on Livestock Wastes*, ASAE Publication PROC-275, p. 306-308, St. Joseph, MI.

#64 Smallbeck, D. R. and M. C. Bromel (1975) Bacterial Analysis and Land Disposal of Farm Waste Lagoon Waters, IN *Managing Livestock Wastes, Proceedings of the 3rd International Symposium on Livestock Wastes*, ASAE Publication PROC-275, p. 318-321, St. Joseph, MI.

#65 Ward, R. L. and C. S. Ashley (1977) Identification of the Virucidal Agent in Wastewater Sludge, *Applied and Environmental Microbiology*, v. 33(4), p. 860-864.

#66 Olsen, J. E., J. B. Jørgensen, and P. Nansen (1985) On the Reduction of *Mycobacterium paratuberculosis* in Bovine Slurry Subjected to Batch Mesophilic or Thermophilic Anaerobic Digestion, *Agricultural* Wastes, v. 13, p. 273-280.

#67 Loehr, R. C. (1984) *Pollution Control for Agriculture*, 2nd ed., Academic Press, Inc., Chapters 6 and 12.

#68 Minnesota Department of Agriculture (1995) *Manure Management Alternatives: A Supplemental Manual*, p. 28-30.

#69 El-Ahraf, A. and W. V. Willis (1996) *Management of Animal Waste: Environmental Health Problems and Technological Solutions*, Praeger Pbl., Westport, CT., Chapter 2.

#70 Willrich, T. L. (1966) Primary Treatment of Swine Wastes by Lagooning, *Management of Farm Animal Wastes: National Symposium on Animal Wastes.*

#71 Krieger, D. J. , J. H. Bond, C. L. Barth (1975) Survival of Salmonellae, Total Coliforms and Fecal Coliforms in Swine Waste Lagoon Effluents, IN *Managing Livestock Wastes, Proceedings of the 3rd International Symposium on Livestock Wastes*, ASAE Publication PROC-275, p. 11-14, St. Joseph, MI.

#72 Cliver, D. O. (1975) Virus Association with Wastewater Solids, *Environmental Letters*, v. 10(3), p. 215-223.

#73 Robertson, A. M., G. A. Burnett, P. N. Hobson, S. Bousfield, R. Summers (1975) Bioengineering Aspects of Anaerobic Digestion of Piggery Wastes, IN *Managing Livestock Wastes, Proceedings of the 3rd International Symposium on Livestock Wastes*, ASAE Publication PROC-275, p. 544-548, St. Joseph, MI.

#74 Roos, K. (1996) unpublished data.

#75 Hashimoto, A. G., Y. R. Chen, and V. H. Varel (1981) *Anaerobic Fermentation of Beef Cattle Manure, Final Report*, Solar Energy Research Institute, Golden, Co.

#76 Pennsylvania Department of Environmental Resources (1986) Field Application of Manure. Harrisburg, PA.

#77 Pennsylvania Department of Environmental Resources (1986a) Swine Manure Management. Harrisburg, PA.

#78 Hobson, P. N. , S. Bousefield, R. Summers, and P. Mills (1980) Anaerobic Digestion of Piggery and Poultry Wastes. *Anaerobic Digestion*,. D. A. Stafford, B. I. Wheatley and D. E. Hughes, *eds*. Applied Science Publishers, London. p. 237-250.

#79 Olsen, J. Elmerdahl, H. E. Larsen and P. Nansen (1985) Elimination of infectious agents by bio-gas digestion of animal manure. (in Danish) p. 275 København.

#80 Burrows, M. R. and J. D. Rankin (1970) *British Veterinary Journal*, v. 126, p. xxxii-xxxiv.

#81 Gudding, R. (1975) The Persistence of *Salmonella typhimurium* in Various Types of Manure With and Without Admixture of Silage Effluent. *Acta Vet. Scand.*, v. 16, p. 115-125.

#82 Dudley, K. J., M. N. Guentzel, M. J Ibarra, B. E. Moore, and B. P. Sagik (1980)

Enumeration of Potentially Pathogenic Bacteria from Sewage Sludges, *Applied and Environmental Microbiology*, v. 39(1), p. 118-126.

#83 Palfi, A. (1973) Survival of enteroviruses during anaerobic sludge digestion, in Proc. 6th Int. Conf. Advances in Water Pollution Research, Jenkins, S.J., ed., Pergamon Press, Oxford, A/13/26/1-6.

#84 Kowal, N.E. (1982) Health Effects of Land Treatment. Microbiological. Health Effects Research Laboratory, EPA-600/1-82-007, USEPA, Cincinnati.

#85 Eisenhardt, A., E. Lund, and B. Nissen (1977) The Effect of Sludge Digestion on Virus Infectivity, *Water Research*, v. 11, p. 579-581.

#86 Sanders, D. A., J. F. Malina, Jr., B. E. Moore, B. P. Sagik, and C. A. Sorber (1979) Fate of Poliovirus During Anaerobic Digestion, *Journal of the Water Pollution Control Federation*, v. 51, p. 333.

#87 Fenters, J. Reed, J. Lue-Hing, C. and Bertucci, J. (1979) Inactivation of viruses by digested sludge components, J. Water Pollut. Control Fed. 51, p.689.

#88 Sagik, B.P., B.E. Moore, and C.A. Sorber (1978) Infectious disease potential of land application of wastewater in State of Knowledge in Land Treatment of Wastewater, V. 1, USGPO, p. 35.

#89 Sattar, S. A. and Westwood, J.C.N. (1979) Recovery of viruses from field samples of raw, digested, and lagoon-dried sludges, Bull. W. H. O., v.57 p.105

#90 Bürger, H. J. and M. Stoye (1978) Identifying the Priority Contaminants Parasitological Problems Associated with Recycling of Animal Excretions, IN *Animal and Human Health Hazards Associated with the Utilization of Animal Effluents*, W. R. Kelly, ED., p. 24-33. C. E. C. EUR 6009 EN.

#91 Peavy, H. S., D. R. Rowe, G. Tchobanoglous (1985) *Environmental Engineering*, McGraw-Hill Book Company, N.Y.

#92 Crook, J. (1985) Water Reuse in California, *J. Am. Water Works Assoc.* v. 77, no. 7.

#93 Nordstedt, R. A., L. B. Baldwin, C. C. Hortenstine (1971) Multistage Lagoon Systems for treatment of Dairy Farm Waste, *Livestock Waste Management: Proc. Int. Symposium on Livestock Wastes*, St. Joseph, MI, p. 77-80.

#94 Current, W. L. (1985) Cryptosporidiosis, *J. Am. Vet. Med. Assoc.*, v. 187, no. 12, p. 1334-1338.

#95 Current, W. L. (1983) Human Cryptosporidiosis, *New England Journal of Medicine*, v. 309, p. 1326-1327.

#96 Tzipori, S. (1983) Cryptosporidiosis in Animals and Humans, *Microbiological Reviews*, v. 47, p. 84-96.

#97 Hill, D. T., H. E. Hamilton, T. A. McCaskey (1980) Operating Characteristics of Two-Cell Lagoon Systems with Recycle Treating Dairy Waste, IN *Livestock Waste: A Renewable Resource, Proceedings of the Fourth International Symposium on Livestock Wastes*, St. Joseph, MI, ASAE, p. 228-231.

#98 Fulhage, C. D. (1980) Performance of Anaerobic Lagoons as Swine Waste Storage and Treatment Facilities in Missouri, IN *Livestock Waste: A Renewable Resource, Proceedings of the Fourth International Symposium on Livestock Wastes*, St. Joseph, MI, ASAE, p. 225-227.

#99 Smith, R. E. (1980) Sludge as a Condition Indicator of Anaerobic Lagoons, IN *Livestock Waste: A Renewable Resource, Proceedings of the Fourth International Symposium on Livestock Wastes*, St. Joseph, MI, ASAE, p. 247-251.

#100 Westerman, P. W., L. M. Safley, Jr. and J. C. Barker (1990) Lagoon Liquid Nutrient Variation Over Four Years for Lagoons with Recycle Systems, IN *Proceedings of the Sixth International Symposium on Agricultural and Food Processing Wastes*, St. Joseph, MI, ASAE, p. 41-49.

#101 Barth, C. L. and J. Kroes (1985) Livestock Waste Lagoon Sludge Characteristics, IN *Agricultural Waste Utilization and Management, Proceedings of the Fifth International Symposium on Livestock Wastes*, St. Joseph, MI, ASAE, p. 660-671.

#102 Koelliker, J. K. and J. R. Miner (1973) Desorption of Ammonia from Anaerobic Lagoons, *Transactions of the ASAE*, vol. 16, p. 148-151.

#103 Doran, J. W., J. R. Ellis and T. M. McCalla (1976) Microbial Concerns When Wastes are Applied to Land, IN *Land as a Waste Management Alternative, Proceedings of the 1976 Cornell Agricultural Waste Management Conference*, R. C. Loehr, ED., Ann Arbor Science Publishers, Ann Arbor, MI, p. 343-361.

#104 Casemore, D. P. (1995) The Problem with Protozoan Parasites, IN *Protozoan Parasites and Water*, W. B. Betts, D. Casemore, C. Fricker, H. Smith, and J. Watkins, EDS., Royal Society of Chemistry, Cambridge.

#105 Addiss, D, G., W. R. MacKenzie, N. J. Hoxie, M. S. Gradus, K. A. Blair, M. E. Proctor, J. J. Kazmierczak, W. L. Schell, P. Osewe, H. Frisby, H. Cicerello, R. L. Cordell, J. B. Rose, and J. P. Davis (1995) Epidemiologic Features and Implications of the Milwaukee Cryptosporidiosis

Outbreak, IN *Protozoan Parasites and Water*, W. B. Betts, D. Casemore, C. Fricker, H. Smith, and J. Watkins, EDS., Royal Society of Chemistry, Cambridge.

#106 Taylor, M. A. (1995) Protozoan Parasites and Water: Veterinary Aspects, IN *Protozoan Parasites and Water*, W. B. Betts, D. Casemore, C. Fricker, H. Smith, and J. Watkins, EDS., Royal Society of Chemistry, Cambridge.

#107 van der Leeden, F. , F. L. Troise, D. K. Todd (1990) *The Water Encyclopedia*, Lewis Publishers, Chelsea, MI.

## ORGANISM INDEX