

FOREWORD

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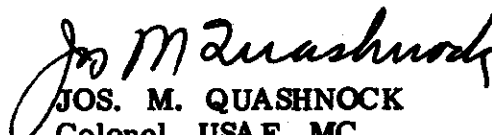
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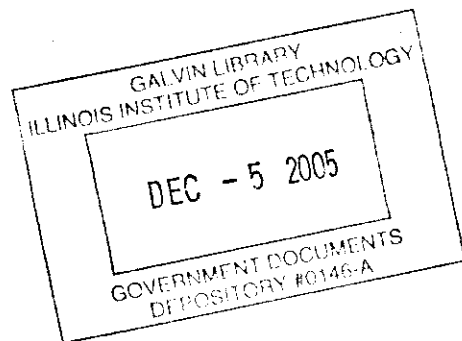
ABSTRACT

Wastes treated in a closed ecological system are discussed according to the parameters affecting composition, quantity, and microorganisms expected in the wastes. Conventional waste treatment processes, activated sludge, trickling filters, and anaerobic digestion are discussed. Microbiological considerations including lists of organisms peculiar to each system and combination of systems are presented and discussed. Results of laboratory scale experiments of conventional systems receiving concentrated waste are evaluated. Using by-products of waste treatment processes (sludge, bacterial bodies) as nutritional supplements for plants, algae, or animals in a nutrient support system for man during an extended space trip is considered. Experimental volume and power requirements are projected to those applicable under space capsule conditions. Limitations imposed on the several systems for extended space travel indicate that solids should be stored and liquids processed by other than microbiological treatment systems for space flights of less than 1 year.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.


JOS. M. QUASHNOCK
Colonel, USAF, MC
Chief, Biomedical Laboratory



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MICROBIOLOGICAL WASTE TREATMENT PROCESSES
IN
A CLOSED ECOLOGY

SUMMARY

Various conventional waste treatment processes used on earth, activated sludge, trickling filters, and anaerobic digestion, are discussed in principle and operation. The experimental status of the proposed photosynthetic-non-photosynthetic, and purely non-photosynthetic systems in a closed space is reviewed.

The amount and quality of human wastes which might be expected in an extended space flight together with the various factors, such as diet and regimen, affecting the formation of wastes are considered and discussed.

Tables listing the variety, grouping and some of the species of non-photosynthetic organisms reported in conventional waste treatment systems are given together with a consideration of source, metabolism and various factors affecting purification performance.

Waste treatment biota which might be expected in a closed space ecology relative to sources, with consideration of those in feces, is presented. Factors which might affect the microbial population in a closed system are considered.

Possible applications of non-photosynthetic systems in the treatment of concentrated human wastes are discussed and projected into a closed, extra-terrestrial, gravity free flight condition. Treatment problems which might arise and newer concepts of treatment which might be considered are examined.

The possibility of using the by-products of waste treatment processes (sludge, bacterial bodies) as nutritional supplements for plants, algae, or animals in a nutritional support system for man in an extended space flight is discussed. A consideration of the chemical composition of bacteria is presented so that some idea of inherent utility of biota as nutritional sources in support systems for man in a closed ecology can be obtained.

Conventional waste treatment processes might be adapted to the treatment of concentrated human wastes so that maximum biological recovery can be achieved within the ecological equilibrium cycle of closed space environment. The possibility of developing treatment processes different from those used on earth, and departing, to some extent, from accepted waste treatment principles should be considered.

The results of several laboratory feasibility experiments handling concentrated raw human wastes by activated sludge, trickling filter, and anaerobic digestion treatment processes are presented and evaluated.

With some reservations, activated sludge, because of its efficiency and speed, shows promise in the treatment of projected human wastes and waste materials in a closed ecology, but must be evaluated and considered further.

Trickling filters, because of the prolonged length of time necessary for conditioning, were not fully evaluated in this project. However, trickling filters represent a more diverse, mixed biota potentially capable of more dependable purification than activated sludge, since the biological combination will resist rapidly changing situations of loading and habitat to a greater degree.

Although anaerobic digestion is effective in the reduction of organic matter of human wastes, the length of time required, the nature of the gases evolved, the nature of the undesirable end products produced, and the necessity for further treatment and handling of residue solids and gases make this process unattractive as a method for the treatment of human wastes in space.

A consideration of thermophilic biological metabolism as compared with mesophilic metabolism of microorganisms and waste treatment systems is presented. Projection of thermophilic treatment processes for the handling of human wastes in a closed space system is discussed.

Summarized Comment

1. The possibility of storing feces should be considered in light of the inherent difficulties which might be encountered during waste treatment procedures.
2. It is impossible that any single species of organism can be depended upon to convert all human wastes into a usable recovery product.
3. It is practically certain that any reserve biota used for the treatment process will have to be carried in preserved form to replace viable cultures aboard the space vehicle. Most of these organisms are not found in bodily wastes at discharge, but are introduced as "contaminants" subsequent to discharge.
4. Under space capsule conditions presently conceived, there are more dependable ways of treating and recovering liquid fractions of waste from urine, cabin waste, and wash waters for limited numbers of personnel than the biological system if reuse of the liquid is the major consideration. However,

for bodily wastes of greater numbers of personnel living at some form of semi-permanent installation, exploration of biological processes should continue.

INTRODUCTION

Populations of the earth have always been faced with the problem of handling and disposing of end products of metabolism and of other organic materials accumulated as part of the living process. Some forms of wastes are largely solid accompanied by moisture while others are the converse. Briefly the methods used in treating wastes of the population include physical, mechanical, biological, and chemical systems.

An extended space trip for a prolonged period of time will only be possible if the space craft can function satisfactorily as a closed balanced ecological system. The possibility of using photosynthetic, biological systems such as algae and plants for atmospheric regulation and food sources has already been considered by several investigators. However, capsule occupants will produce waste products which cannot be handled effectively by photosynthetic systems. Treatment and recovery of these waste products will be necessary since in an enclosed environment of limited mass and payload, waste products, in addition to adding undue storage burdens to already crowded

quarters, are obnoxious and, on an extended time basis, will remove needed elements from the ecological equilibria and make them unavailable and inaccessible for human reutilization.

In the present concept of space flight the capsule will have one to six men for an indefinite period of time within a closed system. The system is required to operate within a pressure range of 0.5 to 1.0 atmosphere with oxygen partial pressure of 160 mm Hg and carbon dioxide content below 1.0%.

Chemical methods will require energy in the form of heat and will result in much of the matter being converted to a form which is biologically unavailable. If it were possible to apply or devise a biological treatment process or processes that would regenerate, recycle, or recover these vital elements so that they could be maintained in a form whereby maximum amounts of the waste materials would be maintained biologically available for reutilization by the human occupants or usable by any of the various related biological support systems, a vital link in the ecological chain of survival would be achieved.

Out of the life work of many scientists there has developed a basic pattern, for earth situations, of treatment methods that were suited to the needs of population. Ultimate treatment of home or domestic wastes has always involved some form of biological treatment as a secondary or stabilization step. Use of biological treatment of sewage solids has been

long standing practice beginning with the privy and culminating in the digestion systems with many controls on temperature, mixing, and proportioning in use currently.

Hence it is logical to examine the feasibility of utilizing microorganisms and the stabilization of organic materials resulting from the bodily discharges, food preparation and other hygienic activities aboard space craft or in out lying space operations.

Microbiological systems include the aerobic methods known generally by the following names:

- Activated Sludge
- Bio-oxidation
- Contact Aeration
- Filtration (trickling filters)
- High Rate Filtration
- Controlled Filtration
- Oxidation Ponds (primarily but not exclusively algal growth)

Each of these processes provides essentially for the intimate association of organic materials, oxygen, and an aerobic biota which depends for its successful growth on the provision of organic nutrient and a favorable habitat suitable to the rapid growth of organisms with a minimum of opportunity for carrying older biota and thus holding endogenous respiration to a minimum.

Digestion as practiced is basically an anaerobic process conducted in either the mesophilic or thermophilic range.

However, the possible development of both aerobic and anaerobic composting of solids utilizing mixing and aeration techniques with temperature control to encourage thermophilic organism activity cannot be ignored.

Some means is required to handle dead cellular material remaining in any of the oxidative processes and to recover any usable products such as vitamin B₁₂, sodium chloride, and perhaps other substances. The path is open to possible combination of physical and biological treatment with thermal conversions and other recovery methods.

It is desirable to catalogue and discuss the various systems, their operations, their advantages and disadvantages, and to compare them and rank them in terms of their possible utility in space operations. Within the objective discussed it is the purpose of this study to examine the various waste treatment processes used and to evaluate these processes relative to possible application in a closed ecological system. This requires consideration of the nature and type of the wastes to be treated, the conditions affecting their formation, and the biota represented.

Observations have been extended to other factors which would affect treatment processes in an extended, extra-terrestrial, gravity free flight.

Biological waste handling as it is conventionally practiced is normally achieved by non-photosynthetic microorganisms, mostly heterotrophic bacteria. Since man requires oxygen and gives off carbon dioxide much investigative time has been devoted to gas exchange systems, either photosynthetic or non-biological chemical methods, which serve to regenerate oxygen and take up carbon dioxide. Because non-photosynthetic systems require oxygen and give off carbon dioxide, such systems have not been seriously considered.

In the course of space capsule waste handling speculation it has been suggested that photosynthetic algae might solve many of the major problems man will encounter in extraterrestrial flight (1). In addition to supplying man's oxygen requirements and consuming carbon dioxide (2, 3, 4, 5, 6), the algae might serve as ready nutrient sources (7, 8) and be used to treat man's wastes (9).

Several operable algal systems have been studied (2, 10, 11, 12, 13) and found capable of satisfying gaseous exchange needs. In addition, various investigators have appraised and examined the edibility and nutritional values of algae (7, 8, 14, 15, 16). Aside from the work of Golueke et al. (9), however, which still presents technical problems and yields an effluent high in coliform count, few, and somewhat limited,

laboratory experiments using photosynthetic systems for waste treatment have been conducted.

THE WASTES

Parameters Affecting Waste Composition

Human waste to be expected from persons operating in a closed ecological system is, of course, dependent upon caloric requirements, nutrient composition and energy expenditure values. Gradwohl (17) has noted additional parameters influencing waste composition. These include:

1. The diet involved.
2. The effectiveness of the individual's digestion.
3. The mucus discharge of the intestinal walls.
4. The biological characteristics of the intestinal flora.
5. Other peculiarities of the individual.

Of the three major waste sources, feces, urine and perspiration, feces is most greatly affected by these parameters (17). Urine apparently is less affected by diet (18), except for a shift from a high protein diet to a low protein diet which causes a sharp decrease in urinary urea (19). Urinary components are also slightly affected by stress (20).

It is difficult to predict precisely, from present knowledge, what the quantitative and qualitative amounts of various fecal elements would be from an individual on a standard diet for an extended period of time.

Gradwohl (17) has made various generalized observations of fecal composition based on dietary intake which have some application:

1. A diet rich in vegetables and starchy foods will increase the weight of the feces, while one rich in protein will decrease it.
2. A meat diet will increase the solids, while a diet consisting mainly of vegetables will increase the watery components.
3. Increased odor of feces is due to the increased amounts of meat ingested in the diet.

Increase in the watery constituent of feces is due to decreased resorption and increased secretions in the intestine. It might be termed a combination process with the increase in transudation in the principal role.

Putrefaction of meat within the digestive tract is said to be the principal cause of increased fecal odor. Odor is less marked in diets consisting mainly of vegetables and almost

non-existent in feces resulting from a milk diet.

The quantity of feces per person is, of course, related to diet and Ingram (21) estimates that 20 to 25 gm of dehydrated fecal material may be expected per man per day.

Taylor et al. (22) has proposed a diet for short term space flights consisting of 2,000 calories containing 49% carbohydrates, 16% protein, and 35% fat. These constituents provide for a semi-solid diet of a balanced nature, yet low in residue. Phillips (23) has recommended that for overall long range physiological well being best nutritional balances indicate a need of 1.07 gm of fat and 2.67 gm of carbohydrate for each gram of protein oxidized, with an approximate minimum of 75.66 gm of fat and 188.79 gm of carbohydrate, plus 70.71 gm of protein required to satisfy the ratios for a total of 1,800 calories per day per man in 335.2 gm of nutrient.

The above values indicate 22.6% fats, 21.0% protein, and 56.4% carbohydrates respectively: values different but, except for fats, comparable with those suggested by Taylor et al. Welch (24) has computed that the metabolism of Phillips' diet would consume about 388 liters of oxygen and liberate about 328 liters of carbon dioxide, and would lead to the excretion of 11 gm of nitrogen in the urine. For these values Welch allowed only minimal physical activity in the space cabin simulator thus providing a sedentary metabolism. For a man engaged in

light physical activity Clamann (25) estimates that the nutrient intake increases from 335.2 gm to 520 gm, and utilizes 602 liters of oxygen and 2,200 gm of water. The wastes expected from a diet based on light physical activity then amount to a total excretion of 60 gm of waste solids, liberation of 400 liters of carbon dioxide, and the reappearance of 2,540 gm of water. In the present studies, the metabolic response of a person engaged in light physical activity has been assumed.

A second essential factor in determining fecal composition is digestibility. McLester and Darby (26) indicate three meanings of digestibility:

1. Percentages of the several nutrients of a food which are available to the body for use as fuel or building material--the "Coefficient of Digestibility".
2. The ease and comfort with which the food is assimilated.
3. The smallness of the residue which the food leaves in the intestine.

The ease and comfort with which a food is assimilated by the gastrointestinal system is accepted as defining digestibility. Perhaps the chief factor which influences this phenomenon can be attributed to the secretory and motor responses which the food calls forth, and the subjective sensation which

it produces. The latter is determined largely by the former (27). These several influences are, in turn, dependent on the physical state of the food, its chemical composition, and its psychological appeal. Idiosyncrasy, however, is often a governing factor.

The completeness with which a food is utilized depends somewhat on its physical state, hence the advantage of cooking. In general, vegetables low in protein leave the stomach rapidly without great change. Boiled vegetables show much more rapid and complete disintegration before leaving the stomach (26).

Miller et al. (27) studied the secretory and motor response produced by various foods, and by different stimulations of the senses. These investigators found anxiety and mental strain to have a decided effect on gastric secretions.

Atwater (28) showed that of the several nutrients contained in a mixed diet, the average amounts utilized were:

Protein	92%
Fat	95%
Carbohydrate	97%

The digestibility of a food element considered in the light of space travel may also be dependent on other factors, such as:

1. The effect of weightlessness on absorption through the intestinal walls.

2. Additional psychological stresses imposed

due to:

- a. Eating in the absence of light.
- b. Boredom.

Mucus discharge of the intestinal walls and the biological characteristics of the intestinal flora also influence digestion, and consequently excretion. Gradwohl (17) has reported that 126 billion bacteria, of a great variety, are eliminated in the feces daily.

Although generalizations can be made as to the composition and amount of feces due to particular combinations of food-stuffs, it would be unwise at present to make a more rigid statement. The wastes resulting from man, being variable from individual to individual, and even variable within the same individual from day to day, make the task of assigning fixed values or even rationalized ranges within which one may find a certain waste material, extremely difficult and impractical.

A sudden, violent muscular contraction, due to a cough, hiccup or sneeze, may cause regurgitation in a weightless, gravity free environment. If this is the case, the waste disposal problem may suddenly be quite different.

It is unfortunate from the research viewpoint that realistic information may have to wait on a situation where actual ingestion, digestion and excretion all occur in a weightless

environment for an extended period of time, i.e., an actual space journey.

Amount and Composition of Wastes

Without actual space experience, information on usual bodily discharges must serve as a guide.

A wide variety of organic and inorganic materials are found in the waste of man, and several reports (17, 21, 29, 30, 31) on the composition of wastes resulting from closed systems and the composition of normal waste products have been published.

Feces

Goldblith and Wick (29) have determined the approximate quantity of fecal components to be processed for a man in one day. These components include:

Bulk (Total)	150	gm	}
Water	99	gm	
Dry Matter	27	gm	
Fats	4.5	gm	
Protein		not generally found	
Nitrogen	1.5	gm	
Carbohydrates		not generally found	
Minerals and Trace Elements	2.1	gm	

Vitamins

B-Vitamins	0.015 gm
Bile Pigments	0.15 gm

The average weight of the feces produced by one man in one day is generally accepted by investigators to be in the range of 100 to 150 gm.

The water content of feces has been determined by many investigators to be in the range 65% to 75% of the bulk weight of the feces. Ingram (21) estimates that dehydrated fecal material would amount to 20 to 25 gm per person per day for normal diet, i.e., a diet not limited by the restrictions imposed by closed space ecology.

Fat elimination has been reported by James (32) to be approximately 4.5 gm per day per man. The major components are palmitic acid, 34.2% bound as the methyl ester and 28.5% as the free acid (designated as bound and free); stearic acid, 25.4% bound and 19.3% free; oleic acid, 17.2% bound and 19.5% free; oleic acid isomers, 8.8% bound and 10.0% free; linoleic acid, 12.2% bound and 2.1% free; and 10 hydroxy stearic acid, 2.0% bound and 3.7% free. There are, in addition to the six fecal fatty acids mentioned here, a number of other fecal fatty acids of lesser importance (see Table 1). It is unlikely that the fecal fatty acids can make any valuable contribution to human

Table 1

Comparison of Composition of Bound, Free, and Total Fatty Acids
in Fecal Lipid for a Normal Human[†]

<u>Acid**</u>	Percentage of Acids in C ₆ - C ₂₀ Range		
	<u>Free</u>	<u>Bound</u>	<u>Total</u>
10:0	0.6	0	0.3
12:0	4.3	2.2	3.3
14:0	8.9	4.4	6.6
Branched 15:0	0.7	1.1	0.9
15:0	1.4	1.1	1.2
16:0	55.2	35.3	45.2
Branched 17:0	0.9	1.4	1.2
17:0	0.4	0.8	0.6
18:0	12.9	31.8	22.3
10-Hydroxy 18:0	0.7	0.9	0.8
14:1	0	0	0
16:1	1.4	2.1	1.8
18:1	6.8	10.7	8.7
Isomer 18:1	3.5	6.5	5.0
18:2	1.3	2.8	2.0
18:3	0	0	0
20:3	Trace*	Trace*	Trace*
20:4	Trace*	Trace*	Trace*
Other unsaturated C ₂₀ acids	Trace*	Trace*	Trace*

[†] From James: see Reference 32 .

* Trace indicates less than 0.5%.

** Number of carbons and double bonds respectively

nutrition due to inherent difficulties in isolation and purification of small quantities within a closed system.

Protein is not generally found in the feces (33) of healthy adults but approximately 1.5 gm of nitrogen is excreted in 24 hours per man (34). The nitrogen content is believed to be due in part to bacteria and in part to small quantities of unabsorbed materials such as unchanged raw vegetables (radishes, cole slaw, pickles, onions, etc.), mucus, digestive fluids, intestinal enzymes, and other alimentary secretions (17, 35).

40
nitrogen
protein

Carbohydrates have been reported in feces but only in minimal quantities (36). The carbohydrates present are assumed to be undigestible cellulose and vegetable fibers which are not affected by alimentary secretions and thus pass through unchanged. For humans cellulose has no nutritional value. Although chemical or enzymatic hydrolysis systems, not found in humans, may convert it to utilizable glucose, the amount of cellulose obtained is diet dependent and consequently ill-defined at this point. Wollager et al. (37) has considered the total caloric value of the feces and found feces to contain only 2.5% to 4.7% of the caloric intake, indicating therefore an exceedingly small caloric source as recoverable nutrient.

Minerals and trace elements are found in the feces. The elements found in the greatest abundance are:

<u>Element</u>	<u>mEq Excreted in 24 hrs (34)</u>	<u>gm/24 hrs (29)</u>
Sodium	5	0.12
Potassium	12	0.47
Calcium	32	0.64
Magnesium	17	0.20
Chloride	3	0.09
Phosphorous	30	0.51
Sulfur	8	0.13

A variety of additional trace elements such as aluminum, arsenic, copper, iron, lead, manganese, silver and zinc are present (38). Several reports (17, 29, 30, 34, 39) have listed the elements in feces. A recent and complete list is that prepared by Goldblith and Wick (29). Nutritionally, the trace elements are utilized most readily as chloride, phosphate and sulfate salts. Gradwohl (17) reports many crystals, phosphates and others.

Vitamins have been reported (38, 40) in the feces in small quantities. The following B vitamins have been detected in the feces over a period of 24 hours.

p-Aminobenzoic Acid	0.246 mg
Biotin	0.133 mg
Folic Acid	0.304 mg
Pantothenic Acid	2.20 mg
Pyridoxin	0.38 mg
Nicotinic Acid	3.63 mg
Thiamine	0.548 mg

Small quantities of Vitamins C, K, E and A are also found, and account for a total of about 0.01% of the feces. The greater part of the fecal vitamin content is believed to be of

bacterial origin. In view of the lack of knowledge of the exact nature of the vitamins as they exist in feces, isolation techniques are, at best, questionable. For this reason and because of the limited quantity found in the feces, human utilization of these vitamins is viewed as impractical within a closed ecological system (29).

Amylase and trypsin are enzymes normally present in feces in small amounts. Other enzymes also found are nucleases, maltase, sucrase, lipase and lysozyme. Lipase usually cannot be detected because it is inactivated by the feces.

Bile pigments are responsible for the color of feces. Daily samples of feces from persons on the same diet will vary in the amount of bile pigments present but weekly totals will be more uniform (34). No nutritional value is assigned to bile pigments.

Amino acids have been reported in feces (35). Among those microbiologically available in the feces are:

<u>Amino Acid</u>	<u>Wt. Present in Feces (ca 900 gm)</u>
Methionine	0.5 - 0.8 gm
Lysine	1.9 - 2.9 gm
Arginine	1.2 - 2.1 gm
Histidine	0.6 - 0.8 gm
Leucine	1.8 - 2.9 gm
Iso-leucine	1.4 - 2.3 gm
Valine	1.5 - 2.6 gm
Threonine	1.4 - 2.2 gm

Indole, skatole, paracresol, para-oxyphenyl proprionic acid, hydrogen sulfide, methane and methyl mercaptan have been reported (17) as some of the constituents causing fecal odor. Additional odor producing materials which may be found are putrescine and cadaverine.

Other fecal components which have been reported (17) include unchanged raw vegetables (such as radishes, cole slaw, pickles, onions, skin of fruit, nuts and berries), mucus, tissue remnants, epithelial cells, muscle fibers, connective tissue, crystals (phosphates and many others), detritus, starch granules, and an enormous variety and number of bacteria.

Urine

A second large source of waste materials is urine. Hawk and Bergeim (30) list a variety of materials one may expect in urine. The major components are water (1,200 gm), urea (30.0 gm), chloride as sodium chloride (12.0 gm), sodium (4.0 gm), sulfur as sulfur dioxide (2.5 gm), inorganic sulfates as sulfur trioxides (2.0 gm), potassium (2.0 gm), and creatinine (1.2 gm). Detailed lists of urine constituents may be found in several publications (39, 40, 41).

Perspiration

A third major source of waste materials is perspiration. Robinson and Robinson (31), and Spector (40), as well as Wallman and Barnett (39), have summarized the chemical components of perspiration and have found a variety of organic and inorganic materials present. The major components are water and sodium chloride.

Skin excretions will include a variety of substances included in sweat and sebaceous excretions. Sweat may contain urea, uric acid, creatinine, lactic acid, ethereal sulphates of phenol and skatol, amino acids, sugars in traces, and albumin in varying amounts (31). Sebaceous gland excretions will contain small amounts of cholesterol, simpler fatty acids, fatty acid esters, albumins, and inorganic salts.

Wash Water Wastes

Until more is known relative to specific uses of wash waters, cleaning necessities, and the amounts and types of liquid and solid materials that will be placed into these solutions, the quantities and composition of wash waters produced can only be estimated. Table 2, adapted from previous waste projections of Ingram et al. (42) will give some idea of the amounts of waste waters which might be expected.

Table 2

Closed Space Occupancy Wash Waters

<u>Source</u>	<u>Description</u>	Estimated Range of Quantity Liters per Capita per Day	
		<u>Min.</u>	<u>Max.</u>
Clothes Washing	Organics, salts, 1% + solids	3.0	4.0
Personal Hygiene	Organics, salts, 1% + solids	1.5	4.5
Cabin Cleansing	Debris, 1% + solids	1.0	5.0

Cleaning Agents

The cleaning agents which might be used in space for various types of cleaning, including laundering, personal washing and cabin cleansing, should be effective in small concentrations while achieving the desired ends of washing, viz., lowering of interfacial tension (wetting), and emulsification of dirt so that rinsing and cleaning may be achieved. The most efficient cleaning agents are synthetic detergents.

Anionic synthetic detergents in the form of alkyl aryl sulfonates (or alkyl benzyl sulfonates) are most commonly used although cationic, non-ionic compounds or even soaps might also be used for cleansing.

Cleaning compounds usually contain alkyl aryl sulfonates in concentrations of 20 to 30 per cent. The remainder of the compound will consist of "builders" to improve detergent action (dirt suspenders), which will represent about 60% of the weight. Builders most frequently used include various phosphate compounds, sodium carboxy methyl cellulose, and perhaps sodium sulfate or sodium carbonate, depending on whether heavy duty or light duty detergents are used. Household cleaning, for example dishwashing, is usually carried out using concentrations of about 0.3% of the packaged detergent for cleaning (43).

The use of detergents, especially anionic, has introduced several problems in conventional waste treatment processes. A

review of these treatment problems, including foaming, and the resistance of these synthetic detergent molecules to biological oxidation can be found in reports of Sawyer (44) or Schmidt (45). Lowering of surface tension imparts fundamental physical and chemical changes in solutions, and will result in bactericidal or bacteriostatic effects (46). Quarternary amines (cationic detergents) are noted for their germicidal properties (47).

A procedure for clothes and personal washings employing minimal water and economical use of cleaning agent has been used in New York University laboratories (48) to produce wash waters similar to those that might be expected in space. Table 3 shows estimated amounts of water and cleaning agent used for clothes washings and personal hygiene.

In clothes washings, and in personal washings, bathing, face and hand washings, the commercial germicidal detergent "Phiso-hex" was used. This is the same cleaning agent used in the simulated space studies at the School of Aerospace Medicine, Brooks Air Force Base, Texas. According to the manufacturer (49), "Phiso-hex" contains about 50% of the detergent Entsufo-n. Entsufo-n contains about 28% solids by weight, or about 14% anhydrous detergent. It is described as being a sodium salt of a polyether sulfonate.

Detailed descriptions of hand, face and bathing procedures may be found in the waste water recovery studies of Ingram (48)

Table 3

Personal Hygiene Wash Water

<u>Type of Washes</u>	<u>Washings/ man/3 day</u>	<u>ml/man/3 day</u>	<u>ml/man/day</u>	<u>ml Detergent man/day</u>
Hand	6	450	150	2
Face	6	450	150	2
Bath	1	2,350	800	4
Clothes	3	9,000	3,000	depends on wash ca 16
Totals		<u>12,250</u>	<u>4,100</u>	24

for the School of Aerospace Medicine. A summary of the amounts of clothes washing water and detergent used can be found in Table 4.

Wash water wastes have been classed into the categories clothes washings, personal hygiene wastes, cabin washings, and food preparation wastes. A brief description of the washings in each category follows:

1. Clothes Washings

Predominantly these waters will be composed of laundry wastes which will include products of skin excretions (sweat and sebaceous glands), dirt particles, and lint suspended in water with a cleaning agent. Also found, however, will be hair, bacteria, substances abraded from the skin, dirt, dust, and various trapped residues from the atmosphere deposited on the skin and rubbed onto the clothes.

2. Personal Hygiene

Personal hygiene water wastes will include bathing waters, hand washings, and oral washings. Essentially bath and hand washings will contain the residues of skin excretions

Table 4
Clothes Washings: A Summary

Wash #	Quantity of Articles Washed	Total Weight Washed gm	Weight of Water Used in Presoak gm	Water Used for Presoak ml	Clean-ser Used for Wash ml	Lost in Presoak ml	Total Volume of Rinses ml	Total Volume of Re-covered Water from Rinses ml	Overall Loss ml
1	1	274.6	290.3	315	7	24.7	1,263	1,218	69.7
2	2	415.7	470.9	501	14	30.1	2,500	2,387	143.1
3	4	606.1	615.0	659	14	44.0	3,500	3,331	213.0
4	3	290.6	308.2	341	6	32.8	3,000	2,950	82.8
5	1	224.1	210.4	233	5	22.6	1,500	1,465	57.6
6	3	735.5	702.7	764	20	61.3	4,000	3,897	164.3
7	2	508.8	490.7	510	14	19.3	3,000	2,963	56.3
8	2	504.5	444.4	491	14	46.6	2,500	2,418	128.6
9	2	543.6	442.6	476	14	33.4	2,500	2,425	108.4
Totals	20	4,103.5	3,975.2	4,290	108	186.5	23,763	23,054	1,023.8

Comments: The amount of clothing washed was roughly equivalent to that which would be worn by one man in 16 days.

ml. water consumed (ml. presoak plus ml. rinses) = 28,053 ml.

ml. recovered as liquid = 23,054 ml.

ml. lost to atmosphere (wt. presoak plus overall loss) = 4,999 ml.

% lost to atmosphere = 17.82 %

concentration of cleanser in recovered water = 0.47 %

ml. water for cleaning clothes/man/day (based on 16.5 man days) = ca 1,700 ml.

water requirements for washing clothes/man/day (ml. H₂O plus atmospheric loss) = 2,003 ml.

ml. water/1,000 gm clothes = 6,836 ml.

of the same nature as clothes washings, but in a more concentrated form.

Oral washings will contain dentifrice and food particles or debris only partially broken down, bacteria, and a mixture of buccal secretions and saliva. Saliva is comprised of inorganic components, organic components and enzymes. Inorganic components include principally chlorides, bicarbonates and sodium, but also potassium and calcium with relatively small amounts of sulfate and phosphate. Organic components include the amylolytic enzyme ptyalin, mucin (a glycoprotein), traces of thiocyanate, and small amounts of urea, glucose, lactic acid, phenols, vitamins, and certain enzymes such as phosphatase and carbonic anhydrase (50).

3. Cabin Washings

Cabin washings are waters resulting from general cabin cleansing. It is not possible to quantitate the amounts of water which would be consumed or produced, or the frequency of this operation. The need for cabin washing, as hitherto suggested (42),

remains a nature of conjecture. The nature of this water would be dependent on the debris, exudates, sloughings, and atmosphere produced by the passengers or by process operations.

4. Food preparation

Food preparation and various stages of cooking and eating will produce waste waters. Most of these wastes will be produced as part of utensil cleansing and will contain particles of food, oils and cleaning agent. The quantity and quality of this water will depend on the character of the diet and the method of preparation and service.

Taylor et al. (22) have suggested a tube type feeding program that would reduce food preparation wastes to an insignificant item. There would be no spoilage, no waste food and no utensil cleansing with the feeding program outlined.

Microorganisms in Wastes

Whereas terrestrial, conventional waste treatment processes depend on organic breakdown of wastes by a diverse

microbial biota derived from a variety of sources including water, soil and air, in space such a diversity of biota may not be possible.

Space treatment systems would have access predominantly to microorganisms discharged from the feces. Other sources of organisms normally resident in or on man might, of course, be considered. Urine as normally excreted is usually sterile ? while oral and skin populations are variable, and their significance in complete purification is questionable.

Microorganisms in Wash Waters

All waters used in cleaning, laundry, personal and even cabin washings to some extent, will contain bacteria whose source will be the human body. It is difficult to predict for the space capsule whether these organisms can or will be killed by the washing agents to be used, or whether the bacteria will be a problem at all. However, as permanent residents of the human body, difficult to remove or sterilize in-situ for prolonged periods of time, all wash waters will contain some bacteria and consideration must be given to their nature, source, and occurrence.

Bacteria and related microorganisms, whose primary source will be the skin, are introduced with bath and laundry washings. A minor addition will be introduced from the mouth and

respiratory tract with oral washings. Bacteria, yeasts, or fungi from both of these sources, in addition to saprophytic organisms which may be found in cabin quarters, will also be found with the cabin washings.

The number and types of bacteria which will be found on the skin are variable. Apparently bacteria may multiply freely on the skin (50), particularly in the sebaceous glands (51, 52). "Resident" microbial flora (as opposed to "transient"), however, have been postulated by Price (53), Lovell (52), and Evans (51). The studies of Lovell show that resident bacteria are situated deep enough within the hair follicles and sebaceous glands so that they cannot be removed by mechanical means without injury to the skin. According to Lovell, generally used antiseptics do not penetrate sufficiently in the deeper recesses of these structures, and even during surgical operations resident bacteria continue to multiply.

The studies of Evans et al. (51) show that a great variability may exist in the numbers of bacteria occurring on any given person, and even from person to person. As an example, few bacterial counts of less than 100 microorganisms per square centimeter were reported. Nearly all of the subjects studied showed counts up to 500 organisms per square centimeter, and some subjects had counts of more than 10,000 organisms per

square centimeter. A few counts in the range of several hundred thousand microorganisms per square centimeter were reported.

Evans et al. (51) have also reported that anaerobic bacteria outnumbered aerobic organisms by a ratio of 10 to 1 in about half of his examinations, and by at least 100 to 1 in one-third of his estimates. The average number of aerobes found was about 350 per square centimeter, while similar counts for anaerobes showed 55,300 per square centimeter. Thirty-nine per cent of the specimens he examined gave counts of over 5,000 anaerobes per square centimeter while aerobes were usually well below this figure.

Propionobacterium acnes was found to be the most numerous organism. The second most numerous organism was Micrococcus epidermis. However, Staphylococcus albus (Micrococcus pyogenes var. albus) and Micrococcus candidus were found to be regularly present. To a lesser extent Micrococcus flavus was observed. Although Evans noted the presence of Staphylococcus aureus (Micrococcus pyogenes var. aureus), he notes the "intergrading" strains of M. albus to M. aureus. In addition, he occasionally found the anaerobic micrococci M. saccharolyticus and diphtheroids.

Evans noted that the sebaceous glands indeed appeared to be the principal site of bacterial growth after examining the total anaerobic bacteria of the sebaceous excretions of the

sternum, neck and face. He verified this by determining the numbers of bacteria on the palms of the hands, where sweat glands are numerous but sebaceous glands are not present, and comparing this with the ear concha, where many sebaceous glands occur but no sweat glands.

The studies of Evans et al. (51) also suggest that failure of an individual to bathe for as long as seven days did not significantly increase the bacterial population of the skin, and that exercise, even when accompanied by sweating, only caused "transient" and minor increases in the bacterial skin flora.

Oral bacteria and, to a lesser extent, bacteria from the respiratory tract will also be introduced with mouth washings. Like fecal and skin bacteria, the kinds and numbers of these organisms are variable from individual to individual (54, 55).

The work of Richardson and Jones (56), and to some extent that of Kraus and Gaston (54), gives an idea of types and distribution. Richardson and Jones indicate that the predominant bacteria of the mouth are anaerobes, numerically in the magnitude of 110×10^6 /ml of saliva (range 10 to 384×10^6). Thirty-five per cent of the total anaerobic population was found to be Streptococci sp. and Veillonella. Aerobe counts gave numerical estimates of 40×10^6 organisms/ml of saliva (range 5 to 114×10^6). These figures are in agreement with

those of Kraus and Gaston (54). Streptococcus salivarius, starch hydrolizers, and Neisseria were found in some quantity. Lower numbers (of the order of 10^3 and 10^2 per ml of saliva) were reported for Fusobacterium, Lactobacillus, Leptotrichia, Candida and coliforms (Alcaligenes and Aerobacter).

Nasal and nasopharyngeal sources would probably contribute minor numbers of microorganisms of significance to the water residue. Aerobic cultures of mucous membranes will reveal a scanty flora, usually white staphylococci and diphtheroids. Thomson and Thomson (57) report many sterile cultures. Watson et al. (58) indicate that small numbers of bacteria (of the order of 10^2 per ml of washings) may be recovered from nasal washings. Anaerobic cultures were found to be consistently higher (as in skin, saliva and feces), and on the order of 35×10^2 (range 1×10^2 to 170×10^2). Aerobic cultures gave comparable counts of 10.5×10^2 per ml (range 0.3×10^2 to 82×10^2). Possibly the predominant organism was Corynebacterium acnes.

Microorganisms in Bodily Discharges

The ecology and relationships of intestinal flora are complex, and changes which have been found to occur in numbers and kind of microorganisms are sometimes difficult to explain (59). The existence of an equilibrium among the different intestinal

microorganisms has been postulated by Wilson and Miles (50), and Zubrzycki and Spaulding (60).

Two main categories of intestinal bacteria are reported; acid producing (saccharolytic), and proteolytic species. The true significance and relationships are not known. Effects of diet on the intestinal flora have been noted by Johansson and Sarles (59).

Literature references are not in agreement as to the types and numbers of bacteria that would be found in the feces, and a wide variation in estimates can be found. Wallman and Barnett (39) have suggested that about one-third the solid weight of feces consists of bacteria, namely E. coli.

Zubrzycki and Spaulding (60) indicate that members of the genus Bacteroides (a gram negative, anaerobic rod) constitute the most numerous group of bacteria in normal human adult fecal flora. This group, together with the enterococci, coliform bacilli, diphtheroids, and lactobacilli, are major bacterial components and will account for more than 99% of total count.

A wide fluctuation in the number and types of minor organisms is also noted. Zubrzycki and Spaulding (60) found that total anaerobic counts on stool specimens averaged about 1×10^9 organisms per gram of feces. Aerobic counts averaged about 1×10^8 organisms per gram of feces with coliforms comprising approximately one-third of the number. Spore formers were

found to be less than 10^4 , and yeasts and staphylococci 10^2 organisms per gram of feces. Pseudomonas, Proteus, Clostridia, and non-lactose fermenting organisms (probably Enterobacteriaceae) were recorded as present in only 20% of the specimens.

The bacterial populations of the intestine are basically anaerobic organisms or at best facultative aerobes. It is unlikely that these organisms can serve further in waste conversion processes. Although the possibility of E. coli survival in other than fecal environment has been demonstrated, there is little evidence to suggest that coliform are other than saprophytic in the exterior environment. Ingram has demonstrated survival of coliform in sea water (61) and has suggested on the basis of exploratory tests (62) that coliform may multiply in an aerobic habitat such as that of the trickling filter. There is little evidence to support the idea that fecal coliform serve effectively as treatment biota in terrestrial sewage treatment processes.

Normal urine is sterile and is therefore not a source of organisms. The organisms found in perspiration or epithelial debris and sebum are mixed cultures, as shown in the discussion of bacteria in wash wastes from various sources. None of these is noted as a predominant waste treatment organism. Hence, it is projected that waste treatment biota must be taken from earth if they are to be used in space waste treatment processes.

WASTE TREATMENT PROCESSES

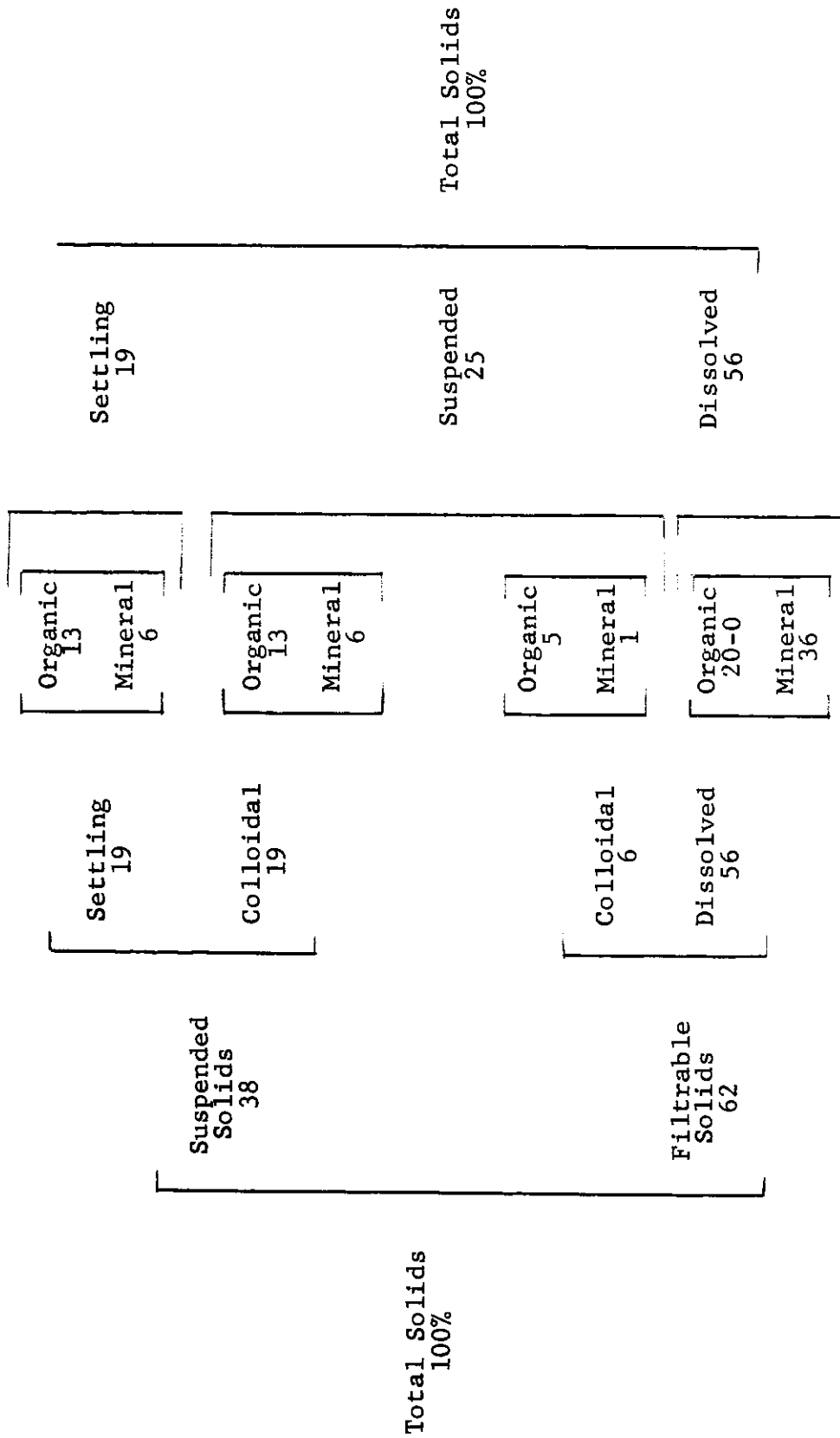
Treatment processes associated with the handling of liquid wastes on earth are multiple in their layout, but are easily reduced to a few basic principles insofar as the accomplishments of treatment are involved.

Domestic wastes are composed of about 99% water and 1% solid matter. The solids are either suspended or dissolved and some of the suspended solids have weight-mass characteristics allowing them to settle in liquid media at atmospheric pressure. In present earth practice it is assumed that from 50 to 60% of the suspended solids (ca 38% of the total) can be removed by settling (ca 0.05# per capita per day). Removals by settling under zero G conditions are precluded without the application of some gravitational force within the space system. Hence, one earth treatment process used to relieve the total treatment loading is seriously restricted in space. It may be projected that some of the so called "primary" treatment processes in which gravity is utilized as a force to obtain separation of waste solids from the liquid fraction are of limited application in space.

A typical presentation of sewage solids relationships is shown in Table 5. It will be noted that about 51% of the solids are organic and the balance is mineral. Experience has

Table 5

Typical Composition of Solids in Domestic Waste



evolved a figure used widely in the waste treatment field that indicates a production of 0.42 \dagger pounds of suspended solids per day per capita.

If domestic sewage content is any criteria, the organic matter amounting to 51% of the total solids constitutes 31% of the suspended solids or 0.13# per capita per day (ca 59 gm) of solids that might be removed by the mechanism of successive filtrations through a series of selected filters of decreasing pore size.

Organic matter requires oxygen in some form for stabilization. Again experience in the waste treatment field has developed the value of 0.17# biochemical oxygen demand (BOD) per capita per day as a basic figure representing the strength of the organic components of waste. This amount of oxygen is required over a five day period to satisfy the oxygen requirements of typical waste. The amount is approximately 68% of the total oxygen required for complete stabilization of organic material contained in the waste subjected to biological forces at 20°C. The requirement for oxygen is exerted by carbonaceous material (largely satisfied within five days), by oxidation of nitrogen derived from nitrate, ammonia and organic nitrogen compounds in the waste (food for Nitrosomonas and Nitrobacter), and inorganic reducing compounds reacting with molecularly dissolved oxygen (63).

Phelps applied the theory of monomolecular reaction to oxygen utilization by organisms in waste and Theriault (64) gave it the form,

$$y_t = L (1 - 10^{-kt})$$

in which y_t = BOD at any time t

t = time in days

L = a constant = ultimate BOD

k = a constant

Orford and Ingram (65) developed a logarithmic BOD equation applicable to the five day period which eliminates many difficulties of the monomolecular equation and avoids many complicated computations.

The equation is expressed

$$y_t = m \log t + b$$

in which y_t = BOD at any time t

m = slope of the line

b = constant (the intercept)

t = time in days

For normal domestic sewage the equation becomes

$$y_t = s (0.85 \log t + 0.41)$$

when s = 5 day BOD intercept of the line

The equation applicable to any sewage may be written as:

$$y_t = S (0.85 \log a t + 0.41)$$

in which S = BOD intercept of the line at $\frac{5}{a}$ days

a = x-axis (log time) intercept of normal domestic sewage ($a = 1$) divided by the x-axis intercept of the observed BOD curve

The carbonaceous BOD curve for standardized domestic sewage may be expressed as:

$$y_t = 100 (0.85 \log 1.0 t + 0.41)$$

If the values for a and S at 20°C are known, the BOD values at any time for any temperature can be determined by using the equation

$$y_t = S_{20} (0.85 \log a_{20} 1.072^{(T - 20)} t + 0.41)$$

in which S_{20} = the constant S at 20°C

T = the temperature in $^\circ\text{C}$ (limits $10^\circ - 30^\circ$)

It is suggested that the Orford-Ingram Logarithmic Equation can be utilized in some modified form for determining more precisely the amount of oxygen required for a biological

treatment system that may be developed for use with wastes developed aboard extraterrestrial space craft.

Most of the so-called "secondary" processes of earth treatment of liquid fractions are biological processes. In principle these processes depend on aerobic biota to attack the complex organic compounds in the waste and progressively, as compounds are converted, to utilize as a source of food the products of metabolism of a series of microorganisms capable of attack on the conversion products. In effect the typical cycles for carbon, nitrogen, phosphorous, sulfur, etc., are made to occur under some form of control. The resulting liquid is stabilized to the point that the mineral form of compound in each cycle may be found.

Viz-- Protein -----> Nitrate

Carbohydrate -----> CO₂

Aerobic Processes

It is obvious that all aerobic processes depend on the presence of oxygen in dissolved form as the easily available form of oxygen to be used as a source of energy by the living organisms. Oxygen is made available in earth treatment processes by passing air through, into, or in intimate contact with liquid and, by transfer to the liquid, oxygen of the air becomes dissolved in the liquid. As long as the oxygen transfer

is equal to or greater than the metabolism requirements of the organisms, the demand is satisfied and organisms continue to utilize the food supply (waste organics).

Biological treatment systems handling domestic wastes contrive to bring an active biota together with the liquid fraction containing both dissolved and suspended solids. The processes have various names depending on the means by which intermingling is accomplished. Activated sludge and bio-oxidation are processes in which the organisms are suspended in a liquid medium containing solids. Air is introduced by mechanical or other means and the mass is kept in a state of turbulence to provide maximum organism mixing. Conventional activated sludge units may be loaded at the rate of 33.3# of BOD per day per 1000 ft³ of aeration tank capacity.

Filtration, high rate filtration, and controlled filtration are processes in which organisms are provided with a bed of inert material on which to grow as a thin film of slime. The waste to be treated is brought to these surfaces as a film flow of liquid. Thus a thin film of water is in intimate contact with a thin film of microorganisms. Air is present in the void space of the medium and is provided in excess of biota requirements by natural draft or forced air flow. Conventional single pass trickling filters may be loaded at the rate of 15# of BOD per day per 1000 ft³ of filter volume. Such filters are

operated at hydraulic loading of 46 to 90 gallons per square foot per day. High rate filters with recirculation may be loaded at the rate of 30# BOD per day per 1000 ft³ of filter volume and range from 460 to 920 gallons per square foot per day. Controlled filtration units may be loaded at rates up to 370# BOD per 1000 ft³ per day with an optimum loading hydraulically of 3,000 to 3,400 gallons per square foot per day.

Contact aeration utilizes ideas of both the above. Plate media are suspended in a liquid and the liquid is moved rapidly by air agitation of the liquid medium over and around the plates to which organisms are attached. Contact aeration units are loaded up to 31.8# BOD per 1000 ft³ of tank capacity per day.

Oxidation ponds are another form of biological processing in which microorganisms are growing in shallow ponds having large surface area. At one time the interchange of oxygen to the liquid was primarily a matter of surface aeration with some assistance in the exposure of air surface due to water currents and minor wind turbulence. Later versions of this system provide for some form of mechanical mixing of air and water by spraying, or pumping combined with some form of aspiration. These ponds have had some degree of success as a "tertiary" treatment following other treatment processes since converted organics could be utilized by algae and algal growth added

oxygen to the liquid more efficiently than surface aeration. Without the benefit of algae the pond becomes a form of contact aeration treatment, limited by the amount of oxygen that can be introduced into the medium. Deeper ponds become anaerobic as settled sludge deposits decompose by fermentation. The range of loading on oxidation and stabilization ponds is variable and dependent on the particular function of the pond. A rate of 50# BOD per acre per day has been reported in the experience of waste treatment with algal oxidation ponds, and from 164 to 173 pounds per acre per day has been reported as satisfactory for the lagooning of raw domestic sewage.

Anaerobic Processes

Organisms growing anaerobically depend on compounds in the medium for oxygen. The process of anaerobic decomposition is referred to generally as digestion and the process in earth treatment is usually reserved for the treatment of solids removed from the liquid fraction of waste. Digestion may be accomplished with biota growing in temperatures 20° to 43°C (mesophilic digestion) or with biota growing in temperatures of 43° to 56°C (thermophilic digestion). A temperature zone ranging from about 37° to 40°C utilizing a mixture of both types of organisms has been applied in digestion practice.

The process is characterized by its extended time requirement. Organics are converted by aerobic organisms in a few hours whereas anaerobic organisms may require days and even months to complete reactions. The reduction process is always accompanied by decomposition end products such as methane, carbon dioxide, ammonia and hydrogen sulphide, the latter being odorous and highly toxic. Likewise, the decomposition process must usually be followed by some oxidative mechanism taking products to stabilization. Digested sludges remain biologically active and demand oxygen for ultimate conversion.

In earth treatment the digestion process has required large storage volumes. Sludge taken from the liquid fraction by settling is taken off the activated sludge process at 98.5 to 99% moisture, off the trickling filter process at 97.5 to 98% moisture, and off primary settling units at 95 to 98% moisture. Secondary settling of the sludges and possible washing (elutriation) or chemical conditioning (with lime and ferric chloride) will permit reduction of the water content to about 90% by decantation. These supernatant liquids are extremely high in organic strength and do not handle well in the aerobic biological processes unless mixed with large quantities of inflowing waste. Well functioning digesters can accept loadings of 0.03 to 0.04 pounds volatile solids per cubic foot of digester per day and require 30 days detention. Continuously

mixed digester units, sometimes referred to as high rate digesters, may be loaded at from 0.1 to 0.2 pounds volatile solids per cubic foot of digester per day and require 10 to 15 days detention according to work of Schlenz reported by Eckenfelder and O'Connor (66).

BIOLOGY OF WASTE TREATMENT

An exposition of theory on the several biological processes can be found in a publication by Eckenfelder and O'Connor (66). Activated sludge, filtration, and digestion have been selected for further study and report under this project. The next sections center on the biological populations associated with waste treatment.

Metabolic Considerations

Waste treatment processes as they are normally carried out are the result of the biochemical activities of a great many different microorganisms working together to destroy or oxidize the organic matter found in wastes to yield energy. The energy thus formed is used in the synthesis of new cellular material which is then removed. The changes in organic matter are brought about by the many different enzyme systems found in microorganisms.

Biochemical attack of the organic molecule occurs in a stepwise, gradual fashion and is not the result of the action of any one bacteria or any one species of any microorganism. Instead, it is probably achieved by the combined activities of a mixed biota consisting of many different organisms living in the same purification unit--each carrying out one or a few biochemical reactions. While one reaction may only be capable of partially degrading the organic molecule, the variety of organisms or enzyme systems continue the attack until molecular degradation is complete.

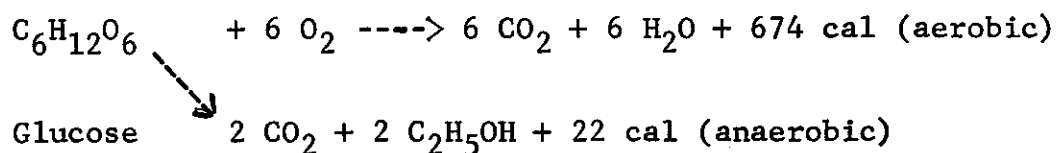
Molecular attack will yield energy which is used by the microorganisms for their life functions: reproduction, locomotion, growth, and even simple existence. Simpler, less complex molecules are produced which are ultimately converted into "end products" which can no longer be attacked by microorganisms and are hence called "stable". Matter found in these molecules, like that contained in the microorganisms, however, is still biologically utilizable by higher organisms and other living systems. The result of the combined activities of these organisms, metabolizing independently yet serving each other, is the ultimate breakdown of the waste molecules.

Depending on whether the treatment process has occurred in the presence of oxygen (aerobic) or in the absence of oxygen (anaerobic), and the operation of the process, different end

products result. Aerobic and anaerobic processes differ basically in the biochemical methods by which organic molecules are broken into the final end products. Aerobic processes are more efficient and rapid, and will produce more oxidized end products such as carbon dioxide, water, nitrates, phosphates, sulfates, etc., whereas anaerobic processes are slower and will produce, besides water and carbon dioxide, more reduced end products such as methane, ammonia and H_2S , and a variety of organic acids.

A simplified overall biochemical visualization of these two processes is shown in Figure 1 (67).

Aerobic systems will yield more energy and thus produce more cells than anaerobic systems. Glucose breakdown will serve to illustrate (68):

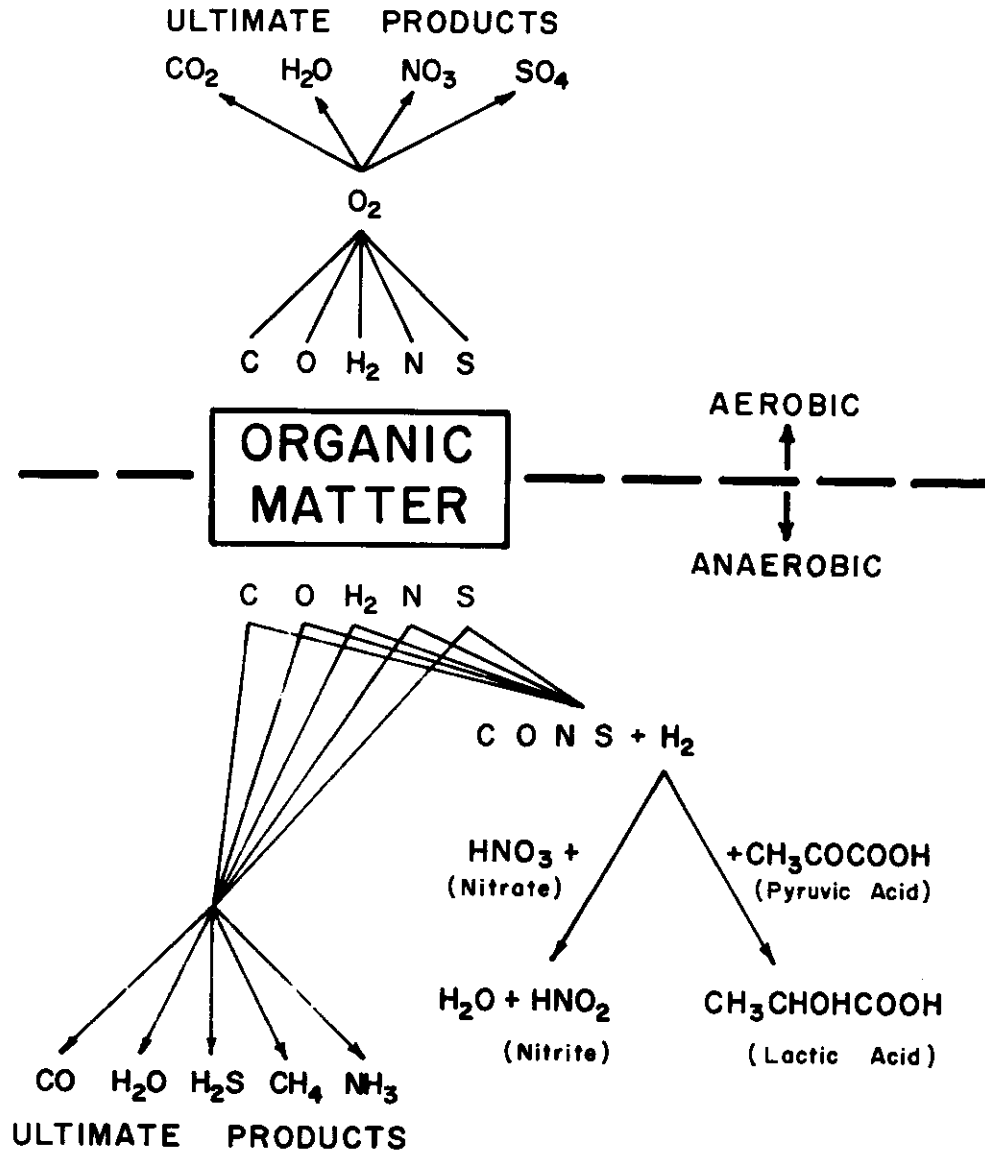


Most sewage bacteria are heterotrophic--deriving their carbon from an organic food source. An idea of the nutrients which can be utilized by bacteria, and their role, is shown in Figure 2 (69).

Biochemical reactions are catalyzed by enzymes which are highly specific in the wide variety of reactions they catalyze.

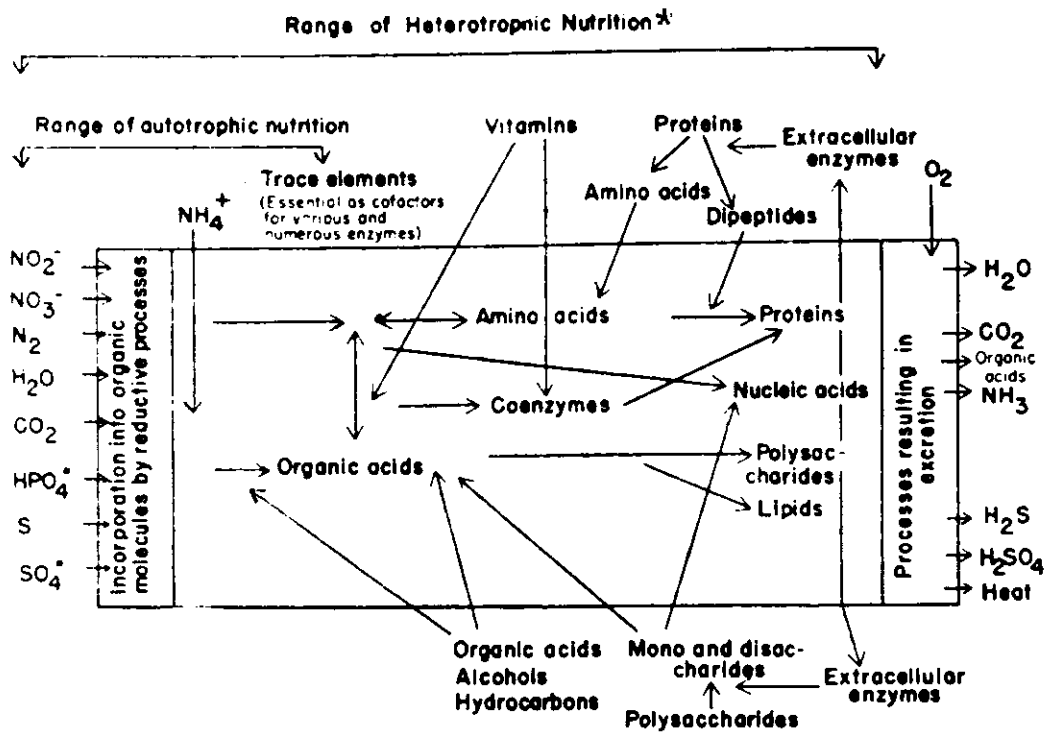
Figure 1

DEGRADATION OF ORGANIC MATTER



After SIMPSON, J.R.
(See Reference 67)

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Figure 2

*Representing nutrients utilized by bacteria and their place in metabolism, after Lamanna and Mallette (see Reference 69).

Enzyme reaction rates, within certain limits, will increase (roughly doubling) with temperature increases of 10°C. pH changes will affect enzymatic rates as will accumulation of certain waste products, chemical salts, heavy metals, physical factors, irradiation, and other factors.

Chemical Composition of Bacterial Cells

A bacterial cell will have about 80% water (range 75 to 90%) and dry matter. The dry matter will consist predominantly of organic substances, mainly protein, however, about 10% of the dry material is mineral. Some of the mineral substances will be combined with organic materials. The absolute and relative amounts of all the components in bacteria will vary considerably with how the bacterial cells are grown. Buchanan and Fulmer (70) have reported dry matter analysis of a few specific organisms and these are shown in Table 6.

Water in bacterial cells occurs in a free state and is also partly bound to other cellular constituents. Bound water does not act as a free solvent and will assume a different physiological role than free water. It can only be totally removed by the application of heat (71).

McKinney (72) estimates that the organic matter of a bacterial cell will be composed approximately of 53% carbon, 29% oxygen, 12% nitrogen, and 6% hydrogen. Much of the organic

Table 6

Gross Chemical Composition of Vegetative Cells*

<u>Organism</u>	<u>Water</u> %	<u>Dry Matter</u>		<u>Carbon</u> as <u>% Dry Wt.</u>
		<u>N</u> %	<u>Ash</u> %	
Acetobacter	98			
Escherichia coli	73	8	9	
Klebsiella pneumoniae	84-88	10-7	30	51
Proteus vulgaris	80	11	8-14 3-7	
Proteus	84			
Pseudomonas aeruginosa	76	10		
Serratia marcescens	86 75 91	11 11	14 9 14	
Bacillus anthracis	80 82 85	6 9	spores 2.0	
"Water" Bacillus	84	10	11	51
"Putrefactive" Bacillus	84		3-5	

*Adapted from Buchanan and Fulmer (see Reference 70).

material is protein (40 to 80%). Carbohydrates (1.5 to 36%) will occur to some extent and lipids will be more variable (0.4 to 39%). Hopkins, et al. (73), have presented analyses of Rhizobium, Clostridium, and Lactobacillus shown in Table 7. Other organic substances include vitamins and pigments.

Most of the bacterial protein may be enzyme protein and will be associated with nucleic acids as nucleoproteins (50 to 90%) (74). Purines and pyrimidines are found associated with nucleic acids as well as pentose sugars and phosphates. All of the commonly known amino acids have been identified in bacteria except diiodotyrosine and the other iodinated types. At least one amino acid, α, ϵ -diaminopimelic acid, has been found only in bacteria and blue green algae. A number of amino acids identified in higher plants are not yet known to occur in bacteria (69).

The amino acid composition of bacterial protein in different bacteria is apparently stable. Freeland and Gale (75) have reported on a number of specific organisms as shown in Table 8. Several organisms including species that would be found in treatment systems were examined. Stokes and Gunness (76) indicate that there is a basic similarity in the type of amino acids present in bacteria although there may be different amounts. The amino acids they found to be stable were

Table 7

Analysis of Bacterial Cells*

	%				
	<u>Moisture</u>	<u>Ash</u>	<u>Protein</u>	<u>Fat</u>	<u>Carbohydrates Calculated</u>
Rhizobium	72	0.8	8.1	4.6	15.5
Clostridium	76	1.1	16.1	0.7	4.1
Lactobacillus	72	2.9	19.3	0.5	2.1

*After Hopkins, Peterson, and Fred (see Reference 73).

Table 8

Amino Acid Content of Bacteria
(% Total Nitrogen)*

<u>Organism</u>	<u>Arginine-N</u>	<u>Lysine-N</u>	<u>Histidine-N</u>	<u>Tyrosine-N</u>	<u>Glutamic-N</u>
E. coli	10.4	7.5	3.3	1.4	5.6
A. aerogenes	10.4	6.8	3.4	1.4	5.6
P. vulgaris	7.8	6.4	4.4	2.1	4.9
Gram Negative	12.2	8.2	2.0	1.7	6.4
B. brevis	10.0	8.0	2.5	2.2	7.3
S. fecalis	5.4	7.6	2.1	0.8	4.4
S. aureus-1	4.8	10.6	1.2	1.8	4.9
S. aureus-2	5.0	9.3	1.3	1.1	4.0
B. subtilis	7.6	7.5	2.5	1.5	5.3

*After Freeland and Gale (see Reference 75).

histidine, arginine, lysine, leucine, isoleucine, valine, methionine, threonine, phenylalanine, and tryptophane.

Nucleic acids are usually found associated with nucleoproteins. The amounts which will be found in a particular bacterial strain will be variable with environment and age. E. coli and other gram negative organisms (74) ranged from 9.7 to 28.2% with more nucleic acids being produced in young cultures.

A dry bacterial cell will contain from 10 to 30% carbohydrate. Carbohydrates can occur as part of the nucleoprotein as pentoses. However, most of the carbohydrates will be found present as complex polysaccharides, free or associated with protein and lipid. The polysaccharides will differ depending on the bacterial species, and may contain nitrogen as amino sugars.

The lipid content of bacteria is variable since lipids may be bound with protein or carbohydrate components of the cell and are subsequently difficult to extract. Some bacteria, however, will deposit fat, while other species will not. According to Knaysi (71), in species that do not deposit fat, the lipid content will probably be below 10% of the dry weight. Fat depositing bacteria (which will not occur in abundance in treatment processes) may contain lipids as high as 40%. Bacterial lipids will consist of free fatty acids, neutral fats and waxes, and phospholipids. Free fatty acids are usually in

abundance, and a gram negative enteric organism such as Salmonella typhosa (also a pathogen) may consist almost entirely of free fatty acids. The fatty acids most commonly found are palmitic, stearic, and oleic acids and lauric, myristic, tetra-cosanic, cerotic, linoleic, butyric, and caproic acids have been reported. Natural lipids consist predominantly of the esters of higher fatty acids with carbohydrates. Glycerol or sterol esters are usually absent, or present only in small quantities. Bacterial lipids will include a variable proportion of phosphatides in which the glycerol has partly or totally been replaced by a carbohydrate (also variable).

The ash content of various bacterial cells will range from 1 to 14%. In addition to the major mineral elements, phosphorous, potassium, sodium, magnesium, chlorine and sulfur, shown in Table 9, many elements, such as iron, aluminum, manganese, copper and boron, will occur in trace amounts. The metals and base elements will occur in both inorganic and organic combinations.

Phosphorous comprises about 50% of the inorganic portion of the mineral elements. As part of organic molecules, phosphorous can exist combined with nucleic acids, phospholipids, and various coenzymes. Magnesium will also occur in organic combinations associated with certain enzymes. Iron is found as part of the cytochrome system. Sulfur occurs in glutathione

Table 9

Composition of Ash from Vegetative Cells*

<u>Organism</u>	<u>%</u>							
	<u>P₂O₅</u>	<u>K₂O</u>	<u>Na₂O</u>	<u>MgO</u>	<u>CaO</u>	<u>SiO₂</u>	<u>Cl</u>	<u>FeO</u>
Acetobacter sp	18	26	--	1	14	8	2	8
Serratia marcescens	36	11	28	7	4	1	5	

*Adapted from Buchanan and Fulmer (see Reference 70).

and certain amino acids. Trace elements may occur in all organic combinations.

Inorganic mineral salts of elements present probably regulate growth, multiplication and other cellular processes such as producing osmotic pressure or affecting colloidal systems or membrane permeability (71).

Ammonia and Other Amine Production

Ammonia is not generally considered to be a problem in conventional waste treatment processes since it occurs only in relatively low concentrations (77) and is readily eliminated or combined with carbon dioxide. Closed system wastes will have relatively high ammonia and urea concentrations. Under normal dietary conditions, fresh urine will contain from 0.3 to 1.2 grams (average: 0.7 gm) of ammonia (78). Urine will also contain about 22.5 gm of urea (39). Feces will contain from 0.25 to 0.88 gm/l of ammonia (34) but very little or no urea.

Known urea splitting bacteria that could be expected in feces, or as aerial contaminants include:

Proteus (all species)

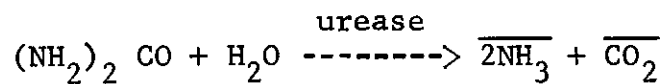
Klebsiella pneumoniae

Aerobacter aerogenes

Pseudomonas aeruginosa

Staphylococcus aureus

These bacteria produce the enzyme urease which readily splits urea into ammonia and carbon dioxide according to the following equation:



Seneca, Peer and Nally (79) report that these organisms attack urea readily and avidly, and increase ammonia levels.

Other sources of ammonia might be considered. Cantarow (78) suggests the feasibility of protein digestion, since feces also contains small amounts of proteolytic enzymes such as trypsin, amylase, nucleases, maltase, lipase and lysozyme.

Ammonia in addition to being toxic to microorganisms effects a pH change. When insufficient carbon dioxide is present for the production of bicarbonates, a high pH, usually in the range 8.6 to 8.8, results. At this pH range most microbiologic metabolic activity is nil.

Snell (80) studied the effect of various ammonium salt concentrations on digestion efficiency and concluded that both urea and ammonium carbonate (4,300 ppm as nitrogen) completely prevent the digestion of seeded feces, but that the same strength of ammonium bicarbonate only retards the rate of digestion by 25 to 50%. Sulfate ion (1.4%) when in combination

with either sodium or ammonium ion was found to be completely inhibitive even when the pH was adjusted to 7.0. It is thought that the strong alkaline ions, sodium and potassium, are more inhibitive than ions such as ammonium or calcium. A combination of ions from a strong acid and a weak base, as well as from a strong base and a weak acid, are found to be more inhibitive than those salts resulting from a combination of a strong acid and a strong base or from a weak acid and a weak base.

McCarty and McKinney (77, 81) have also studied salt toxicity as affecting digestion. According to the equations for the equilibrium constant of free ammonia versus the ammonium ion

$$\frac{(\text{NH}_4^+) (\text{OH}^-)}{(\text{NH}_3) (\text{H}_2\text{O})} = 1.85 \times 10^{-5}$$

and the P_w equation at 35°C

$$\frac{(\text{H}^+) (\text{OH}^-)}{(\text{H}_2\text{O})} = 2.09 \times 10^{-14}$$

the ammonia concentration, upon combining the two equations, is

$$(\text{NH}_3) = 1.13 \times 10^{-9} \frac{(\text{NH}_4^+)}{(\text{H}^+)}$$

Consequently, the lower the pH the higher is the ammonium concentration required to produce a given free ammonia concentration. Assuming ideal conditions, it has been calculated that when the free ammonia concentration exceeded 150 mg/l, digestion stopped completely.

The studies of McCarty and McKinney are in agreement with those of Snell--that the more alkaline the cation involved in the salt added the greater is the inhibition of digestion. It has been pointed out that if these salts were added gradually rather than on a "slug" feed basis, digester toxicity is decreased (77, 81). It is evident from these studies that digester efficiency is sharply curtailed when sufficient buffer capacity is not maintained.

Although ammonia production may be the greatest source of digester toxicity, other toxins, present in the wastes, may contribute to digester toxicity as well as to human toxicity. The toxins include cadaverine, putrescine, histamine and decarboxylation products of amino acids. Decarboxylation is the result of bacterial enzyme systems found among intestinal flora. According to Gale (68), bacterial decarboxylases are adaptive enzymes formed in large quantities only when the organisms are grown in an acid medium depending on the organism (pH 2.5 to 5.5).

The overall elimination of amino acids in the feces, however, is relatively small (35); pH values are higher than the values given by Gale as optimum; and the substrate, i.e., feces, is of a mixed nature rather than specific. It follows that the production of the toxins, putrescine, cadaverine and histamine, would be minimal if produced at all.

Hanke and Koessler (82) report that histamine has actually been found but in a concentration of 6 to 20 mg per 500-600 gm of feces.

Hacker et al. (83) have reported on 14 amino acids found in sewage sludge. The amount of acids found is compared with the amount found in egg protein (see Table 10). All nine essential amino acids were considered ^{essentially} inadequate as a food supplement for swine and poultry with the possible exception of threonine.

Microorganisms

Microorganisms found in both aerobic and anaerobic treatment processes enter with the waste water that originates in wastes contributed directly by man, or infiltration wash from the soil and other extraneous sources. Many of the microorganisms found are not essential to purification. Only a few are essential to the reduction of BOD and the clarification of waste.

Table 10

Amino Acid Content of Sewage Sludge*

<u>Amino Acids</u>	<u>Percentage of Material in</u>	
	<u>Sludge</u>	<u>Egg Protein</u>
Arginine	1.1	3.35
Histidine	0.44	1.34
Isoleucine	1.4	4.26
Leucine	1.8	5.48
Lysine	1.3	3.95
Methionine	0.5	1.52
Cystine	0.2	0.61
Phenylalanine	1.6	4.87
Tyrosine	0.7	2.13
Threonine	1.5	4.56
Tryptophan	0.25	0.76
Valine	2.0	6.08
Glycine	1.55	4.71
Glutamic	2.9	8.8
Moisture	4.5	
N. dry basis	5.26	
Ash dry basis	38.0	

*Taken from Hacker, et al (see Reference 83).

In both aerobic and anaerobic systems the nature of the microbial flora will be determined by the nature of the waste and other operational factors.

A large number of microorganisms have been reported as present in both activated sludge and trickling filters. Both activated sludge units and trickling filters contain biota consisting of essentially similar groups of organisms.

Tables 11 and 12 list the microorganisms reported in activated sludge units and trickling filters, and Table 13 is a compilation of reported organisms common to both processes. While the organisms listed represent a methodical search, it does not follow that all organisms present in the aerobic processes have been listed.

Zoogleal bacteria, or floc-forming bacteria, are probably the most abundant forms of bacteria present in both activated sludge and trickling filter growths. Under certain circumstances, depending on relative surface changes and energy levels (72), many bacteria might be able to form floc.

Both activated sludge units and trickling filters will support filamentous bacteria, filamentous fungi, and many different species of protozoa. Rotifers, nematodes, worms, tardigrades, water mites, annelids, insect larvae and snails usually grow, sometimes in large numbers. Snails have been

Table 11

Microorganisms Reported in Activated Sludge

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
<u>Bacteria</u>		
Achromobacterum liquefaciens	McKinney & Weichlein 1953	Appl. Microbiology, 1, 259
Achromobacterum sp.	Allen, L. A. 1944	J. Hyg. Cambridge, 43, 424
Aerobacter aerogenes	Butterfield 1935	Pub. Health Repts., 50, 671
Alkaligenes faecalis	McKinney & Weichlein 1953	op. cit.
Bacillus mycooides	Lackey & Wattie 1940	Sew. Wks. J., 12, 669
Bacillus cereus	McKinney & Horwood 1952	Sew. Ind. Wastes, 24, 117
Beggiatoa	Buswell 1932	Sew. Wks. J., 3, 363
Bacillus megatherium	McKinney & Weichlein 1953	op. cit.
Bacillus subtilis	Courmont	Compt. Rendu., 170, 75, 976, 1134
Chromobacterium (Flavobacterium)	Allen, L. A. 1944	op. cit.
Crenothrix polyspora	Buswell & Long 1923	J. Amer. Water Wks. Assn., 10, 309
Escherichia coli	Courmont	op. cit.
Escherichia freundi	McKinney & Weichlein 1953	op. cit.
Escherichia intermedium	McKinney & Horwood 1952	op. cit.
Flavobacterium lueve	McKinney & Weichlein 1953	op. cit.
Flavobacterium solare	McKinney & Weichlein 1953	op. cit.
Klebsiella pneumonia	McKinney & Weichlein 1953	op. cit.
Neisseria catarrhalis	McKinney & Weichlein 1953	op. cit.
Nitrobacteria	Russell, R.	Univ. of Ill. Bull. State Water Survey, No. 13, 348
Nitrosomonos sp.	Russell, R.	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Nocardia actinomorpha	McKinney & Horwood 1952	op. cit.
Paracolobactrum aerogenoides	McKinney & Horwood 1952	op. cit.
Pseudomonas sp.	Lackey & Wattie 1940	op. cit.
Pseudomonas pavonacea	McKinney & Weichlein 1953	op. cit.
Pseudomonas perlurida	McKinney & Weichlein 1953	op. cit.
Pseudomonas tralucida	McKinney & Weichlein 1953	op. cit.
Pseudomonas salopium	McKinney & Weichlein 1953	op. cit.
Sphaerotilus sp.	Buswell & Long 1923	op. cit.
Sphaerotilus dichotomus	Buswell & Long 1923	op. cit.
Zooglea ramigera	Johnson 1914	J. Econ. Biol., <u>9</u> , 105
<u>Fungi</u>		
Achlya	Chicago; Edwards, G. P.	not reported
Allerschierii boydii	Cooke & Kabler 1955	Publ. Hlth. Repts., <u>70</u> , 689
Aspergillus fumigatus	Cooke & Kabler 1955	op. cit.
Cladothrix dichotoma	Smit 1934	Sew Wks J., <u>6</u> , 1041
Geotrichum candidum	Cooke & Kabler 1955	op. cit.
Geotrichoides paludosus	Smit 1934	op. cit.
Zoophagus insidians	Cooke & Ludzack 1953	Sew & Ind Wastes, <u>30</u> , 1490
<u>Protozoa</u>		
<u>Sarcodina</u>		
Amoeba sp.	Buswell & Long 1923	J. Amer. Water Wks. Assn., <u>10</u> , 309
Amoeba proteus	Lackey 1938	Ecological Monographs, <u>8</u> , (<u>4</u>), 501
Amoeba radiosa	Lackey 1938	op. cit.
Amoeba striata	Lackey 1938	op. cit.
Amoeba tachypoda	Lackey 1938	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Amoeba valkampfia	Edwards, G. P.	Not published
Amoeba verrucosa	Lackey 1938	op. cit.
Arcella sp.	Edwards, G. P.	Not published
Arcella vulgaris	Tomlinson 1939	The Surveyor, <u>95</u> (No. 2469), 655
Centrophyxis aculaeta	Lackey 1938	op. cit.
Chlamodophrys minor	Lackey 1938	op. cit.
Chlamodophrys stercorea	Lackey 1938	op. cit.
Cochliopodium bilibosum	Lackey 1938	op. cit.
Diffflugia globosa	Lackey 1938	op. cit.
Diplophrys sp.	Lackey 1949	Sew. Wks. J., <u>21</u> , 659
Euglypha alveolata	Lackey 1938	op. cit.
Euglypha tuberculata	Tomlinson 1939	op. cit.
Hartmanella hyalina	Agersborg & Hatfield 1929	Sew. Wks. J., <u>1</u> , (4) 411
Nuclearia simplex	Lackey 1938	op. cit.
Protamoeba primitava	Agersborg & Hatfield 1929	op. cit.
Trinema lineare	Lackey 1938	op. cit.
Valkampfia albida	Agersborg & Hatfield 1929	op. cit.
Valkampfia guttula	Agersborg & Hatfield 1929	op. cit.
Valkampfia limax	Agersborg & Hatfield 1929	op. cit.
<u>Mastigophora</u> (Flagellates)		
Anisonema sp.	Agersborg & Hatfield 1929	op. cit.
Anisonema ovale	Lackey 1938	op. cit.
Astasia sp.	Agersborg & Hatfield 1929	op. cit.
Bodo sp.	Lackey 1938	op. cit.
Bodo agilis	Lackey 1938	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Bodo caudatus	Agersborg & Hatfield 1929	op. cit.
Bodo globosus	Lackey 1938	op. cit.
Bodo lens	Lackey 1938	op. cit.
Bodo ovatus	Lackey 1938	op. cit.
Bodopsis godboldi	Lackey 1938	op. cit.
Cercobodo caudatus	Lackey 1938	op. cit.
Cercobodo crassicauda	Lackey 1938	op. cit.
Cercobodo longicauda	Lackey 1938	op. cit.
Cercobodo ovatus	Lackey 1938	op. cit.
Cercomonas sp.	Agersborg & Hatfield 1929	op. cit.
Chilomonas sp.	Agersborg & Hatfield 1929	op. cit.
Chilomonas paramecium	Lackey 1938	op. cit.
Cythomonas truncata	Lackey 1938	op. cit.
Dinomonas vorax	Agersborg & Hatfield 1929	op. cit.
Distigma proteus	Agersborg & Hatfield 1929	op. cit.
Entosiphon sulcatum	Lackey 1938	op. cit.
Euglena sp.	Agersborg & Hatfield 1929	op. cit.
Euglena gracilis	McKinney & Gram 1956	Sew & Ind Wastes, 28, 1229
Euglena quartana	Lackey 1938	op. cit.
Hexamitus crassus	Lackey 1938	op. cit.
Hexamitus inflatus	Lackey 1938	op. cit.
Mastigamoeba longifilum	Lackey 1938	op. cit.
Mastigamoeba reptans	Lackey 1938	op. cit.
Monas sp.	Agersborg & Hatfield 1929	op. cit.
Menoidium incurvum	Lackey 1938	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Monas amoebina	Lackey 1938	op. cit.
Monas minima	Lackey 1938	op. cit.
Monas obliqua	Lackey 1938	op. cit.
Monas vivipara	Lackey 1938	op. cit.
Monas vulgaris	Lackey 1938	op. cit.
Notosolenus orbicularis	Lackey 1938	op. cit.
Oicomonas sp.	Agersborg & Hartfield 1929	op. cit.
Oicomonas arcellata	Lackey 1938	op. cit.
Oicomonas socialis	Lackey 1938	op. cit.
Oicomonas steinii	Lackey 1938	op. cit.
Oicomonas termo	Lackey 1938	op. cit.
Peranema sp.	Agersborg & Hartfield 1929	op. cit.
Peranema ovalis	Lackey 1938	op. cit.
Petalomonas irregularis	Agersborg & Hatfield 1929	op. cit.
Physomonas sp.	Agersborg & Hatfield 1929	op. cit.
Pleuronema jaculans	Agersborg & Hatfield 1929	op. cit.
Polytoma uvella	Lackey 1938	op. cit.
Rhynchomonas nasuta	Lackey 1938	op. cit.
Tetramitus pyriformis	Lackey 1938	op. cit.
Trepomonas agilis	Lackey 1938	op. cit.
Trepomonas rotans	Lackey 1938	op. cit.
<u>Infusoria (Ciliates)</u>		
Acineta sp.	Buswell & Long 1923	op. cit.
Amphileptus sp.	Baines et al 1953	Sew & Ind Wks, <u>25</u> , 1023
Aspidisca costata	Agersborg & Hatfield 1929	op. cit.
Aspidisca lynceus	Lackey 1938	op. cit.
Aspidiscus turrita	Lackey 1938	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Blepharisma sp.	Agersborg & Hatfield 1929	op. cit.
Bursaria sp.	Agersborg & Hatfield 1929	op. cit.
Carchesium sp.	Buswell & Long 1923	op. cit.
Carchesium polypinium	Lackey 1938	op. cit.
Chilodon sp.	Agersborg & Hatfield 1929	op. cit.
Chilodon cucullulus	Agersborg & Hatfield 1929	op. cit.
Chilodonella cucullulus	Lackey 1938	op. cit.
Chilodonella uncinatus	Lackey 1938	op. cit.
Cinetochilum margaritaceum	Lackey 1938	op. cit.
Coleps hirtus	Lackey 1938	op. cit.
Colopoda inflata	Lackey 1938	op. cit.
Colpidium sp.	Agersborg & Hatfield 1929	op. cit.
Colpidium campylum	Lackey 1938	op. cit.
Colpidium colpoda	Lackey 1938	op. cit.
Cyclidium glaucoma	Lackey 1938	op. cit.
Didinium nasutum	Lackey 1938	op. cit.
Dileptus gigas	Lackey 1938	op. cit.
Epistylis plicatilis	Lackey 1938	op. cit.
Eupoltes sp.	Buswell & Long 1923	op. cit.
Eupoltes charon	Agersborg & Hatfield 1929	op. cit.
Eupoltes harpa	Lackey 1938	op. cit.
Eupoltes patella	Lackey 1938	op. cit.
Frontonia sp.	Buswell & Long 1923	op. cit.
Glaucoma scintillans	Lackey 1938	op. cit.
Hexotricha sp.	Agersborg & Hatfield	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Holophyra discolor	Lackey 1938	op. cit.
Holophyra nigricans	Lackey 1938	op. cit.
Lagynus simplex	Lackey 1938	op. cit.
Lembus infusorium	Lackey 1938	op. cit.
Lembus pusillus	Lackey 1938	op. cit.
Lionotus sp.	Lackey 1938	op. cit.
Lionotus fascicola	Lackey 1938	op. cit.
Linnotus lamella	Lackey 1938	op. cit.
Loxocephalus granulosa	Lackey 1938	op. cit.
Loxophyllum maleagris	Lackey 1938	op. cit.
Loxodes opaca	Sladeczek 1957	Scientific Papers, Inst. of Chem. Tech., Prague
Metopus sp.	Sladeczek 1957	op. cit.
Metopus sigmoides	Agersborg & Hatfield 1929	op. cit.
Microthorax sulcatus	Lackey 1938	op. cit.
Monadinium sp.	Horosawa 1950	Sew. & Ind. Wastes, <u>22</u> , 959
Opercularia sp.	Johnson, J. W. H. 1914	J. Econ. Biol., <u>9</u> , 105
Oxytricha sp.	Agersborg & Hatfield 1929	op. cit.
Oxytrichia falla	Lackey 1938	op. cit.
Paramoecium caudatum	Lackey 1938	op. cit.
Paramoecium putrinum	Lackey 1938	op. cit.
Podophyra sp.	Buswell & Long 1923	op. cit.
Pyxidium sp.	Buswell & Long 1923	op. cit.
Rhabdostylus	Sladeczek 1957	op. cit.
Spathidium spathula	Lackey 1938	op. cit.
Spirostoma teres	Lackey 1938	op. cit.

<u>Organisms</u>	<u>Reported by</u>	<u>Reference</u>
Stentos sp.	Edwards, G. P.	Not published
Stylonichia mytilus	Lackey 1938	op. cit.
Stylonichia pustulata	Lackey 1938	op. cit.
Styonychia sp.	Sladeczek 1957	op. cit.
Tachysoma sp.	Saladecek 1957	op. cit.
Tetrahymena gelii	McKinney & Gram 1956	op. cit.
Trochilopsis opaca	Lackey 1938	op. cit.
Vorticella sp.	Buswell & Long 1923	op. cit.
Vorticella campanula	Lackey 1938	op. cit.
Vorticella microstoma	Agersborg & Hatfield 1929	op. cit.
Vorticella nebulifera	Lackey 1938	op. cit.
Vorticella telescopia	Agersborg & Hatfield 1929	op. cit.
Zeothamnium sp.	Buswell & Long 1923	op. cit.

Table 12

Microorganisms Reported in Trickling Filters

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
<u>Bacteria (including filamentous)</u>		
Actinomyces spp.	Calaway 1957	Sew. & Ind. Wastes, <u>29</u> , 1
Aerobacter aerogenes	Calaway et al. 1952	Sew. & Ind. Wastes, <u>24</u> , 642
Alcaligenes spp.	Calaway et al. 1952	op. cit.
Alcaligenes bookeri	Calaway et al. 1952	op. cit.
Alcaligenes faecalis	Calaway et al. 1952	op. cit.
Bacillus alvei	Calaway et al. 1952	op. cit.
Bacillus cereus	Calaway et al. 1952	op. cit.
Bacillus circulans	Calaway et al. 1952	op. cit.
Bacillus megatherium	Calaway et al. 1952	op. cit.
Bacillus violaceus	Buswell 1928	Chapt. XXII, The Chem of Water and Sewage Treatment, The Chem Catalog Co., N. Y. C.
Bacillus pumilus	Calaway et al. 1952	op. cit.
Bacillus subtilis	Calaway et al. 1952	op. cit.
Beggiatoa sp.	Rettger 1906	Eng News Record, <u>61</u> , 459
Beggiatoa alba	Buswell 1928	op. cit.
Chromobacterium janthinum	Calaway et al. 1952	op. cit.
Cladothrix sp.	Holtje 1943	Sew. Wks. J., <u>15</u> , 14
Cladothrix dichotoma	Nauman 1933	Zentr. Bakteriol Parasit 2, Abs 85
Colon bacillus	Buswell 1928	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Coli-aerogenes		Mass. State Brd. of Hlth., 1887-1915, Ann. Rept's, Fr. J. Hyg., <u>47</u> , 303 (1949)
Escherichia coli	Boyce, MacConkey & Hill 1902	Roy Comm on Sew. Disp., 2nd Report Sew & Ind Wastes, <u>24</u> , 117
Escherichia coli, intermedium	McKinney et al 1952	op. cit.
Flavobacterium spp.	Calaway et al 1952	op. cit.
Flavobacterium aquatile	Calaway et al 1952	op. cit.
Flavobacterium balustinum	Calaway et al 1952	op. cit.
Flavobacterium devorans	Calaway et al 1952	op. cit.
Leptothrix ochrocae	Nauman 1933	op. cit.
Magneta bacillus	Buswell 1928	op. cit.
Nitrobacter sp.	Holtje 1943	op. cit.
Nitrosomonas sp.	Holtje 1943	op. cit.
Nocardia spp.	Calaway et al 1952	op. cit.
Rhizobium radicicola	Buchanan 1909	Centrb1 f. Bakt, <u>22</u> , 37
Sphaerotilus sp.	Cox 1921	Eng. News Record, <u>87</u> , 720
Sphaerotilus natans var carnea	Johnson 1914	J. Econ. Biol., <u>9</u> , <u>105</u>
Sphaerotilus natans var compacta	Johnson 1914	op. cit.
Sphaerotilus natans var natans	Johnson 1914	op. cit.
Sphaerotilus natans var uva	Johnson 1914	op. cit.
Spirillum sp.	Buswell 1928	op. cit.
Streptococcus faecalis	Allen, Brooks, & Williams 1949	J. Hyg., <u>47</u> , 303
Streptomyces spp.	Calaway et al 1952	op. cit.
Thiothrix nivea	Buswell 1928	op. cit.
Zooglea filipendula	Butterfield et al 1937	Pub Hlth Rpts US, <u>52</u> , 387
Zooglea ramigera	Johnson 1914	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
<u>Fungi</u>		
Absidia spp.	Cooke & Hirsch 1958	Sew. & Ind. Wastes, <u>30</u> , 138
Absidia corymbifera	Cooke 1959a	Ecology, <u>40</u> , 273
Absidia cylindrospora	Cooke 1959a	op. cit.
Acreminium sp.	Cooke 1959b	Proc. 13th Ind. Waste Conf., No. 96, Vol. 43, pp. 26
Allescheria boydii	Cooke & Kabler 1955	Publ Hlth Rpts, <u>70</u> (7) 689
Alternaria tenuis	Cooke & Hirsch 1958	op. cit.
Ascodesmis microspica	Cooke & Hirsch 1958	op. cit.
Ascoidea rubescens	Tomlinson 1946	Proc. Inst. Sew. Purif., Pt. 1
Aspergillus spp.	Cooke & Hirsch 1958	op. cit.
Aspergillus candidus	Cooke & Hirsch 1958	op. cit.
Aspergillus chevalieri	Cooke 1959b	op. cit.
Aspergillus clavatus	Cooke & Hirsch 1958	op. cit.
Aspergillus flavipes	Cooke & Hirsch 1958	op. cit.
Aspergillus flavus	Cooke & Hirsch 1958	op. cit.
Aspergillus fumigatus	Cooke & Kabler 1955	op. cit.
Aspergillus niger	Cooke & Hirsch 1958	op. cit.
Aspergillus ochraceous	Cooke & Hirsch 1958	op. cit.
Aspergillus syndowii	Cooke & Hirsch 1958	op. cit.
Aspergillus terreus	Cooke 1959a	op. cit.
Aspergillus ustus	Cooke 1959a	op. cit.
Aspergillus versicolor	Cooke & Hirsch 1958	op. cit.
Aspergillus wentii	Cooke & Hirsch 1958	op. cit.
Botrytis vulgaris	Buswell 1928	op. cit.
Cephalosporium spp.	Cooke & Hirsch 1958	op. cit.
Chaetomium globosum	Cooke & Hirsch 1958	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Cladosporium cladosporoides	Cooke & Hirsch 1958	op. cit.
Conidiobolus villosus	Cooke 1959b	op. cit.
Coniothyrium spp.	Cooke & Hirsch 1958	op. cit.
Coniothyrium fuckelii	Cooke & Hirsch 1958	op. cit.
Dictyochyus spp.	Hausman 1923	N. J. Agr. Exp. Sta., Bul. 390
Dematiaceae spp.	Cooke & Hirsch 1958	op. cit.
Epicoccum nigrum	Cooke & Hirsch 1958	op. cit.
Fusarium spp.	Cooke & Hirsch 1958	op. cit.
Fusarium aquaeductum	Rettger 1906	Eng News, 61, 459
Fusarium auriantiacum	Buswell 1928	op. cit.
Fusarium oxysporum	Cooke & Hirsch 1958	op. cit.
Fusarium roseum	Cooke & Hirsch 1958	op. cit.
Fusarium solani	Cooke & Hirsch 1958	op. cit.
Geotrichum (Oospora) candidum	Rettger 1906	op. cit.
Gliocladium catenulatum	Cooke 1959b	op. cit.
Gliocladium roseum	Cooke & Hirsch 1958	op. cit.
Gliomastix convulata	Cooke & Hirsch 1958	op. cit.
Leptomitius lacteus	Kolkwitz 1903	Ber. Deutsch. Bot. Ges., 19, 288
Margarinomyces heteromorphyum	Cooke & Hirsch 1958	op. cit.
Memnoniella echinata	Cooke & Hirsch 1958	op. cit.
Moniliaceae spp.	Cooke & Hirsch 1958	op. cit.
Monilia sitophila	Cooke & Hirsch 1958	op. cit.
Mucor circenelloides	Buswell 1928	op. cit.
Mucor fragilis	Cooke & Hirsch 1958	op. cit.
Mucor mucedo	Bell 1926	Surveyor, 70, 561

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Mucor plumbeus	Cooke & Hirsch 1958	op. cit.
Mucor racemosus	Buswell 1928	op. cit.
Mucor saturninus	Cooke 1959a	op. cit.
Myrothecium verrucaria	Cooke & Hirsch 1958	op. cit.
Oospora sp.	Ray Noldson 1942	Proc. Inst. Sew. Purif., Pt. 1
Oospora fragrans	Cooke & Hirsch 1958	op. cit.
Oospora lactis	Buswell 1928	op. cit.
Oidium sp.	Hausman 1923	op. cit.
Oidium lactis	Bell 1926	op. cit.
Paecilomyces varioti	Cooke & Hirsch 1958	op. cit.
Penicillium spp.	Hausman 1923	op. cit.
Penicillium brevi-compactum	Cooke 1959b	Proc. 13th Purdue Ind. Waste Conf. Series No. 96, Vol. 43, No. 3, pp. 26-45
Penicillium chrysogenum	Cooke 1959a	op. cit.
Penicillium clavigerum	Cooke & Hirsch 1958	op. cit.
Penicillium Corylophilum	Cooke 1959b	op. cit.
Penicillium cyclopium	Cooke 1959a	op. cit.
Penicillium digitatum	Cooke 1959a	op. cit.
Penicillium expansum	Cooke & Hirsch 1958	op. cit.
Penicillium funiculosum	Tiegs 1934	Vom Wasser, <u>13</u> , 78
Penicillium frequentans	Tiegs 1934	op. cit.
Penicillium herquei	Cooke & Hirsch 1958	op. cit.
Penicillium implicatum	Cooke 1959a	op. cit.
Penicillium janthinellum	Cooke & Hirsch 1958	op. cit.
Penicillium lilacinum	Cooke & Hirsch 1958	op. cit.
Penicillium luteum	Cooke & Hirsch 1958	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Penicillium martensii	Cooke & Hirsch 1958	op. cit.
Penicillium nigricans	Cooke & Hirsch 1958	op. cit.
Penicillium ochloro-chloron	Cooke & Hirsch 1958	op. cit.
Penicillium oxalicum	Cooke & Hirsch 1958	op. cit.
Penicillium palitans	Cooke 1959a	op. cit.
Penicillium piscarium	Cooke 1959a	op. cit.
Penicillium purpureogenum	Cooke & Hirsch 1958	op. cit.
Penicillium roquefortii	Cooke 1959a	op. cit.
Penicillium stiptitatum	Cooke & Hirsch 1958	op. cit.
Penicillium variable	Cooke & Hirsch 1958	op. cit.
Penicillium velutinum	Cooke & Hirsch 1958	op. cit.
Penicillium veridicaum	Cooke 1959b	op. cit.
Penicillium vermiculatum	Cooke 1959a	op. cit.
Peziza omphalodes	Cox 1921	op. cit.
Phoma spp.	Cooke 1959a	op. cit.
Pilobulus oedipus	Cox 1921	op. cit.
Pullaria pullulans	Cooke & Hirsch 1958	op. cit.
Pythium spp.	Hausman 1923	op. cit.
Rhizopus spp.	Cooke & Hirsch 1958	op. cit.
Rhizopus arrhizus	Cooke 1959a	op. cit.
Rhizopus nigricans	Cooke 1959a	op. cit.
Rhizopus oryzae	Cooke 1959b	op. cit.
Rhizopus rhizopodiformis	Cooke 1959a	op. cit.
Rhodotorula spp.	Cooke 1959a	op. cit.
Rhodotorula glutinis	Cooke & Hirsch 1958	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Sepedonium spp.	Tomlinson 1946	op. cit.
Selenospora sp.	Butcher 1932	Trans Brit Mycol Soc, <u>17</u> , 112
Sphaeronema spinella	Cooke 1959a	op. cit.
Sporotrichum pruinatum	Cooke & Hirsch 1958	op. cit.
Sporotrichum lanatum	Buswell 1928	op. cit.
Stachybotrys atra	Cooke & Hirsch 1958	op. cit.
Stemphylium consortiale	Cooke & Hirsch 1958	op. cit.
Subbaromyces splendens	Cooke & Hirsch 1958	op. cit.
Syncephalastrum racemansum	Cooke 1959a	op. cit.
Torula rosea	Bell 1926	Surveyor, <u>70</u> , 561
Trichoderma viride	Cooke & Hirsch 1958	op. cit.
Trichothecium roseum	Cooke 1959b	op. cit.
Verticillium spp.	Cooke & Hirsch 1958	op. cit.
Verticillium lateritium	Cooke & Hirsch 1958	op. cit.
White yeasts	Cooke & Hirsch 1958	op. cit.
<u>Protozoa</u>		
<u>Sarcodina</u>		
Actinophrys spp.	Lackey 1926	N. J. Agr. Sta. Bull. 427
Actinophrys sol	Lackey 1926	op. cit.
Actinosphaeraeium spp.	Lackey 1924	N. J. Agr. Sta. Bull. 403
Actinosphaeraeium eichhornii	Lackey 1926	op. cit.
Amoeba spp.	Hausman 1923	op. cit.
Amoeba guttula	Hausman 1923	op. cit.
Amoeba limax	Hausman 1923	op. cit.
Amoeba proteus	Hausman 1923	op. cit.
Amoeba radiosa	Lackey 1924	op. cit.
Amoeba verrucosa	Lackey 1926	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Amoeba villosa	Frye & Becker 1929,	Sew. Wks. J., <u>1</u> , 286
Arcella spp.	Hausman 1923	op. cit.
Arcella dentata	Lackey 1926	op. cit.
Arcella vulgaris	Lackey 1926	op. cit.
Centrophyxis spp.	Hausman 1923	op. cit.
Centrophyxis aculeata	Lackey 1926	op. cit.
Chlamodophrys spp.	Lackey 1926	op. cit.
Chlamodophrys stercorea	Lackey 1926	op. cit.
Cochliopodium spp.	Lackey 1924	op. cit.
Cochliopodium bilimbosum	Lackey 1926	op. cit.
Diffflugia spp.	Hausman 1923	op. cit.
Diffflugia pyriformis	Lackey 1926	op. cit.
Diplophrys archeri	Heukelekian 1948	Sew Wks J, <u>20</u> , 1032
Distigamoeba gruberi	Frye & Becker 1929	op. cit.
Euglypha spp.	Hausman 1923	op. cit.
Euglypha alveolata	Lackey 1926	op. cit.
Hartmanella hyalina	Lackey 1926	op. cit.
Pamphagus nutabilis	Lackey 1926	op. cit.
Protamoeba primitiva	Lackey 1926	op. cit.
Rhapidophrys	Lackey 1924	op. cit.
Trinema spp.	Hausman 1923	op. cit.
Trinema lineare	Lackey 1924	op. cit.
Valkhampfia albida	Lackey 1926	op. cit.
Valkhampfia guttula	Lackey 1926	op. cit.
Valkhampfia limax	Lackey 1926	op. cit.
Vampyrella laterita	Frye & Becker 1929	op. cit.

<u>Organism</u>	<u>Author</u>	<u>Reference</u>
<u>Mastogophora (Flagellates)</u>		
Anthophysis vegetans	Frye & Becker 1929	op. cit.
Astasia	Lackey 1924	op. cit.
Bodo caudatus	Frye & Becker 1929	op. cit.
Bodo lens	Frye & Becker 1929	op. cit.
Bodo mutabilis	Frye & Becker 1929	op. cit.
Cercobodo sp.	Frye & Becker 1929	op. cit.
Cercobodo longicauda	Frye & Becker 1929	op. cit.
Cercobodo ovatus	Frye & Becker 1929	op. cit.
Distigma sp.	Lackey 1924	op. cit.
Distigma proteus	Frye & Becker 1929	op. cit.
Entosiphon sp.	Lackey 1924	op. cit.
Entosiphon sulcatus	Lackey 1924	op. cit.
Euglena sp.	Lackey 1924	op. cit.
Euglena gracilis	Lackey 1924	op. cit.
Euglena intermedia	Lackey 1924	op. cit.
Heteronema sp.	Lackey 1924	op. cit.
Mastagamoeba sp.	Lackey 1924	op. cit.
Menoidium	Lackey 1926	op. cit.
Monas sp.	Frye & Becker 1929	op. cit.
Notosolenus	Lackey 1924	op. cit.
Oicomonas socialis	Frye & Becker 1929	op. cit.
Peranema sp.	Lackey 1924	op. cit.
Peranema trichophora	Frye & Becker 1929	op. cit.
Petalomonas	Lackey 1926	op. cit.
Tetramitus sp.	Frye & Becker 1929	op. cit.
Trepomonas agilis	Frye & Becker 1929	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
<u>Infusoria (Ciliates)</u>		
Amphileptus spp.	Lackey 1924	op. cit.
Amphisia spp.	Lackey 1924	op. cit.
Aspidisca spp.	Hausman 1923	op. cit.
Blepharisma	Hausman 1923	op. cit.
Bursaria	Hausman 1923	op. cit.
Carchesium spp	Lackey 1924	op. cit.
Chaenir	Hausman 1923	op. cit.
Chilodon spp.	Hausman 1923	op. cit.
Chilodon cucullus	Frye & Becker 1929	op. cit.
Cinetochilum spp.	Lackey 1926	op. cit.
Cinetochilum margaritaceum	Frye & Becker 1929	op. cit.
Coenomorpha	Hausman 1923	op. cit.
Colpidium spp.	Hausman 1923	op. cit.
Colpidium striatum	Lackey 1924	op. cit.
Colpidium tetrahymena	Calaway 1957	Sew. & Ind. Wastes, <u>29</u> , 1
Colpoda spp.	Hausman 1923	op. cit.
Colpoda inflata	Frye & Becker 1929	op. cit.
Cyclidium spp.	Hausman 1923	op. cit.
Cyclidium glaucoma	Frye & Becker 1929	op. cit.
Epistylis	Holtje 1943	op. cit.
Euplotes spp.	Hausman 1923	op. cit.
Euplotes charon	Frye & Becker 1929	op. cit.
Frontonia	Hausman 1923	op. cit.
Glaucoma spp.	Hausman 1923	op. cit.
Glaucoma scintillans	Frye & Becker 1929	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Halteria spp.	Hausman 1923	op. cit.
Holophyra spp.	Hausman 1923	op. cit.
Lacrymaria	Hausman 1923	op. cit.
Lembus spp.	Lackey 1924	op. cit.
Leucophyrs	Calaway 1957	op. cit.
Lionotopsis spp.	Hausman 1923	op. cit.
Lionotus spp.	Hausman 1923	op. cit.
Lionotus fascicola	Frye & Becker 1929	op. cit.
Loxodes spp.	Lackey 1924	op. cit.
Loxophyllum spp.	Hausman 1923	op. cit.
Macrymaria spp.	Lackey 1926	op. cit.
Metopus spp.	Hausman 1923	op. cit.
Microthorax spp.	Lackey 1924	op. cit.
Nassula spp.	Lackey 1924	op. cit.
Opercularia spp.	Crozier 1933	Science, <u>58</u> , 424
Opercularia berberina	Frye & Becker 1929	op. cit.
Oxytrichia spp.	Hausman 1923	op. cit.
Oxytrichia pellionella	Frye & Becker 1929	op. cit.
Paramoecium spp.	Hausman 1923	op. cit.
Paramoecium caudatum	Frye & Becker 1929	op. cit.
Prorodon teres	Frye & Becker 1929	op. cit.
Pleuronema spp.	Lackey 1924	op. cit.
Pleuronema chrysalis	Frye & Becker 1929	op. cit.
Pleurotricha lanceolata	Frye & Becker 1929	op. cit.
Podophyra spp.	Lackey 1924	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Prorodon spp.	Lackey 1924	op. cit.
Prorodon teres	Frye & Becker 1929	op. cit.
Saprophilus	Hausman 1923	op. cit.
Spirostomon spp.	Hausman 1923	op. cit.
Stentor spp.	Hausman 1923	op. cit.
Stichotricha	Hausman 1923	op. cit.
Stylonochia spp.	Lackey 1924	op. cit.
Stylonochia pustulata	Frye & Becker 1929	op. cit.
Uroleptus spp.	Lackey 1924	op. cit.
Uronema spp.	Lackey 1924	op. cit.
Uronema marina	Frye & Becker 1929	op. cit.
Vorticella spp.	Hausman 1923	op. cit.
<u>Rotatoria (Rotifers)</u>		
Euchlanis dilatata	Frye & Becker 1929	op. cit.
Philodina roseola	Frye & Becker 1929	op. cit.
Rotifer vulgaris	Frye & Becker 1929	op. cit.
<u>Polychaeta worms (Polychaete worms)</u>		
Polychaeta spp.	Cooke 1959a	op. cit.
<u>Oligochaeta (Round Worms)</u>		
Aelosoma spp.	Reynoldson 1939	op. cit.
Aelosoma hemprici	Wilson 1943	Upper Miss River Basin Sanit Aggr'mt Bull, pp. 111
Dendrobaena subricunda	Hawkes 1960	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Dero spp.	Wilson 1943	op. cit.
Dero limosa	Wilson 1943	op. cit.
Eisenia foetida	Terry 1951	J. & Proc Inst Sew Purif, pt. 1, 16
Enchytraeus albidus	Cooke 1959a	op. cit.
Limnodrilus spp.	Holtje 1943	op. cit.
Lumbricillus spp.	Reynoldson 1939	Surveyor, 95, 2456
Lumbricillus rutilus	Welch 1914	Bull Ill Lab Nat Hist No 10
Lumbricillus lineatus	Reynoldson 1939	op. cit.
Lumbricus rubellus	Reynoldson 1939	op. cit.
Pristina spp.	Wilson 1943	op. cit.
Pristina longisetata	Hawkes 1960	op. cit.
Tubifex	Holtje 1943	op. cit.
<u>Gastrotrichia</u> (Gastrotrich worms)		
Chaetonotus spp.	Frye & Becker 1929	op. cit.
<u>Nematoda</u> (Nematode Worms)		
Diplogaster strictus	Peters 1930	J. Helminth, 8, 165
Diploscapter coronata	Peters 1930	op. cit.
Dorylaimus saporophilus	Peters 1930	op. cit.
Rhabdites	Peters 1930	op. cit.
<u>Annelidia</u> (Annelid Worms)		
Aelosoma spp.	Hausman 1923	op. cit.
Aelosoma hemprichi	Frye & Becker 1929	op. cit.
Naias	Hausman 1923	op. cit.
Pristina	Hausman 1923	op. cit.
Tartigrada	Hausman 1923	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
<u>Turbellaria</u> (Flat Worms)		
Stenostomun leucops	Frye & Becker 1929	op. cit.
<u>Crustacea</u>		
Cyclops spp.	Cooke 1959a	op. cit.
<u>Tartigrada</u>		
Macrobiotus	Lackey 1924	op. cit.
<u>Snails</u>		
Lymnea globra	Reynoldson 1939	op. cit.
Lymnea humilis modicella	Ingram 1958	Sew & Ind Wastes, <u>30</u> , 821
Lymnea pereger	Hawkes 1960	op. cit.
Physa anatina	Brown 1937	Amer. Midland Naturalist, <u>18</u> , 251
Physa cubensis	Lohmeyer 1955	Sew & Ind Wastes, <u>29</u> , 89
Physa helei	Ingram 1958	op. cit.
Physa integra	Ingram 1957	Biol Symp in Water Pollut Robt Taft Sanit Engr C'tr Apr

Table 13

Microorganisms Common to Activated Sludge
and Trickle Filters

Bacteria and filamentous forms

Aerobacter aerogenes
Alcaligenes faecalis
Bacillus cereus
Bacillus megatherium
Bacillus subtilis

Beggiatoa sp.
Chromobacterium sp.
Cladothrix
Escherichia coli
Escherichia coli, intermedium

Flavobacterium sp.
Nitrobacter
Nocardia sp.
Sphaerotilus sp.
Zooglea ramigera

Fungi

Allerscherii boydii
Aspergillus fumigatus
Geotrichum candidum

Protozoa

Sarcodina

Amoeba sp.
Amoeba proteus
Amoeba radiosa
Amoeba tachypoda
Amoeba verrucosa

Arcella sp.
Arcella vulgaris
Centrophyxis aculeata
Chlamodophrys stercorea
Cochliopodium bilimbosum

Diffflugia sp.
Diplophrys sp.
Euglypha sp.
Euglypha alveolata
Hartmanella hyalina

Protamoeba primitiva
Trinema sp.
Valkhampfia albida
Valkhampfia guttula
Valkhampfia limax

Mastigophora (Flagellates)

Bodo sp.
Bodo caudatus
Bodo lens
Cercobodo sp.
Cercobodo longicauda

Cercobodo ovatus
Distigma proteus
Entosiphon sulcatus
Euglena sp.

Mastigamoeba sp.
Menoidium sp.
Monas sp.
Notosolensus sp.
Oicomonas sp.

Oicomonas socialis
Peranema sp.
Petalomonas sp.
Tetramitus sp.
Trepmonas sp.

Trepmonas agilis

Ciliates

Amphileptus sp.
Aspidisca sp.
Blepharisma sp.
Bursaria
Carchesium sp.

Chilodon sp.
Chilodon cucullulus
Cinetochilum margaritaceum
Colpoda sp.
Colpoda inflata

Cyclidium glaucoma
Epistylis sp.
Euplotes sp.
Euplotes charon
Frontonia

Glaucoma sp.
Glaucoma scintillans
Holophyra sp.
Lembus sp.
Lionotus sp.

Lionotus fascicola
Loxodes sp.
Metopus sp.
Microthorax sp.
Opercularia sp.

Oxytrichia sp.
Paramoecium sp.
Paramoecium caudatum
Podophyra sp.
Stylonichia sp.

Stylonichia pustulata
Vorticella sp.

suggested to be functional as scavengers (84) in film removal, while psychoda larvae may only be of nuisance value.

Since algae are photosynthetic, no attempt has been made to list them.

Table 14 offers an incomplete list of organisms associated with anaerobic digestion processes. It is generally assumed that anaerobic digestion is carried out predominantly by bacteria although protozoa have been recovered from anaerobic processes. The role of yeasts and fungi has not been investigated. It is known that fungi are essential to composting operations.

An American Public Works Association Committee (85) reports research that indicates that no pure culture of organisms can compare with a mixed culture in the aerobic composting of manure and other organic matter. Compost is known to contain many types of bacteria, actinomycetes and fungi.

Bacteriological studies of anaerobic digestion have been tedious, slow and controversial. It is probable that, as with composting, anaerobic digestion is the result of the combined activities of several types of organism including facultative bacteria.

Table 14

Organisms Reported in Anaerobic Digesters

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Anaerobic Bacteria		
Bacillus endorhythmos	Buck et al. 1954	Sew. & Ind. Wastes, <u>26</u> , 164
Bacillus fosicularum ?	Omeliansky 1899	Arch. Science Biol., <u>7</u> , 411
Bacillus methanizens ?	Omeliansky 1899	op. cit.
Methane bacterium formicicum	Mylorie & Hungate 1954	J. of Microbiol., <u>1</u> , 155
Methanobacterium omelianski	Barker 1936	Arch. f. Mikrobiologie, <u>7</u> , 420
Methanobacterium propionicum	Stradtman & Barker 1957	J. Bact., <u>61</u> , 67
Methanobacterium sohngenii n. sp.	Stradtman & Barker 1957	op. cit.
Methanobacterium suboxidans	Stradtman & Barker 1957	op. cit.
Methanococcus mazei n. sp.	Barker 1936	op. cit.
Methanosarcina	Buswell 1947	Sewage Wks. J., <u>19</u> (1) 28
Sphaerotilus ?	Agersborg & Hatfield 1929	Sew. Wks. J., <u>1</u> , (4) 411
Streptococcus diploodus	Buck et al. 1954	op. cit.
Sporovibrio desulfuricans	Buck et al. 1954	op. cit.
Protozoa		
<u>Sarcodina</u>		
Amoeba guttula	Lackey 1924	N. J. Agr. Sta. Bull. 403
Amoeba limax	Lackey 1924	op. cit.
Amoeba proteus	Lackey 1924	op. cit.
Amoeba radiosa	Lackey 1938	Ecological Monographs, <u>8</u> , (4), 501
Amoeba striata	Lackey 1938	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Amoeba verrucosa	Lackey 1938	op. cit.
Chlamodophys minor	Lackey 1938	op. cit.
Chlamodophys stercorea	Lackey 1925	N. J. Agr. Exp. Sta., Bull. No. 417
Cochliopodium bilimbosum	Lackey 1938	op. cit.
Dimastigamoeba gruberii	Lackey 1925	op. cit.
Euglypha alveolata	Lackey 1924	op. cit.
Hartmanella hyalina	Lackey 1925	op. cit.
Nuclearia simplex	Lackey 1938	op. cit.
Trinema lineare	Lackey 1925	op. cit.
Valkampfia albida	Lackey 1925	op. cit.
Valkampfia fragilis	Lackey 1932	Biol. Bull., 63, 287
Valkampfia guttula	Lackey 1925	op. cit.
Valkampfia limax	Lackey 1925	op. cit.
Valkampfia minuta	Lackey 1932	op. cit.
<u>Mastigophora (Flagellates)</u>		
Anisonema ovale	Lackey 1938	op. cit.
Anthophysa vegetans	Lackey 1924	op. cit.
Bodo sp.	Lackey 1924	op. cit.
Bodo angustus	Lackey 1925	op. cit.
Bodo agilis	Lackey 1938	op. cit.
Bodo caudatus	Lackey 1925	op. cit.
Bodo glissans	Lackey 1932	op. cit.
Bodo globosus	Lackey 1938	op. cit.
Bodo lens	Lackey 1932	op. cit.
Bodo minimus	Lackey 1938	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Bodo mutabilis	Lackey 1925	op. cit.
Bodo ovatus	Lackey 1925	op. cit.
Bodopsis godboldi	Lackey 1938	op. cit.
Cercobodo sp.	Lackey 1924	op. cit.
Cercobodo caudatus	Lackey 1938	op. cit.
Cercobodo crassicauda	Lackey 1925	op. cit.
Cercobodo longicauda	Lackey 1925	op. cit.
Cercobodo ovatus	Lackey 1925	op. cit.
Cercomonas sp.	Lackey 1924	op. cit.
Chilomonas paramecium	Lackey 1932	op. cit.
Chlamydomonas sp.	Lackey 1925	op. cit.
Clautriavia parva	Lackey 1925	op. cit.
Cyathomonas truncata	Lackey 1932	op. cit.
Dinomonas vorax	Hausman 1923	N. J. Agr. Exp. Sta., Bul. 390
Distigma proteus	Lackey 1925	op. cit.
Entosiphon sulcatum	Lackey 1924	op. cit.
Euglena deses	Lackey 1938	op. cit.
Euglena gracilis	Lackey 1924	op. cit.
Euglena intermedia	Lackey 1925	op. cit.
Euglena mutabilis	Lackey 1925	op. cit.
Euglena polymorpha	Lackey 1925	op. cit.
Euglena quartana	Lackey 1938	op. cit.
Euglena viridia	Lackey 1938	op. cit.
Helkesimastix faecicola	Lackey 1938	op. cit.
Heteronema sp.	Lackey 1925	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Heteronema acus	Lackey 1932	op. cit.
Hexamitus crassus	Lackey 1938	op. cit.
Hexamitus inflatus	Lackey 1925	op. cit.
Mastigamoeba longifilum	Lackey 1925	op. cit.
Mastigamoeba radiosa	Lackey 1932	op. cit.
Mastigamoeba reptans	Lackey 1924	op. cit.
Mastigamoeba viridis	Lackey 1932	op. cit.
Mastigella simplex	Lackey 1925	op. cit.
Menoidium incurvum	Lackey 1925	op. cit.
Monas sp.	Hausman 1923	op. cit.
Monas amoebina	Lackey 1925	op. cit.
Monas minima	Lackey 1925	op. cit.
Monas obliqua	Lackey 1938	op. cit.
Monas vivipara	Lackey 1938	op. cit.
Monas vulgaris	Lackey 1932	op. cit.
Notosolenus sp.	Lackey 1924	op. cit.
Notosolenus orbicularis	Lackey 1924	op. cit.
Oicomonas sp.	Lackey 1924	op. cit.
Oicomonas socialis	Lackey 1925	op. cit.
Oicomonas steinii	Lackey 1938	op. cit.
Oicomonas termo	Lackey 1932	op. cit.
Peranema ovalis	Lackey 1938	op. cit.
Peranema trichophorum	Lackey 1924	op. cit.
Petalomonas carinata	Lackey 1925	op. cit.
Petalomonas mediocanellata	Lackey 1925	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Physomonas sp.	Hausman 1923	op. cit.
Platytheca microspora	Lackey 1925	op. cit.
Pleuromonas jaculans	Hausman 1923	op. cit.
Polytoma uvella	Lackey 1938	op. cit.
Prowazekia sp.	Lackey 1924	op. cit.
Rhynchomonas nasuta	Lackey 1938	op. cit.
Salingoeca marssonii	Lackey 1925	op. cit.
Synura uvella	Lackey 1938	op. cit.
Tetramitus decissus	Lackey 1925	op. cit.
Tetramitus pyriformis	Lackey 1932	op. cit.
Tetramitis variabilis	Hausman 1923	op. cit.
Trepomonas agilis	Lackey 1924	op. cit.
Trepomonas rotans	Lackey 1938	op. cit.
<u>Infusoria (Ciliates)</u>		
Aspidisca costata	Lackey 1924	op. cit.
Blepharisma	Hausman 1923	op. cit.
Carchesium lachmanni	Lackey 1938	op. cit.
Coenomorpha	Agersborg & Hatfield 1929	Sew. Wks. J., 1, (4) 411
Cinetochilum margaritaceum	Lackey 1938	op. cit.
Chilodon sp.	Lackey 1924	op. cit.
Chilodonella cucullulus	Lackey 1938	op. cit.
Colpoda sp.	Hausman 1923	op. cit.
Colpoda aspera	Lackey 1938	op. cit.
Colpoda cucullus	Hausman 1923	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Colpoda inflata	Lackey 1924	op. cit.
Colpidium sp.	Lackey 1925	op. cit.
Colpidium campylum	Lackey 1938	op. cit.
Colpidium colpoda	Lackey 1938	op. cit.
Cyclidium glaucoma	Lackey 1925	op. cit.
Drepanomonas revoluta	Lackey 1938	op. cit.
Dysteriopsis minuta	Lackey 1938	op. cit.
Glaucoma scintillans	Lackey 1924	op. cit.
Hexotrichia sp.	Lackey 1924	op. cit.
Hexotrichia caudatum	Lackey 1925	op. cit.
Holophyra sp.	Hausman 1923	op. cit.
Lionotus sp.	Hausman 1923	op. cit.
Lionotus fasciola	Lackey 1924	op. cit.
Lionotus lamella	Lackey 1938	op. cit.
Metopus sigmoides	Lackey 1923	op. cit.
Opercularia sp.	Lackey 1924	op. cit.
Opercularia berberina	Lackey 1925	op. cit.
Oxytricha sp.	Lackey 1924	op. cit.
Oxytrichia fallax	Lackey 1938	op. cit.
Paramecium caudatum	Lackey 1938	op. cit.
Paramecium putrinum	Lackey 1924	op. cit.
Plagiophyla nasuta	Lackey 1925	op. cit.
Pleuronema chrysalis	Lackey 1925	op. cit.
Prorodon sp.	Lackey 1925	op. cit.
Saprodinium sp.	Lackey 1924	op. cit.

<u>Organism</u>	<u>Reported by</u>	<u>Reference</u>
Saprodinium putrinum	Lackey 1925	op. cit.
Trinema compressa	Lackey 1925	op. cit.
Vorticella sp.	Lackey 1924	op. cit.
Vorticella microstoma	Lackey 1938	op. cit.

Activated Sludge

The treatment cycle is carried out in an activated sludge unit by many aerobic organisms: those capable of utilizing organic compounds as they would be found in the waste material proper, and those that can attack partially converted organic molecules. Microorganisms found in activated sludge systems can be regarded as members of a complex ecological system and include a wide variety of bacteria (including filamentous forms), fungi, protozoa, and smaller metoza^s such as rotifers and nematode worms.

Many bacteria found in activated sludge systems will form slimes or gelatinous materials which coalesce to form flocs or flocculating particles. These flocs adsorb organics and facilitate biochemical oxidation of the colloidal organic matter in sewage. Various floc forming bacteria have been reported and isolated (86), however, Zooglea ramigera is considered to be the most active organism responsible for the metabolism of unconverted organic substrates (87) in this process. Other non floc forming organisms are capable of oxidation and their enzyme systems are unimpaired (88).

Trickling Filters

A trickling filter is usually a highly efficient and complex bio-ecological community representing a wide variety of

organisms with a broad spectra^{of} of metabolizing powers. The microbial population must be built up and gradually adapted to the substrate. Initially, the filter media is coated with a slime matrix material consisting predominantly of zooglear bacterial forms, mostly Zooglea ramigera, but in which filamentous bacteria or other micro-flora are also actively growing.

Besides a multiplicity of bacteria (including filamentous forms), numerous fungi, various kinds and species of protozoa, rotifers, and scavenger organisms (or grazing fauna) including higher forms of life such as oligochaete worms and insects are also found.

As a general rule, nitrifying organisms will be found in greater abundance in trickling filters than in activated systems.

A trickling filter bed consists of a succession of communities established at different levels of the filter associated with corresponding different degrees of purification. Unlike the activated sludge system, trickling filters can withstand greater shock loads and are more resistant to changes.

Anaerobic Digestion

In the anaerobic digestion process water-binding organic solids are attacked by bacterial enzyme systems and the sludge or waste mass will be reduced in volume.

Both facultative and obligate anaerobes appear to be responsible for the anoxic digestion of the waste conglomerate.

It is generally agreed that two groups of bacteria are required for anaerobic digestion and the production of methane. One group of organisms, the acid producing bacteria, will degrade the organic molecules and produce organic acids, alcohols and aldehydes (the facultative bacteria). The second group, the methane producing bacteria, use end products of the acid producers to form methane, carbon dioxide or other gases.

In addition, thio-bacteria break down sulfates. Protozoans may occur in great numbers or not at all. Their function in anaerobic decomposition is not clear. The most numerous are the flagellates Trepomonas, Tetramitis, Trigonomonas; the amoeba, Vahlkampfia and Hartmanella; and ciliates, Metopus, Trinema and Saprodinium.

TREATMENT PROCESSES FOR CLOSED ECOLOGICAL SYSTEMS

Photosynthetic--Non-Photosynthetic Systems

Two types of algal-bacterial waste treatment plants have been described which are capable of treating human wastes and serve as gas exchange units in a closed environment.

In the system first constructed by Golueke, et al. (9), separation was maintained between the algal unit and the bacterial unit. In a later modification, algae were grown together with the bacteria (10, 89) and there was no separation.

Through photosynthesis algae convert carbon dioxide to oxygen and new algal materials. Bacteria in turn convert complex compounds in liquid and solid wastes to carbon dioxide, ammonia and various other degradation products. Since it is the bacteria which achieve the greatest work in waste treatment, oxygen must be supplied to them.

In the first method above, two separate units are maintained; an algal unit and a bacterial unit. The oxygen-rich gas released by the algal culture is passed through the bacterial culture in much the same manner as that of the activated sludge process. The carbon dioxide released by bacterial decomposition of the organic matter is in turn passed through the algal culture.

The apparatus in which separate algal and bacterial cultures were maintained produced effluents having BOD's of 10 to 20 ppm even though influent BOD's were as high as 1,000 to 2,000 ppm. Removal of influent volatile dissolved solids reached 91% when the input of feces was one gram (dry weight) per liter per day, and of urine, 50 ml per liter per day. Although 99% of the influent coliforms were removed, the

effluent still had counts as high as 1.5×10^5 per ml. When the dosage was increased to two grams feces per liter per day and to 100 ml urine per liter per day, removal was negligible and the cultures failed. In this system the authors proposed that the algal culture would receive the liquid waste and the bacteria the solid waste.

In two later reports Oswald et al. (89) and Golueke (10) have described the operation of a two phase waste treatment system using both photosynthetic and non-photosynthetic biological elements. In practice, an algal suspension, probably Chlorella sp., is passed through an activated (bacterial) sludge culture and the sludge allowed to settle in a "quiescent" chamber. Aeration is applied in a separate chamber (the "active" chamber). The chambers are housed in the same unit and are interconnected. Gaseous interchange is mutual and the free gases from one chamber are used to agitate and aerate the other chamber. Algae remain suspended. Bacteria, it is stated, will convert complex compounds contained in the liquid and solid wastes to carbon dioxide, water, ammonia, and various degradation products. Through photosynthetic activity, algae convert bacterial decomposition products, as well as the carbon dioxide, to new algal material and in so doing, release oxygen which could be used by a capsule crew and by bacteria.

Water which remains is suitable for reuse as a suspension medium.

In an annual report Oswald et al. (89) present the results of six experiments. Varying amounts of feces suspended in sewage (from 1.53 to 3.92 gm dry weight/1/day) and 35 ml of urine were added to this system. The system was found capable of processing three grams (dry weight) of feces per liter per day. The authors estimated this would require three gallons of culture per individual fed on a low residue diet. The effluent, however, was found to contain sufficient levels of nitrogen which would require an additional gallon of culture; was apparently not altogether satisfactory; and resulted in sufficient turbidity to interfere with light transfer in the algal system.

The authors believed that part of the difficulties might be resolved by proper design modifications. They did, however, feel that this system indicated waste treatment by a simple operation in which volume requirements were minimum.

In a later publication (10) a more complete report on the unit was given. BOD removal was found to be 98% at loadings of 721 mg per liter per day, and dissolved volatile solids removal ranged from 50 to 70%. Maximum permissible loading rate was found to be one gram (dry weight) of feces per liter per day. Although the authors considered the removal of organic

solids sufficiently extensive within 24 hours to permit the use of reclaimed water, the extent of the conversion of suspended solids to final breakdown products of gas, water and minerals was considered very limited. The authors suggested this might be due partly to the presence of roughage materials, such as lignin and cellulose.

Experiments with this unit are in progress (1962), and the authors report effluents having BOD's of 8 to 10 ppm with loadings of 1.8 to 2.0 gm (dry weight) feces and 30 ml of urine per liter per day.

Non-Photosynthetic Systems

Aerobic

For an activated sludge system Pipes (90) calculates the concentration of suspended solids for wastes produced in a closed ecological system to be around 4,000 mg/l. The aeration period may range from 2 to 24 hours. The minimum feasible aeration time, however, is about two hours. Longer aeration times, he notes, achieve greater degrees of oxidation. At an aeration period of six hours the aeration vessel would have a volume of about three liters per person allowing space occupied by gaseous oxygen. The composition vessel, however, would probably have a volume of about five liters per person, enough

to hold twelve hours waste production. Optimum temperature is 35°C with a wide variation; optimum pH 7.0 to 8.0 (range 6.5 to 9.5). A concentration of 1 mg/l dissolved oxygen must be maintained in the mixture. The maximum efficiency which may be attained is in the range of 95 to 98% oxidation of the organic matter, and with longer aeration periods (24 hours) and high suspended solids concentration in the mixed liquor, it is possible, he says, to recover 90% of the carbon fed in the form of carbon dioxide and 80% of the nitrogen fed as nitrate. The assimilation and oxidation of the waste will require 0.5 to 1.3 gm of oxygen per gram of organic matter in the waste (72 to 186 gm oxygen per day per person). The amount of carbon dioxide produced will be about 0.96 gm per gram of oxygen utilized. The waste sludge produced will range from 0.1 to 0.65 gm per gram of organic matter in the waste with 0.25 "representing a reasonable figure".

Chapman (91) and Bogen et al. (92) have reported results based on observations using a miniature activated sludge plant and Warburg microrespirometer studies. The human wastes treated are described as a material some 300 times as concentrated as ordinary domestic sewage. Prior to addition to the pilot plant, the microorganisms were pre-conditioned for a period of time by the addition of gradual increment doses of the undiluted human wastes until the mixture was capable of

receiving one-third its volume of wastes per day. This conditioned cellular mass was then used to start the activated sewage plant. Batch procedures were used.

Both mesophilic and thermophilic studies were made. In addition to direct observations made on the pilot plant, cells harvested from this pilot plant were studied using a Warburg respirometer. A bimodal temperature response was found. Maximum efficiency was found to be, however, in the mesophilic range (maximum biochemical oxidation was found between 30° and 32°C). At 30°C with continuous flow and adequate aeration, a three day retention period was calculated, requiring a culture volume of 0.5 cu ft which the authors estimate sufficient to handle the wastes of a four man crew. At this temperature, the reaction rate constant indicated that 90% of the added organic matter was oxidized in less than four hours. From the data they estimate 97 to 98% of the dissolved organic matter added in the form of wastes was removed from solution. Forty per cent or more of the added organic matter, they conjecture, could be destroyed by the process or perhaps found useful as a nutritional supplement for animals or plants. Gases produced from the activated system will be primarily carbon dioxide and ammonia which could be passed through plant systems prior to passage into the cabin. The sewage, they say, will trap obnoxious gases and ozone will be decomposed instantly by the system.

Anaerobic

A theoretical consideration of anaerobic digestion in a closed ecology is presented by Pipes (90), who bases his calculations on the production of 250 gm of solids in 10 liters of wastes containing 1.45% organic solids per person. On this basis he calculates a loading of about 14.5 gm of organic solids per person. On this basis he calculates a loading of about 14.5 gm of organic matter per day (0.9 cu ft organic matter per cubic foot per day). Conventional loadings, he notes, range up to 0.5 pounds organic matter per cubic foot per day, but it is possible that satisfactory treatment could be obtained at higher loadings. The highest degree of treatment obtainable by anaerobic digestion is about 90% reduction of volatile matter (93). The amount of gas produced is approximately 10 cu ft per pound (0.626 l per gm) of organic matter digested (94). Thus, a digester, he concludes, operating under conditions assumed, would digest about 13 gm of organic matter per liter per day. The composition of the gas expected is about 70% methane and 30% carbon dioxide with traces of hydrogen, hydrogen sulfide, ammonia, and some volatile organic compounds (95).

Pote et al. (96) have described, in a preliminary report, a thermophilic (49°C) anaerobic digestion apparatus designed to process human waste to provide nutrients for growth of food

and algae. The combined capacity of the two digester bottles used in the unit is 15 liters. Digestion was initiated by addition of four liters of supernatant material taken from a domestic sewage disposal plant. Loading was reported to be 0.06 to 0.08 pounds of volatile solids per cubic foot per day, with a 25 day detention period. Further work will be done and the investigators plan addition of animal and plant parts to the digester to determine adjustments needed to satisfy their use as food for plants or algae. The investigators state, "The operations indicate that expectations as to digestion rates and parameters will be fulfilled". An "objectionable" residue from the digestion will be produced amounting to about one liter per person per day with a solids concentration of around 12%.

By combining the investigations of Hurwitz (97) and Rose (98) a comparison can be made of the amino acids found in activated sludge and the requirements of man. These values are given in Table 15. Hurwitz in feeding experiments on pigs, chickens, and lambs, came to the conclusion that sludge in amounts greater than 3% was of little value as an adjuvant. The tabular comparison suggests that the amino acids of activated sludge may supply some but not all of man's requirements. At a sludge intake of 100 gm (dry) per day there would still be deficiencies in leucine, lysine, phenylalanine and

Table 15
A Comparison
The Amino Acid Content of Sludge and Man's Requirements

<u>Amino Acid</u>	<u>Sludge Amino Acids*</u>	<u>Essential Amino Acid**</u>		<u>Deficiency***</u> at <u>Safe Value</u> gms/day
	<u>gm/100 gm dry</u>	<u>Requirements</u> <u>of Man</u> gms/day	<u>Safe</u> <u>Value</u>	
Arginine	1.04 - 1.26			
Histidine	0.41 - 0.50			
Isoleucine	0.91 - 2.20	0.70	1.40	Q.S. 0.49
Leucine	1.58 - 2.03	1.10	2.20	0.17 0.62
Lysine	0.92 - 1.33	0.80	1.60	0.27 0.68
Methionine	0.45 - 0.65	0.10	0.20	Q.S. Q.S.
Cystine	0.18			
Phenylalanine	1.20 - 2.00	1.10	2.20	0.20 1.00
Tyrosine	0.70			
Threonine	1.15 - 2.20	0.50	1.00	Q.S. Q.S.
Tryptophan	0.22 - 0.34	0.25	0.50	0.16 0.28
Valine	0.18 - 2.77	0.80	1.60	Q.S. 1.42
Glycine	1.55 - 1.71			
Glutamic Acid	2.89			

*From Hurwitz (see Reference 99).

**From Rose (see Reference 100).

***Q.S.: Quantity sufficient to satisfy requirement at 100 gm sludge intake.

tryptophan. Further, with the limited amount of sludge solids that could be made available it is improbable that 100 gm dry solids per day per man would ever become available in purified form. In a closed space regimen the isolation of desirable substances from objectionable or toxic materials offers many difficulties and further research is required to establish the justification, if any, for the equipment that may be needed to reclaim a minor dividend on food mass substitutes.

EXPERIMENTAL WORK

As a means of checking on some of the experimental and theoretical projections which have been presented in previous sections of this report, a series of experimental, exploratory, laboratory investigations was undertaken.

Two basic aerobic treatment processes, activated sludge and trickling filtration, and anaerobic digestion treatment in mesophilic and thermophilic ranges were selected.

Both of the aerobic processes are known to provide a high degree of treatment and produce a liquid fraction relatively free of waste impurities and stabilized to a point of mineralization that could provide nutrient for algal growth. The effluents might be easily treated by some form of fine filtration or by one of the distillation processes to produce potable water for drinking, and conceivably could be used with minimal

additional treatment for space cabin usage of water of lesser quality as suggested by Ingram et al. (42).

Digestion has been advanced by Buswell (99) as a means of pre-treatment of highly concentrated, toxic, or otherwise hard to manage industrial wastes. The mixtures of cabin wastes are highly concentrated and not materially different in physical aspects from sludges taken in primary conventional treatment units. They most nearly resemble the contents of the privy, a primitive treatment unit wherein digestion and composting succeed, over long periods of time, in reducing volume and liquifying some of the materials.

The following series of experiments was carried out to provide some idea of the problems, the manner of handling and the possible need for some form of pre-treatment or supplementary treatment during the biological progression.

A. Activated Sludge using

1. Undiluted urine-feces blend.
2. Tap water dilutions of urine-feces blend.
3. Simulated space cabin wash water dilutions of urine-feces blend.

B. Trickling Filter using

1. Settled sewage dilution of urine-feces blend.

- C. Anaerobic Digester in mesophilic range using
 - 1. Digested activated sludge and urine-feces blends.
 - 2. Primary sludge and urine-feces blend.
- D. Anaerobic Digester in thermophilic range using
 - 1. Digested sludge and urine-feces blends.

Activated Sludge Experiments

Procedure

Aeration experiments were set up using four-liter conical flasks equipped with porous gas diffusers. The experimental apparatus is diagrammed in Figure 3. All samples were aerated continuously with sufficient vigor to effect gentle mixing, except when samples were being taken for analyses. To minimize evaporation loss the flasks were covered with aluminum foil.

In the first set of experiments (AS 1 and AS 2)

- 1. Undiluted urine-feces blend (3 liters) was set up to be fed once a day with 50 ml of fresh urine-feces mixture. In the experiment only one subsequent addition was made. Biological examinations and BOD analyses were performed three times a week.
- 2. Undiluted urine-feces blend (3 liters) was set up as above, however, no additional

AERATION

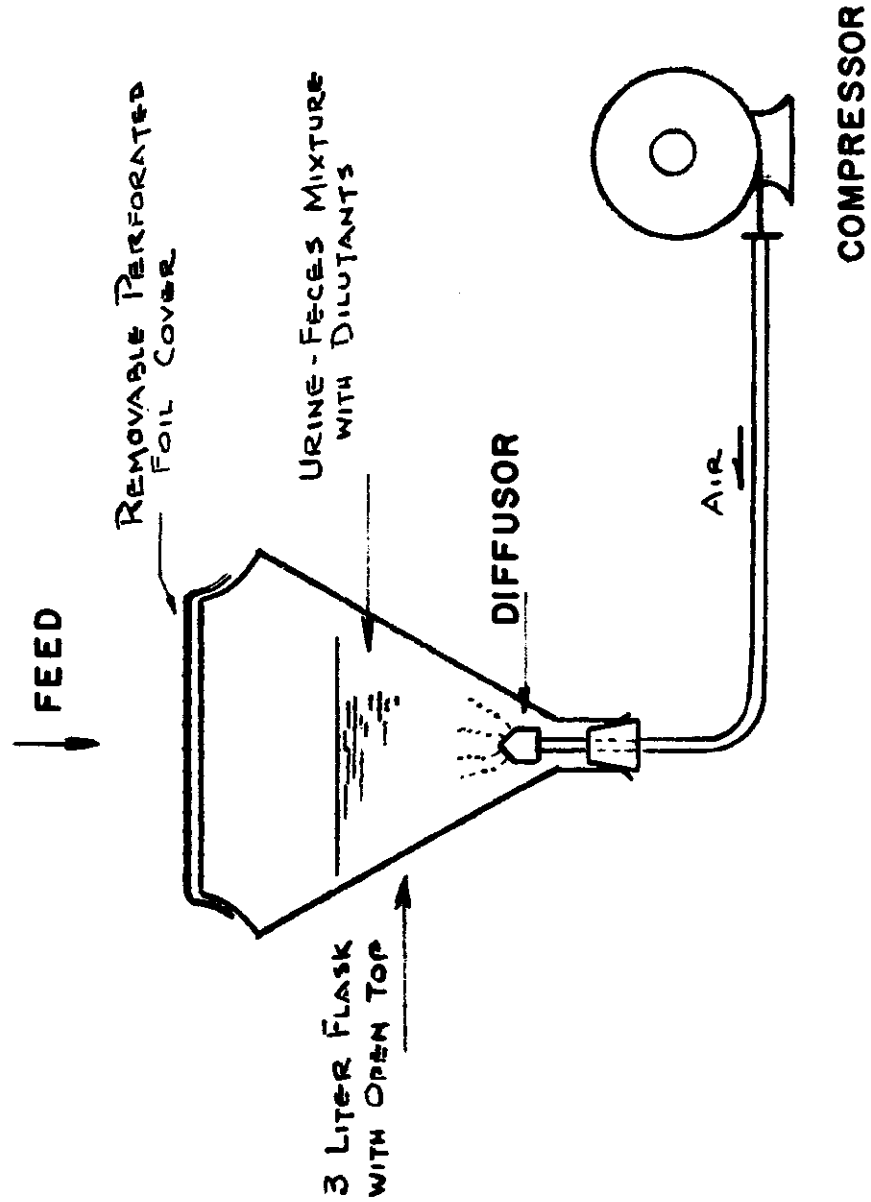


Figure 3

urine-feces blend was added. Samples were taken intermittently for BOD determination and examined for the presence of biological flora.

In another series (AS 3) urine-feces blend was diluted with tap water (1:5, 1:20, 1:27, 1:40). BOD analyses and biological analyses were run, on the average, three times a week.

In the third series (AS 4) urine-feces blend was diluted with wash waters (1:5, 1:20, 1:27, 1:40). BOD analyses and biological analyses were made, on the average, three times a week.

Wastes consisting of urine (1,500 ml) and feces (135 gm) were blended in a Waring blender and diluted with wash waters of the following composition: hand washings (150 ml), face washings (75 ml), and clothes washings (ca 1,700 ml). Urine was collected in beakers and stools were collected in plastic bags. Hand and face washings with "PhisoHex" as a cleansing agent were collected over a basin. "PhisoHex" has been used as a cleansing agent in closed space ecology studies at the School of Aerospace Medicine, Brooks Air Force Base, Texas.

According to the manufacturer, the preparation contains 14% of the anhydrous detergent Entsufof. Entsufof is an anionic surface active agent, the sodium salt of a polyether

sulfonate. "PhisoHex" also lists the following components: lanolin, cholesterol, hexachlorophene and petrolatum.

Clothes washings were prepared according to a standardized procedure for clothes washings used by our laboratory (48).

Urine-feces mixture, and hand and face washings were stored in the refrigerator prior to compounding. At the time of compounding urine was blended with the feces alone (using the Waring blender) for ten minutes, then poured into the receiving vessel. After blending and just prior to aeration, the mixture of hand and face washings was added to the urine-feces blend. The compounded mixture was immediately poured into the aerating flasks and aeration was started. The volume of each aerating flask was three liters.

Identical sampling techniques were rigidly maintained. Immediately preceding each sampling, aeration was discontinued for four or five minutes. The mixture was allowed to settle, thus reducing the possibility of collecting floccular aggregates with a representative sampling of the supernatant. Initial BOD determinations for the raw urine-feces mixture were found to be in the range of 18,000 ppm; raw wash waters had a value of 1,375 ppm BOD.

The activated sludge experiments are shown in tabular form in Table 16.

Table 16
Activated Sludge Experiments

<u>Run No.</u>	<u>Conditions</u>		
AS 1	Undiluted feces-urine blend, 50 ml fed daily.		
AS 2	Undiluted feces-urine blend set initially, no additions.		
AS 3	Urine-feces blend, diluted with tap water.		
	<u>Dilution</u>	<u>Urine-Feces Blend</u> ml	<u>Tap Water</u> ml
AS 3-1	1:5	600	2400
3-2	1:20	150	2850
3-3	1:27	112.5	2887.5
3-4	1:40	75	2925
AS 4	Urine-feces blend diluted with wash waters.		
	<u>Dilution</u>	<u>Urine-Feces Blend</u> ml	<u>Wash Water</u> ml
AS 4-1	1:5	600	2400
4-2	1:20	150	2850
4-3	1:27	112.5	2887.5
4-4	1:40	75	2925

Trickling Filter Experiment

Time limitations on the experimental work phase in turn limited the extent of experimentation with trickling filter loadings. A simple experiment was designed to introduce a surface organic loading about six times greater than loading applied in controlled filtration to explore the ability of the filter to work under extreme organic loadings.

A bench scale filter, as shown diagrammatically in Figure 4, was constructed. Aeration studies had demonstrated that recirculation of effluent as a diluent to the feces-urine mixture would be necessary. The filter was conditioned for the run with settled domestic sewage. The feed rate on the filter was initiated at 300# BOD per 1000 ft³ per day and increased to 2,190# BOD per 1000 ft³ per day. The hydraulic loadings corresponding were 0.37 and 2.7 gallons per ft² per minute.

During the run the experimental unit was modified as shown in Figure 5. The organic loading was increased to 7,700# BOD per 1000 ft³ per day while maintaining the hydraulic loading at 2.7 gallons per ft² per day.

One and one-half gallons of the raw urine-feces mixture was diluted with 54 gallons of water to give a 2.7% dilution of the urine-feces mixture. The diluted waste mixture had a BOD value of 495 mg/l.

**TRICKLING FILTER
CONDITIONING PHASE**

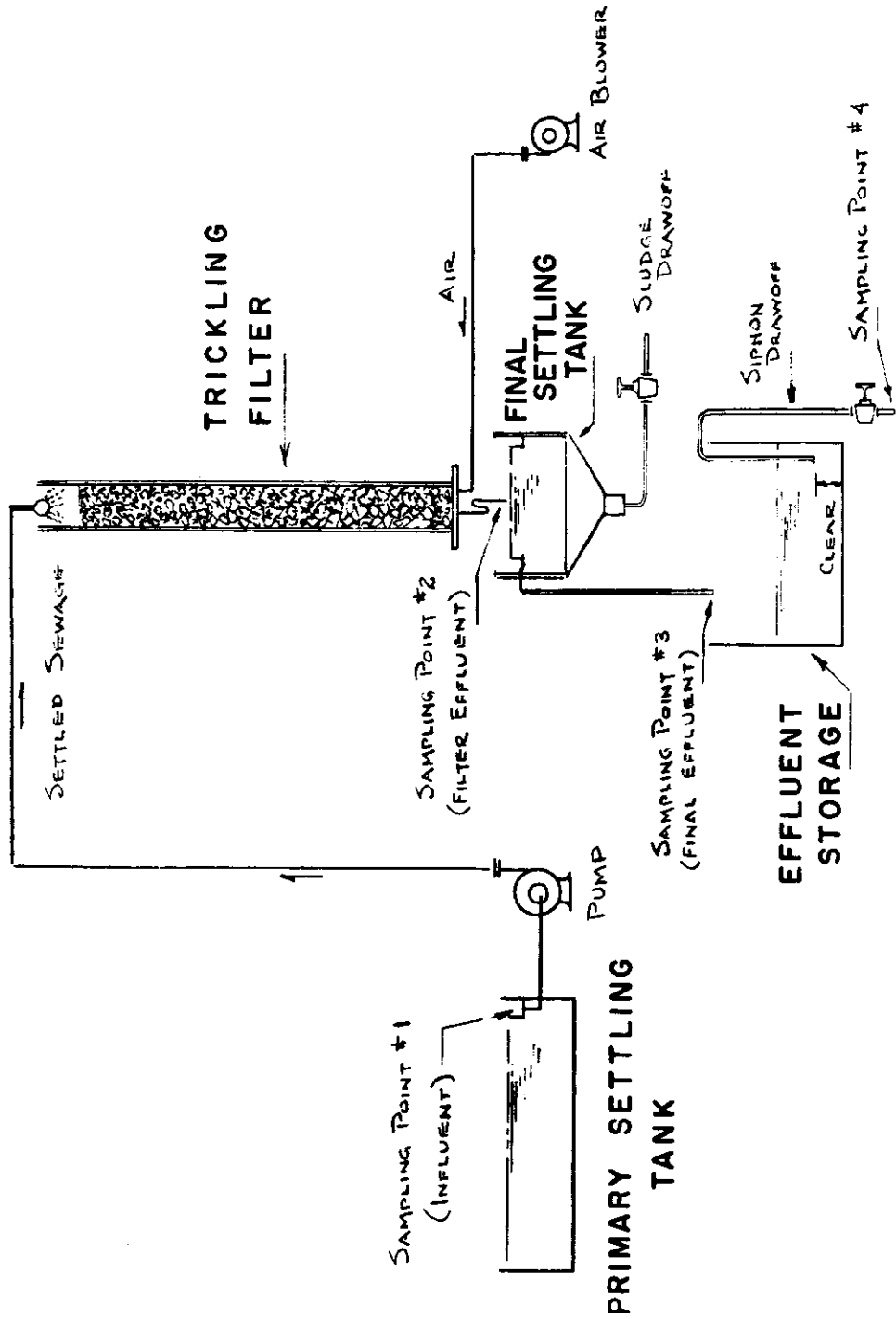


Figure 4

TRICKLING FILTER

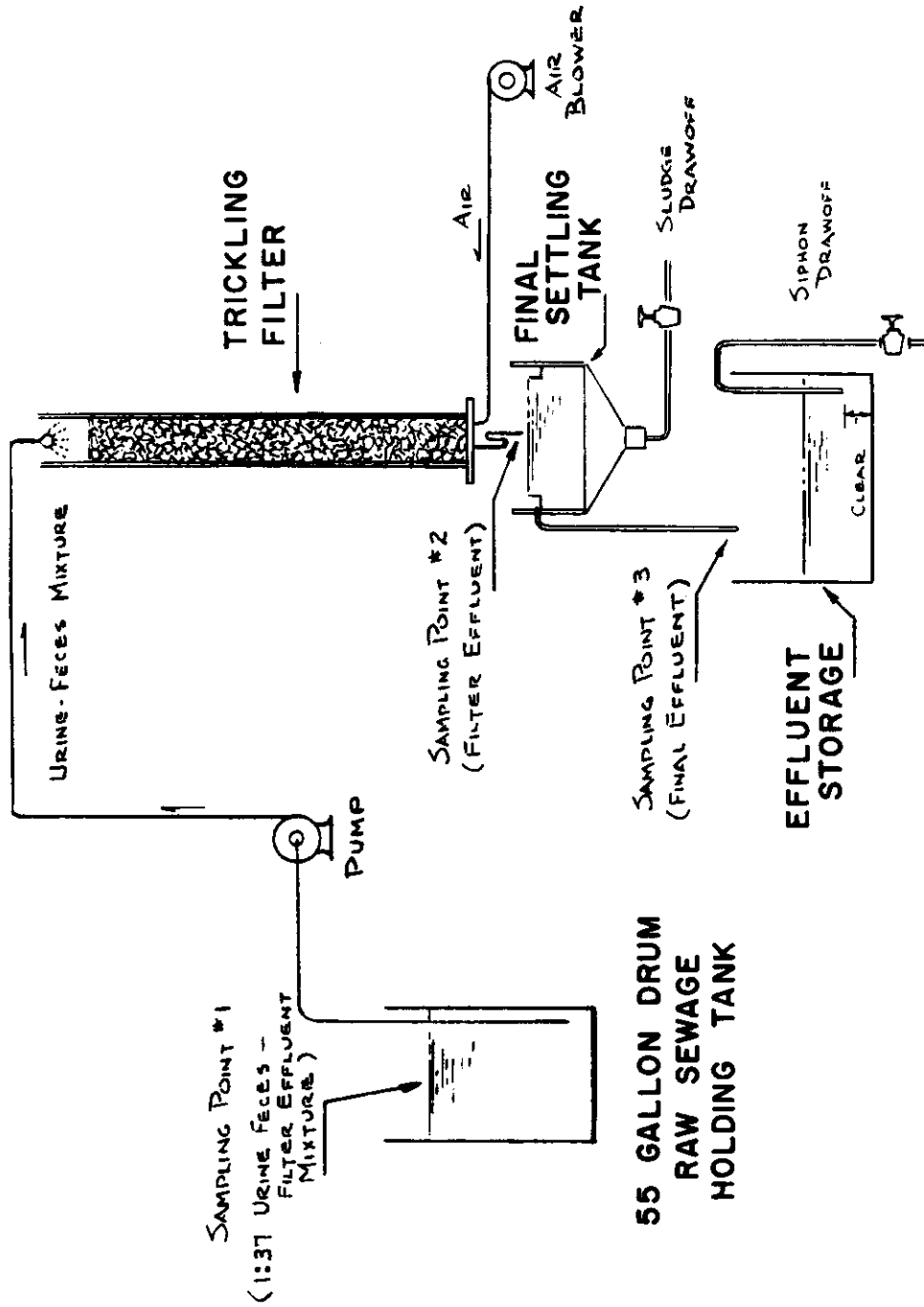


Figure 5

BOD samples were collected from the filter flow at influent, filter effluent, settling tank effluent and exit of storage tank to indicate performance of the filter at the beginning of the experimental run.

The experimental run was performed as follows: Immediately prior to transferring the feed hose from the raw sewage to the urine-feces drum the mixture was stirred thoroughly and sampled. Thereafter the mixture was stirred every half hour. Special care was taken to agitate vigorously enough so that any particles which may have settled on the bottom of the drum would be re-suspended. It was assumed that twenty minutes after feeding of the urine-feces mixture began hose lines, filter, and all storage units had been purged, and the first sample was taken. Thereafter samples were taken at 1 hour, 2-1/2 hours, 4-1/2 hours and 7-1/2 hours.

Anaerobic Digester Experiments

Procedure

In all a total of 14 digesters were set up. Of these, three were initiated at $28^{\circ}\pm$ C, five were incubated at 30°C , and two each were incubated at 35°C , 45°C and 60°C .

All digesters were started using an initial seed inoculum of digested sludge obtained from one of the local New York City

sewage treatment plants. As a general rule, digested sludge was used for seed, however, two experiments were performed using primary sludge. All of the digester sludges used as seeds were obtained from digesters operating at mesophilic temperatures.

In all of these digester experiments a urine-feces mixture was used based on the approximate output of one man per day (21). This mixture consists of 135 gm of feces (usually fresh, but at times frozen feces was used) to which 1,500 ml of urine was added. The mixture, blended with a Waring blender, was usually prepared in batches of four or five liters, stoppered and stored in a refrigerator until it was to be used. Before additions to digesters the mixture was brought to the temperature of the digester.

The digesters (see Figure 6) consisted of a main digester bottle with a minimum sludge capacity of three liters. For feed, withdrawals and gas collection, two exits were provided into the digester vessels to assure minimum of loss and/or disturbance of the digesting mixture. Connected to the digester was a gas holder vessel and a displacement bottle for measuring evolved gas volumes. At the time of gas measurements the liquid level in the three vessels was equalized and the connecting stop-cocks were closed off. Urine-feces feeding and

ANAEROBIC DIGESTION

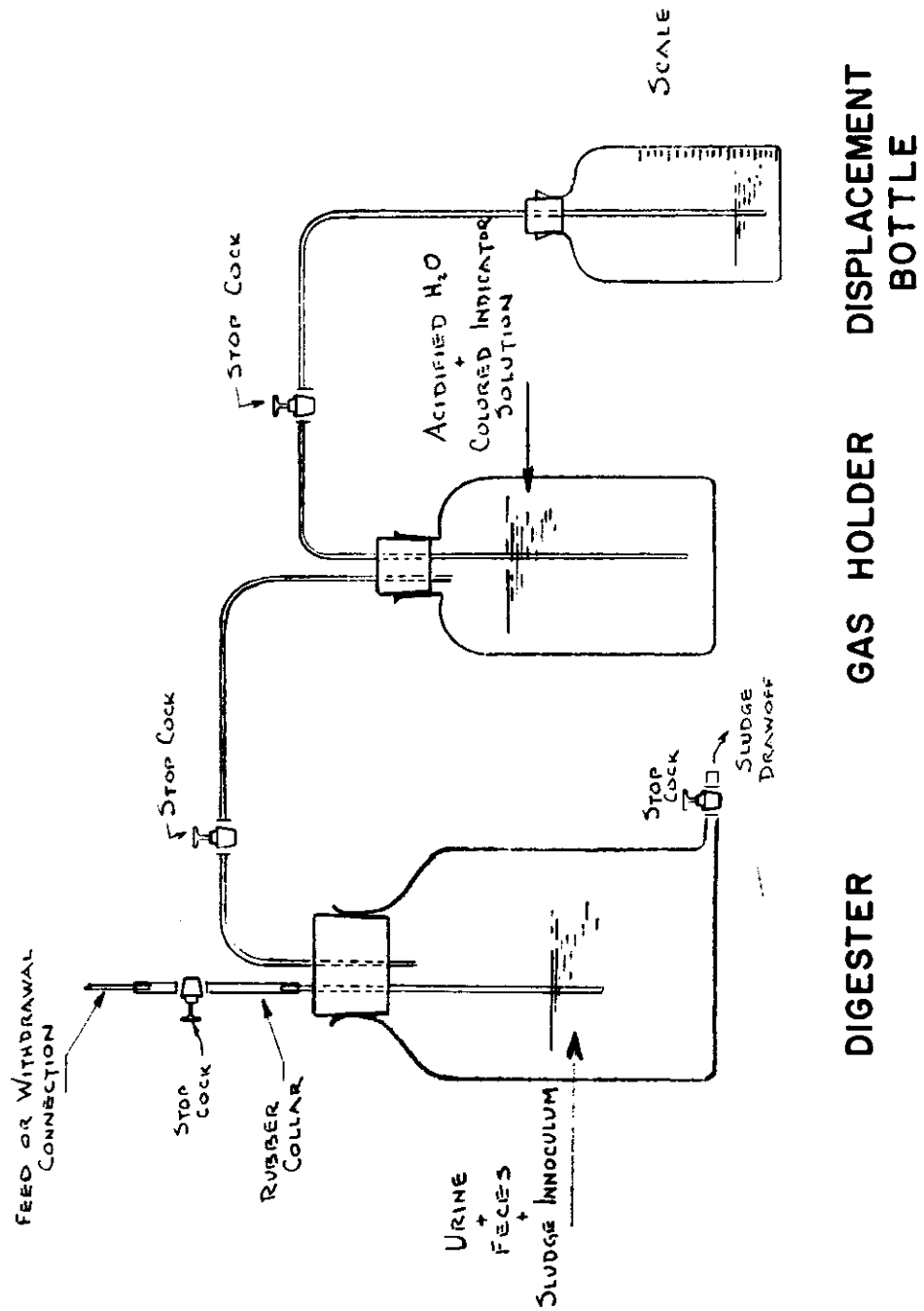


Figure 6

gas volume measurements were usually done daily, at about the same hour.

With the exception of three studies at $28^{\circ}\pm$ C, all digester experiments were conducted in standard laboratory incubators with temperature controls of $\pm 0.5^{\circ}$ C.

Digesters are usually operated in mesophilic temperature ranges, from 80° F to 100° F (27° C to 38° C) with optimum temperature given of about 90° F to 95° F (32° C to 35° C) (67). Higher temperatures are usually considered to be thermophilic and optimum temperatures are given as 120° F to 130° F (49° C to 54° C). For these experiments 45° C and above was considered to be within the thermophilic temperature range. Low temperature digesters might conceivably be used; however, previous experience indicates that the results are not particularly favorable. Phelps (100) and Bloodgood (101) have observed that digestion "practically ceases" at 50° F (10° C) or less.

Digester experiments were set up at mesophilic (M series) and thermophilic (T series) temperatures using different ratios of seed and urine-feces blend. A summary of the several experiments performed can be seen in Table 17.

All of the digesters were set into operation in the same manner. The digester vessel was used for mixing the sludge seed and the urine-feces. Initially, total solids, volatiles and pH were determined for both the sludge and the urine-feces.

Table 17
Anaerobic Digester Experiments

<u>Experiment</u>	<u>Temperature</u> °C	<u>Initial Sludge*</u> ml	<u>Initial Urine-Feces</u> ml	<u>Total Length of Run</u> days
<u>MESOPHILIC</u>				
M-1	28+ (warm room)	115	3,000	2
M-2	28+ (warm room)	100	1,500	2
M-3	28+ (warm room)	86.6	650	3
M-4	30	1,000	500	60
M-5	30	1,000**	500	35
M-6	30	700**	100	7
M-7	30	750	50	28
M-8	30	500	100	4
M-9	35	1,500	75	18
M-10	35	1,500	50	27
<u>THERMOPHILIC</u>				
T-1	60	750	750	4
T-2	60	500	100	4
T-3	45	2,000	100	131
T-4	45	2,000	50	5

*Digested sludge unless otherwise noted.
**Primary sludge.

Immediately after mixing the digester was closed off and the siphon was put into operation. Gas was collected over acid water to which an indicator (modified methyl-orange) was added. Precautions were taken to disturb the digesters as little as possible when withdrawing digest. After feeding the digester was shaken thoroughly to reduce stratification. Total solids and volatiles were run on each batch of urine-feces used for inoculum until it was assured that a more or less consistent mixture was being achieved. The average of five batches used in the experiments was 5.71% solids, 27.8% ash, and 72.4% volatiles. The range on solids was 5.44 - 6.06%; on ash, 23.6 - 31.3%; and on volatiles, 68.7 - 77.4%.

Digester loading ranged from 0.196 to 0.311 pounds of volatile solids per ft³ of digester capacity.

Bacteriological specimens for examination were collected directly from active digesters. Samples were streaked onto B.H.I. agar¹ (Brain Heart Infusion Agar, Difco), and incubated aerobically and anaerobically. Cultivation, at the beginning, was attempted using various media: B.H.I. Agar, Tryptose Glucose Extract Agar, Nutrient Agar, Levine's E.M.B., and a compounded media consisting of a basic salt mixture with yeast extract and urine-feces (1 and 2%). B.H.I. Agar, however, was found to be slightly more favorable for recovery and growth over other media used.

Colonies generally did not develop sufficiently in less than 48 hours to be picked for isolation.

Standard procedures (102) were used for staining and biochemical tests.

EXPERIMENTAL RESULTS

Activated Sludge

In the activated sludge units set up to receive concentrated wastes (raw urine-feces blend), three liters of urine-feces blend represent the wastes resulting from one man for two days. A summary of the analytical results of all activated sludge runs (AS 1 through AS 4) is shown in Tables 18, 19 and 20.

Experiments AS 1 and AS 2 were continued for 57 days; experiment AS 3 was maintained for 49 days, and AS 4 for 44 days. Though AS 1 and AS 2 showed a reduction of about 85% from the original concentration, the resulting mixture still contained a BOD of approximately 3,300 mg/l. From the standpoint of time involved for BOD reduction and the quality of the final product, the treatment of concentrated wastes by the activated sludge method is deemed unreasonable.

The diluted samples (AS 3 series) showed BOD reductions in the range of 85% to 95%. Actual BOD's of the final products

Table 18
Analyses Experiments AS 1 and AS 2

<u>Days</u>	<u>AS 1</u>		<u>AS 2</u>	
	<u>BOD</u> mg/l	<u>%</u> <u>Removal</u>	<u>BOD</u> mg/l	<u>%</u> <u>Removal</u>
0	18,000	0.0	18,000	0.0
1	17,400	3.3		
2	17,600	2.2	15,400	14.5
3	15,000	16.7		
4	16,600	7.8		
9	18,500*	-	15,500	13.9
10	15,600	13.3		
11	18,400*	-		
29	7,900	56.1		
30	6,600	63.3		
31	5,200	71.1		
36	5,700	68.3	3,900	78.8
37	5,000	72.2		
38	3,250	81.9		
43	2,850	84.2		
44	3,100	82.8	3,600	80.0
45	2,400	86.7		
57	3,625	80.0	3,225	82.1

*Irregularities are probably due to dilution error.

Table 19

Analyses Experiment AS 3

Days	1:5		1:20		1:27		1:40	
	AS 3-1		AS 3-2		AS 3-3		AS 3-4	
	BOD mg/l	% Removal	BOD mg/l	% Removal	BOD mg/l	% Removal	BOD mg/l	% Removal
0**	3,600	0	900	0	600	0	450	0
1	2,800	22.2	720	20.0	454	24.3	312	30.7
2	2,960	17.8	630	30.0	772*		283	37.1
3	3,040	15.6	555	38.3	340	43.3	175	61.1
21	1,400	61.1	635	29.4	380	36.7	210	53.3
22	960	73.3	400	55.6	297	50.5	207	54.0
23	1,300*	63.9	450	50.0	256	57.3	160	64.4
28	740	79.4	235	73.9	94	84.3	220	51.1
29	930	74.2	333	63.0	240*	60.0	123	72.7
30	690	80.8	256	71.6	95	84.2	102	77.3
35	570	84.2	143	84.1	65	89.2	59	86.9
36	550	84.7	110	87.8	55	90.8	18*	96.0
37	520	85.6	114	87.3	65	89.2	48	89.3
49	565	84.3	94	89.6	59	90.2	28	93.8

*Irregularities are probably due to dilution error.

**Computed BOD value.

Table 20

Analyses Experiment AS 4

Days	1:5		1:20		1:27		1:40	
	AS 4-1		AS 4-2		AS 4-3		AS 4-4	
	BOD mg/1	Removal %	BOD mg/1	Removal %	BOD mg/1	Removal %	BOD mg/1	Removal %
0**	4,700	0	2,206	0	1,991	0	1,791	0
1	4,950	--*	1,100	50.1	1,810	9.1	920	48.6
2	7,500	--*	1,630	26.1	--	--	1,220	31.9
3	2,700	42.6	1,240	43.8	--	--	700	60.9
8	1,850	60.6	490	77.8	966	51.5	495	72.4
9	2,750	41.5	670	69.6	940	52.8	340	81.0
10	1,650	64.9	410	81.4	--	--	430	76.0
15	1,400	70.2	360	83.7	640	67.9	225	87.4
16	--	--	220	90.0	467	76.5	160	90.5
17	1,250	73.4	250	88.7	493	75.2	195	89.1
29	1,175	75.0	430	80.5	367	81.6	213	88.1
30	950	79.8	400	81.9	367	81.6	245	86.3
32	800	83.0	250	88.7	387	80.6	215	88.0
37	734	84.4	273	87.6	367	81.6	165	90.8
44	737	84.3	277	87.4	220	89.0	196	89.1

*Irregularities probably due to dilution error.

**Computed BOD value.

were in the range of 28 to 565 mg/1 with the lesser values in the higher dilutions. Extrapolations to zero dilution also demonstrate that greater BOD reduction was obtained in the highest dilution (1:5 = 2,825 mg/1 BOD; 1:20 = 1,880 mg/1 BOD; 1:27 = 1,770 mg/1 BOD; 1:40 = 1,120 mg/1 BOD).

Experiment AS 3 was maintained for 49 days and showed BOD removal of about 85% after 35 days.

Experiment AS 4 was maintained for 44 days. BOD removal reached 85% in 1:40 and 1:20 dilutions in about 15 days and in 1:5 and 1:27 dilutions after 37 days. Actual BOD of the final product (750 to 200 mg/1) was somewhat higher than that of experiment AC 3 (tap water dilutions). The initial BOD was also higher since wash waters used for dilution had a BOD of 1,375 mg/1.

Extrapolating results to zero dilution (1:5 = 3,700 mg/1 BOD; 1:20 = 5,550 mg/1 BOD; 1:27 = 5,720 mg/1 BOD; 1:40 = 7,850 mg/1 BOD), it can be observed that a greater degree of purification was obtained in AS 4-1, the 1:5 dilution. This is a reversal in purification compared with the AS 3 series.

The concentration of detergent in the wash waters was 0.47% or 4.7 ml detergent per liter of wash water (see Table 4). The 1:5 dilution contained 11.3 ml of detergent; the 1:20 dilution 13.4 ml detergent; the 1:27 dilution 13.6 ml detergent; and the 1:40 dilution 13.8 ml of detergent. That is, the amount

of detergent was increased 22% over the dilution series and may have inhibited bacterial growth in the higher dilutions.

Figure 7 shows the per cent BOD removal vs. time in days of continuous aeration for the AS 4 series of wash water dilutions of urine-feces blend.

According to Pipes (90) an activated sludge unit will require 0.5 to 1.3 gm of oxygen per gram of organic matter oxidized. The urine-feces blend used in the present studies contained 5.7% solids, of which about 72% were volatile. Oxygen demand calculations based on these values indicate that for each liter of waste (41 gm organic matter) 20.5 to 53.3 gm of oxygen would be required, or for 1,635 ml waste (representing discharge of one man per day) 34.3 to 87.2 gm of oxygen are necessary.

The resulting BOD in the highest dilutions of the series AS 4 were still too high to permit water usage without a "tertiary" treatment. If increasing dilutions showed decreasing BOD's, it could be argued that a suitable end product could be obtained by working at a 1:60 or higher dilution. The implied detrimental influence of detergent tends to negate this means of obtaining a lower BOD in the final product. Furthermore, as has been shown in the entire series, the length of time required to obtain a reasonable removal efficiency is indicative of an excessive amount of storage volume to complete

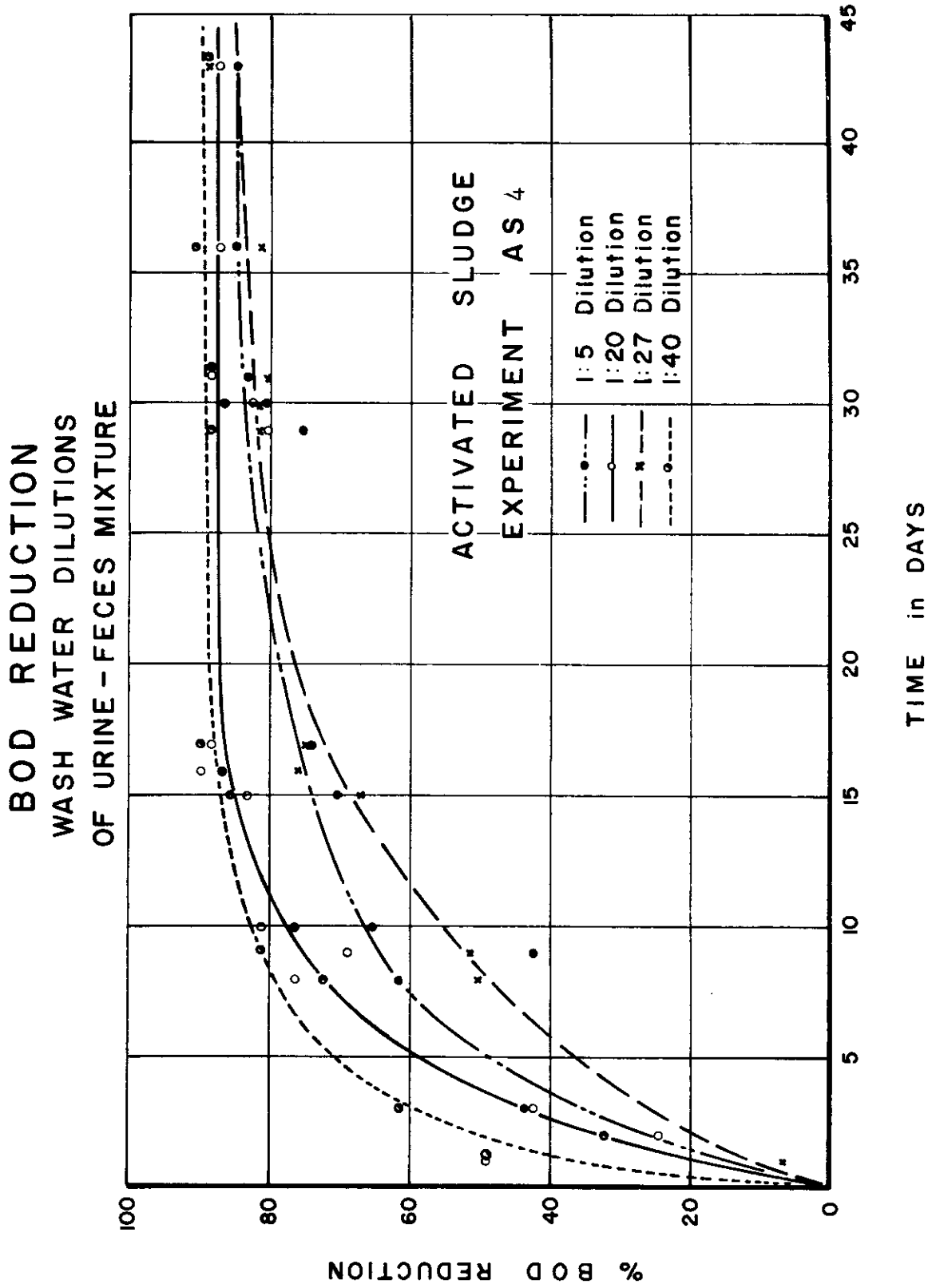


Figure 7

a water cycle from consumption through treatment and back to consumption. Assuming a satisfactory product on completion of activated sludge treatment, the transient storage volume would be not less than 74 liters per man (ca 163# of water). That is, about 20 gallons of water per man would have to be carried as supply over the space of time required for treated water replacement to become available.

It is suggested that raw metabolic wastes of man may not be adequately treated by the activated sludge process without resorting to dilutions more nearly resembling domestic sewage. Though the per cent BOD removal increased on dilution in the present experiments, the nature of the final product indicated essentially that conditions compatible with required biological activity were not reached. The process might be suitable as a treatment to provide a nutrient substrate for subsequent algal treatment and growth as demonstrated by Golueke (10). It is also suggested that further experimentation with feces excluded from the wastes may present a more favorable potential for the method.

Trickling Filter

The conditioned filter showed a reduction of 75% BOD with the applied BOD being 141 mg/l. Table 21 shows the rapid response of the filter to application of a mixture of seeded

Table 21

Trickling Filter Performance
Treating
Urine-Feces--Settled Sewage*

<u>Time Sampled</u>	<u>BOD ppm</u>		<u>% Reduction</u>	
	<u>Filter Effluent</u>	<u>Settled Effluent</u>	<u>Filter Effluent</u>	<u>Settled Effluent</u>
0 + 20 min.	453	--	8.5	--
0 + 1 hr.	454	--	8.3	--
0 + 2.5 hrs.	469	349	5.3	29.5
0 + 4.5 hrs.	468	395	5.5	20.2
0 + 7.5 hrs.	477	374	3.6	24.4

*Mixture BOD Applied = 495 ppm = 7,700 #/10³ ft³/day. Flow rate = 2.7 gal/ft²/min.

sewage and feces-urine blend having a BOD of 495 mg/l. Failure in the flow due to clogging of the pump prevented a continuation of the experiment. It was obvious, however, that the filter would not operate at high efficiency with the extremely high loading that was tried out. As with the activated sludge process it appears that either greater dilutions of waste must be used or that feces must be eliminated from the mixture. One suggested line of exploration has been opened, namely the thorough blending of feces with waste waters and removal of the solids by some form of centrifugation prior to aerobic biological treatment. More dilution could be obtained by recirculating the filter effluent. Time limitations prevented any extended exploration of this method since each experimental run requires the establishment of a ripened (biologically active) filter as a condition of the investigation. This type of experimentation requires about one month per test run to obtain dependable information on performance. Since this method has not been adequately studied it is suggested that further investigations may be profitable in revealing potentialities of the method.

With some conditioning it might be expected that a trickling filter bed might eventually be developed which could handle highly concentrated wastes such as we have attempted to treat. However, a considerable amount of work would have to

be done relative to a development of a satisfactory biota which can (a) survive in a concentrated urine-feces mixture, and (b) oxidize this concentrated waste.

Anaerobic Digestion

Analytical results of experiments with fourteen digesters are shown in Tables 22 and 23. Ten are in the mesophilic range (the M series) and four in the thermophilic range (the T series). Of the fourteen only two were found to function with any degree of satisfaction. A comparison of gas yields of the several experiments is shown in Table 24. The mesophilic unit M-10 and the thermophilic unit T-3 both showed evidence, by gas evolution measurements, of continuing operation. Though each of these units gave gas, the correlation of gas produced to volatile solids added was not firmly established.

The relative amount of sludge-seed inoculum used to start a digester appears to be important for a satisfactory digestion of urine-feces mixture (see Table 17). Small sludge seedings, at ratios of less than 1:1 volatiles (sludge:urine-feces), do not appear adequate for initiation of digester activity and no digester receiving lesser ratios survived. On the other hand, five digesters out of ten having sludge seedings greater than 1:1 volatiles failed to produce gas after the first few days.

Table 22
Summary of Analyses: Mesophilic Digestion Experiments

Experi- ment No.	Raw Materials										
	Volume		Solids %		Volatile %		Additions		Removals		
	Sludge* ml	Urine- Feces ml	Sludge	Urine- Feces	Sludge	Urine- Feces	Mixture	Urine-*** Feces ml	Digester*** Substrate ml		
M-1	115	3,000	6.19	5.44	31.70	68.7	5.47	31.70	67.13	0	0
M-2	100	1,500	3.43	5.44	57.13	68.7	5.31	57.13	68.23	0	0
M-3	87	650	3.43	5.73	57.13	77.4	5.46	57.13	75.90	80	80
M-4	1,000	500	3.43	5.73	57.13	77.4	4.20	57.13	66.57	1,980	1,680
M-5	1,000**	500	9.90	6.06	78.08	74.4	8.62	78.08	77.18	1,400	650
M-6	700**	100	9.90	6.06	78.08	74.4	9.42 ^c	78.08	77.18 ^c	350	0
M-7	750	50	8.21	5.64	42.83	69.1	8.05 ^c	42.83	43.98 ^c	750	450
M-8	500	100	8.21	5.64	42.83	69.1	7.78	42.83	46.00	300	0
M-9	1,500	75	6.90	5.61	49.32	72.15	6.84	49.32	50.22	1,895	0
M-10	1,500	50	6.90	5.61	49.32	72.15	6.86	49.32	49.93	2,180	460

Note: Volume of Mixture = Volume Sludge + Volume Urine-Feces.

* Digested sludge unless otherwise noted.

** Primary sludge.

*** Total added or removed during run according to experimental procedure.

c Calculated.

Table 23
 Summary of Analysis: Thermophilic Digestion Experiments

Experi- ment No.	Raw Materials										
	Volume		Solids %			Volatile %			Additions		Removals
	Sludge* ml	Urine- Feces ml	Sludge	Urine- Feces	Mixture	Sludge	Urine- Feces	Mixture	Urine- Feces** ml	Digester Substrate** ml	
T-1	750	50	8.21	5.64	8.05	42.8	69.1	43.95	100	0	
T-2	500	100	8.21	5.64	7.78	42.8	69.1	45.98	200	0	
T-3	2,000	100	6.80	5.61	6.74	49.32	72.15	49.79	12,760	12,585	
T-4	2,000	50	6.81	5.61	6.77	49.32	72.15	49.70	125	0	

*Digested sludge unless otherwise noted.

**Total added or removed during run according to experimental procedure.

Table 24
Comparative Gas Yields of Digesters

Digester Run	Raw Material				Ratio A/B	Volatiles Solids	Activity days	Gasification		Operation*
	Volume		Urine-Feces ml	Volume				Total Volume ml	Average Production ml/day	
	A	B								
M-1	115	3,000	1:26	1:50	1	100	100	Q		
M-2	100	1,500	1:15	1:29	0	0	0	Q		
M-3	87	650	1:8	1:16	1	250	250	Q		
M-4	1,000	500	2:1	1:1	56	3,720	66	Q		
M-5	1,000**	500	2:1	7:2	34	3,080	91	Q		
M-6	700**	100	7:1	12:1	3	1,000	333	Q		
M-7	750	50	15:1	27:2	24	1,250	52	Q		
M-8	500	100	5:1	9:2	1	160	160	Q		
M-9	1,500	75	20:1	17:1	15	7,135	476	Q		
M-10	1,500	50	30:1	25:1	27	21,795	807	S		
T-1	750	50	15:1	25:2	2	1,060	530	Q		
T-2	500	100	5:1	9:2	2	1,025	513	Q		
T-3	2,000	100	20:1	17:1	129	102,725@	796	Q		
T-4	2,000	50	40:1	34:1	1	3,000	3,000	Q		

*Q = Quit, digester ceased gasification; S = Stopped experiment.

** Primary sludge used.

@ Feeding urine-feces substrate to the digester was terminated after 56 days of operation; gasification continued for an additional 73 days.

Other factors including initial temperature, stabilization, and digester handling could affect digestion activities. These variables could not be examined adequately in the present exploratory work.

Mesophilic digesters having sludge:urine-feces volatiles concentrations of 27:2 and greater were apparently able to start digestion and gasification. However, thermophilic digester, T-4, did not start digesting and gasification activity with a sludge:urine-feces volatiles ratio of 34:1.

Immediately after blending urine-feces mixtures had a pH of about 7.0 or slightly lower (in the pH range of urine), but seldom below 6.6. Storage of urine-feces blend, even when refrigerated, was found to result in a rise in pH, to about 8, in a short period of time (two or three days). The blended mixture was more or less consistent in composition and gave the appearance of a thick, syrupy slurry. Solids and volatile contents of the urine-feces did not vary greatly from batch to batch. On the basis of the exploratory results of experiment T-3 it has been estimated that the unit digester capacity required might be as much as 11.8 liters per man to hold an equilibrium in a satisfactorily operating anaerobic digester (thermophilic range).

During 23 consecutive sampling days 6,070 ml of the digested material, containing 228.6 gm of dry solids, were

Projection of Volume and Power Requirements

It is evident that any biological treatment system must be supported by powered equipment including mechanical movers (pumps), and pressurizing equipment. No accurate prediction of the power requirement can be offered without greater refinement in the choice and design development of one or more of the methods. The power requirements anticipated at this writing include a source of energy for:

1. Pressurizing the entire treatment system as a closed system.
2. Mechanical or other separation of solids from liquids by filtration or rotational means prior to biological treatment. (Note that feces solids should not enter the treatment system as other than dissolved or colloidal solids.)
3. Movement of materials along the line of flow, i.e.,
 - (a) Liquid fraction to biological unit, thence to solids-liquid separation, thence to recirculation and mixing with additions of waste to be treated or to secondary treatment as a recovery system for potable and other water use.
 - (b) Gaseous fractions to biological units and away from units, separatory actions, subsequent gas purification, admixtures with oxygen supply as required by process.

lubricant is applied to decrease the frictional coefficient between two objects, boundary lubrication should become dependent on the pressure exerted rather than the load involved. A monomolecular film may consequently be all that is required for sufficient lubrication. As mechanical pressure is increased the vapor pressure of a liquid is also increased. As the vapor pressure is increased under conditions of reduced pressure of the surrounding atmosphere, boiling will occur more rapidly than it will at normal atmospheric pressures. The term "boiling," in a zero gravity situation would seem to be a misnomer, since boiling is dependent on the density gradients caused by gravity to separate the boiling "bubble" from the liquid. Consequently, "boiling" as such would not occur, but rather, at the point of origin of the "boiling" bubble, a minute "explosion" would take place. "Explosions" would tend to disengage large numbers of molecules from each other, perhaps in the form of aerosols or droplets which, unless completely sealed off, would enter space capsule atmosphere in an erratic fashion and cause additional problems in environmental comfort and air purification control.

lubrication is used. Frictional coefficients depend on the load or pressure at the contact of two surfaces. Fluid friction exists when the oil film is thick enough so that the surface regions are essentially independent of each other and the coefficient of friction depends on the hydrodynamic properties, especially the viscosity (109) of the oil.

As the load is increased and relative speed between moving surfaces decreases, the intersurface lubricating film becomes thinner and more contact occurs between the surface regions. Consequently the coefficient of friction increases from the low values associated with fluid friction to a value intermediate between fluid friction and unlubricated surface friction. This type of lubrication, i.e., where the nature of the surface region becomes important, is known as boundary lubrication. The area of actual contact is undoubtedly only a small fraction of the total area of apparent contact, so that only occasional patches of film are put under mechanical pressure (load). Since most of the load is being supported by the boundary film itself, the load is said to be "floating" on pressurized film (109).

In a zero gravity situation such frictional forces exist only in a pressurized situation, i.e., where two objects are mutually held in contact by a pressurized force. As a

Any of the processes discussed require controls and ancillary mechanized equipment. Zero gravity conditions impose limits on many of these.

Gas bubbles, as well as being inhibitive in the nutrient assimilation transport phenomena of microorganisms, are also inhibitive in any battery type power system where a gas is formed at one or both of the electrodes. The bubbles formed in these batteries will remain in contact with the electrodes and act as resistances, sharply curtailing electrical efficiency and may lead to a deterioration of the electrode itself.

Liquid level devices will malfunction. Manometers, for example, will not function due to the lack of "weight" of the indicating fluid. Mechanical devices dependent on springs will have different characteristics due to the spring assuming new equilibrium positions under gravity conditions other than 1 G.

Since there will be no convective movement of air due to thermal density gradients, problems will be encountered in treatment equipment cooling. Though equipment cooling may be handled via an air-conditioning system, the malfunction of the air-conditioning system could easily lead to a series of overheating breakdowns in other equipment.

Moving parts require some form of lubrication to reduce frictional effects. Two limiting conditions exist where

Another system, based on the principle of the oxygen transfer in the lungs may be especially applicable to the trickling filter modification of a forced feed system. Where the filter itself would be completely full of liquid wastes, a filter bed consisting of a large number of porous micro capillary membranes into which a continuous supply of pure oxygen is fed, may have merit. Then, as the organisms grow on the bed of micro capillaries, oxygen is fed directly to the organisms through the porous bed itself. Problems may arise in keeping the slime matrix layer of microorganisms thin enough so that the oxygen might be supplied adequately to all organisms.

Anaerobic systems are not oxygen dependent and the problems of oxygen supply are therefore eliminated.

Zero gravity will have the same effect on limiting the transport phenomena at the cell membrane surface of the organisms as has been noted for the aerobic process. Assuming that an operation such as mechanical agitation may overcome the difficulty of membrane transport, the separation of the gases from the liquid medium may then be overcome by a system of porous membranes retaining the liquid and permitting passage of the vapors.

less adhering qualities and force both solids and liquids outward toward the periphery at some stage of treatment. The process is further complicated by the need to separate solid, liquid, and gaseous components within the system. Pressurized gases and vapor phase liquids might be passed through a porous membrane while trapped solids and liquids might be removed from the peripheral surfaces by negative pressure (siphon) devices.

Solid-liquid separations for either activated sludge or "trickling" filter method could be continued by means of plate filters or increases in rotational force to achieve inertial separation. Earth experience with such sludges indicates that about 15% solids content can be obtained by these methods. To drive off additional moisture from the solids it is probable that some evaporative process at temperatures and pressures low enough to prevent the formation of volatilized gases would be required. Laboratory experience with space cabin wastes (48) suggests that the evaporative processes can accomplish such separations at about $35\pm^{\circ}\text{C}$ and 20 mm pressure with minimum ammonia carryover. Ultimately the residues of the process would become available for nutrient extraction or would be non usable debris to be placed in storage.

including the material involved; the polarity or non-polarity of the adsorbate or absorbent; the concentration of the adsorbate; and the velocity of the flow of the wastes over the purifying absorbent (the porous bed of microorganisms).

In the light of the preceding discussions, it is suggested that for any of the systems to function within the parameters of closed space ecology, a completely closed system must be employed. A good example of such a system is the human circulatory system and its pump, the heart. This system is known to function under the conditions of zero gravity.

In a system of zero gravity, "trickling" is impossible. Consequently, design modifications for a positive flow of waste or for a rotating filter to induce gravity would be necessary. Such modifications will require power not contemplated in earth practice.

If wastes are forced over the filter bed in some manner overcoming the zero gravity condition, conventional methods of oxygen supply are not applicable. An induced counter flow system, where the oxygen is supplied under pressure and injected together with waste containing dissolved organics into a rotating filter might serve to bring both nutrient and oxygen to biota anchored to filter media. Variations of rotational speed might be used to dislodge older biota having

Essentially, the adsorption to be expected in waste treatment systems is that of the organic waste constituents onto a porous floc of organisms so that consequent attack and absorption may take place by the organisms. The organic molecules adsorbed will be non electrolytes for the most part.

The non electrolytes that have been studied include fatty acids, aromatic acids, esters and other single functional group compounds plus a great variety of more complex species such as porphyrins, bile pigments, carotenoids, lipids and others (109).

The behavior of a given system may be predicted qualitatively by thinking of adsorption as constituting a distribution of an adsorbate between two phases, the solution phase and the solid. The solution, adsorbent and adsorbate are then classified qualitatively according to their polarity, i.e., as to whether they are essentially polar or non-polar. The rule is that a polar adsorbate will tend to prefer that phase which is more polar, i.e., it will be strongly adsorbed by a polar adsorbent from a non-polar solution (or solvent) and vice versa. The converse should then be true for a non-polar adsorbate.

Wastes contain a great variety of polar and non-polar organic materials. These will be adsorbed and subsequently assimilated at different rates affected by many factors

In the absence of convective transfer the exchange of materials occurs by molecular diffusion only, a relatively slow process.

In the absence of density gradients caused by gravity, metabolic waste products of microorganisms may diffuse through the cell wall but have no rapid mechanism for removal away from exterior surfaces. Hence accumulations in the immediate vicinity of the cell wall may inhibit absorption mechanisms necessary for the vital life functions of the organism.

Activated sludge is dependent on gravity separation of liquid-solid mixtures and without a created separatory force or mechanism such as a filter the liquid fraction could not be removed from the solid.

The trickling filter process is also dependent on gravity as well as an oxygen supply. At 1 G the waste material is allowed to trickle over the filter media which acts as the support for the microbial population. The organic matter present is rapidly adsorbed by the slime matrix of organisms then absorbed by the purifying biota.

Adsorption, a prime mechanism of both activated sludge and trickling filter processes, is not affected by zero gravity or atmospheric pressure change. Adsorption is best understood as monomolecular adsorption with competition between solute and solvent for the surface of the solid.

breaking of foams, and the like would be difficult if not impossible without an imposed gravitational field."

The question arises as to whether there is a change in the physical chemistry of liquids and gases as a function of G forces and changes in atmospheric pressure. Transfer phenomena are believed to be based on these facts.

Aerobic treatment processes depend on the utilization of dissolved oxygen by microorganisms. The concentration of gas in a liquid is in equilibrium with gas in the gaseous medium in accordance with Henry's Law, and if the equilibrium condition is changed the rate of oxygen absorption is changed. Oxygen will enter or leave the liquid medium as a function of the undersaturation of oxygen. The rate varies directly as the area of liquid exposed to the gas phase, inversely as the volume of the water and according to the gradient of undersaturation. Thus the amount of dissolved oxygen available for biota is directly affected by absorption phenomena.

At atmospheric pressure and 20°C, 0.03144 gm of oxygen will dissolve in 100 gm of aqueous solution. If the pressure is increased, more gas will dissolve (i.e., carbonated water is made by the addition of carbon dioxide to water under pressure). When pressure is released, the gas comes out of solution. Likewise, if the pressure is decreased below 1 atmosphere the dissolved gases present will evolve (outgassing).

to 5μ by 0.7, not usually found in chains. Spores were ellipsoid, terminal and subterminal with only a slight swelling of the sporangium. Growth on B.H.I. gave a smooth, whitish, grey colony. Glucose (and maltose to a minor degree) was fermented with no gas production. Other sugars, as a rule, were not utilized. Gelatin was not hydrolyzed, citrate was not utilized, and the acetyl-methyl carbinol reactions were negative.

Gordon and Smith (107) have described this organism as intermediate between mesophilic and thermophilic spore-formers.

TREATMENT LIMITATIONS IMPOSED UNDER SPACE CONDITIONS

Standard waste treatment processes functioning in a normal environment, i.e., on earth, have certain reasonably well-defined restrictions on operation. Aerobic systems, i.e., activated sludge and trickling filters, are affected by temperature, oxygen supply and loading (both organic and hydraulic). Anaerobic systems do not need aeration. However, some means of maintaining a sludge suspension is required.

During extended space travel, additional operating parameters--the effect of zero gravity, air pressure and limited supply of oxygen must also be considered.

Dole (108) states, "Operations such as distillation (for water recovery), water electrolysis (for oxygen production),

1 μ in length. Staining was irregular at times, but once growth was established found to be gram negative and clearly coccoid. Colonies were very small, pinpoint and smooth. Milk was liquified and sugars were attacked to varying degrees. Both rods isolated were non-motile. Morphologically the two organisms were indistinguishable, however, they differ in their ability to ferment carbohydrates, and in gas production.

Based on liquification, motility and carbohydrate fermentations, the bacteria are probably either Bacteroides ovatus or Bacteroides convexus, according to Bergey (103).

The gram positive rod isolated from digester M-5 was found to be a member of the family Bacillaceae, and as per Bergey, identified as Bacillus circulans.

Only one organism was isolated from thermophilic digesters (digester T-3). The organism was isolated approximately two weeks after the digester had been in operation.

This bacterium can be described as a gram positive, spore forming, motile anaerobe. Classification, according to Bergey (103), is substantiated by reference to Group 2 of Allen's studies (104), the classification of Smith, Gordon and Clark (105), and Knight and Proom (106), and place this organism in the genus and species Bacillus coagulans.

Characteristically this organism may be described as a large, motile, gram positive rod producing vegetative rods 2

Facultative organisms were encountered more frequently than strict anaerobes. Two such organisms were definitely identified. Only two strict anaerobes--Bacteroides sp., which may really be biochemical variants of the same organism--were isolated, and these proved to be slow growing, rather fastidious organisms. Fungi, yeasts and actinomycetes were not observed. Neither was there evidence of Clostridia. As a general rule, the organisms isolated were not active carbohydrate fermenters, and dispelled very foul odors with anaerobic incubation. Persistency of bacterial species during the operational span of life of the several digester runs was normally not observed but the digester M-10, until its termination, repeatedly yielded the same bacterial types with a staphylococcus (Micrococcus pyogenes var. albus) predominant. There is no ready explanation for the presence of this normally aerobic contaminant.

Four mesophilic bacterial isolates were made on the active digesters. Three were rods, two gram negative and one gram positive. The gram negative rods were taken from digester M-5 and M-7. The gram positive rod was isolated from digester M-5.

Both gram negative rods appear to fall in the group of bacteria loosely described as Bacteroides. Characteristically these organisms may be described as being small rods with rounded ends, appearing almost coccoid at times and less than

Within the first few days after starting a digester a wide morphological representation of different bacterial forms could be seen. Noteworthy were many gram positive or irregularly stained rods of varying sizes, some as long as 5μ or even more, occurring singly or as streptobacilli, and resembling members of the family Bacillaceae. Many organisms of this type had both spherical and cylindrical spores, enlarged and unenlarged, with the sporangium positioned either centrally or terminally. Diphtheroid-like forms were also seen, as were great numbers of gram negative rods of varied sizes and shapes--some almost coccoid, tiny, elongate and even thinly curved. Gram positive cocci were observed less frequently. Few fungi or yeast like forms were seen. No protozoans or other higher forms of life were observed.

Cultivation

Isolation of bacteria, especially in older digesters, proved to be difficult. The organisms were found to be slow developers. Many times overgrowth and contamination occurred--in one instance a yeast overgrew the entire plate, giving the typical odor associated with yeast fermentation. On another occasion the organism, after isolation, changed biochemically and even morphologically from its original form--apparently adjusting to growth conditions of the medium provided.

laboratory, provided material that was added as a supplemental seed source in this experiment, but no beneficial effect was noted.

Over a span of a year several attempts at prolonged biological digestion of urine have been made at our laboratories. None of these have proven successful. Approaches have included batch urine digestion, daily urine dosings, addition of sewage seed, serial urine digest transfers, agitation, serial urine-sludge transfer, addition of nitrates, and cultivation of microbial flora. Manipulations over a period of several months brought the pH so low (pH 2.0) that any survival of a microbial population capable of achieving biological degradation was considered unlikely. Eventually even chance microbial contaminants could no longer be seen microscopically, and these experiments were abandoned.

Bacteriology

Samples from newly started digesters examined by direct microscopy revealed a heterogeneous, diverse bacterial population. As the digester continued to operate the bacterial population became less diverse, numerically smaller, and slowly changed in character from a facultative-aerobic population to one almost completely anaerobic or facultative-anaerobic.

removed from the digester. If the digestion process were to provide 100 gm dry solids per man per day to satisfy amino acid requirements (see Table 15) about 2,650 ml of digester material output per day would be needed. It seems unlikely that digester materials could provide more than 60 gm per day per man using normal output figures and assuming 100% availability as purified sludge solids. If one considers that the dietary regimen of an extended space flight may produce only a minimal amount of feces solids, then even less digested solids will be available. These experiments strongly suggest that utilization of sludge as an amino acid source is not quantitatively feasible.

Usually a well digested sludge from a satisfactorily operating sludge tank is black with a tarry, but not unpleasant or offensive in odor. In all of the present digestion experiments, after a period of a few days the color began to change from black, imparted by the original sludge seed, to a darker brown color more characteristic of the feces. The odor became increasingly bad, foul and offensive in both the mesophilic and thermophilic digesters. No substantive difference was noted in these odors, both being equally offensive. The odor of raw urine-feces was mild by comparison.

A urine-digest seed was used in experiment M-3. Related experiments on anaerobic digestion of urine, underway in the

- (c) Solids fraction movement to, through, and from separatory processes, thence to storage or secondary treatment for utilization as nutrient.
4. Automation of flow controls, valves, and monitoring equipment.
 5. Temperature control of process and heating or cooling of wastes to maintain selected temperature ranges (i.e., thermophilic, mesophilic, or psychrophilic growth conditions).

A gross projection of the power requirement for each of the biological processes explored in the laboratory is offered here solely as an indication of the minimum that might be anticipated in order to accomplish the basic operations:

	<u>Watts per Man</u>
Activated Sludge	1000 †
Trickling Filter	200 †
Mesophilic Digestion	100 †
Thermophilic Digestion	200 †

Likewise a gross projection of the cubage requirement for the biological process only is offered as an indication of the minimum that might be anticipated in order to accomplish

the basic operation:

	<u>Cubic feet/Man</u>
Basic Storage (Aeration) (includes requirement for recirculation)	40-50
Activated Sludge (including separation of solids by settling)	2+
Trickling Filter (includes separation of solids by settling)	0.7+
Mesophilic Digestion (includes subsequent sep- aration of solids by settling)	0.4+
Thermophilic Digestion (includes separation of solids by settling)	0.4+

Both the power and cubage unit figures offered must be taken as exploratory and preliminary estimates subject to change when research dealing with more specific development of method rather than overall feasibility is undertaken.

At this stage of investigation no reliable information on equipment weight can be offered.

The methodology of gas handling and purification has not been explored. It is assumed that waste gas cleaning and handling will be incorporated into the processes for cleaning

and handling space capsule air. Carbon dioxide and any gaseous products of digestion or distillation such as methane, hydrogen, sulphide, and ammonia, must be removed from the cabin atmosphere.

DISCUSSION AND COMMENT

The possibility of storing feces should be considered in light of the inherent difficulties which might be encountered during waste treatment procedures.

Both literature and experimentation point up the fact that feces is neither a productive source of water, an adequate source of essential amino acid diet supplement, nor a source of organisms that might contribute significantly to the purification of wastes.

Introduction of feces solids into the waste treatment cycle guarantees a material increase in oxygen supply requirements aboard space craft, adds significantly to the treatment requirements, and makes treatment of wastes by biological means impractical.

Assuming that further research established some bases of need for extracting free water and colloidal and dissolved solids from feces, it would be necessary to incorporate

grinding or maceration equipment into a closed operation where outgassing of highly objectionable gases would have to be controlled. Likewise specially developed separatory equipment would be needed to remove a solids fraction somewhat viscous in character and possessed of extremely poor liquid separation characteristics.

Intestinal flora have little value as purification biota. Unless adequate means have been taken prior to take-off to purge flight personnel of pathogenic organisms, the possibility of crew infection or infestation would raise problems of aseptic handling of the wastes. It is probable that any technique designed to kill pathogens would also affect saprophytic organisms thus reducing or eliminating biota and negating values in biological treatment potential that might ensue.

The problems of handling fecal waste are many and the apparent benefits are few. It has been estimated that compacted and frozen feces (as discharged) will require storage space of about 2.0 cubic feet per man per year. It is suggested that the probable logistics of any biological treatment process handling feces solids will not prove favorable for a lesser period of time.

It is impossible that any single species of organism can be depended upon to convert all human wastes into a usable recovery product. The metabolic mechanisms of purifying biota are complex. It appears to be well established that no one organism is capable of converting either proteins or carbohydrates directly to inorganic end products. Progressions in conversion are aided by enzyme systems that are in turn dependent on the abilities of organisms to attack nutrient. In aerobic treatment processes Z. ramigera is probably the most important single organism initiating the conversion of a waste nutrient complex.

The presence of ciliates has been associated with rise in nitrite peak in controlled filtration (110). The rise in protozoan population presumably results in increased ammonia production. Ammonia is then converted to nitrite through bacterial activity. Protozoans appear to function as bacterial scavengers using both young and old organisms as a food source. Protozoans also utilize cleavage products of proteins, carbohydrates, and lipids as nutrient. It has been shown that in earth biological processes the higher levels of stabilization are not attained solely in the presence of bacteria. It is not

conclusive that protozoans are mandatory organisms, but it is significant that the ciliates (particularly the stalked ciliates) are found in partly purified waters following the initial attack, and their predominance has been associated with treated waters approaching the nitrite stage of organic conversion. The protozoans may well be essential in the preparation of dissolved organics for final attack by nitrifying bacteria. In any event evidence that a single organism in isolated culture can convert bodily wastes to relatively organic-free water is absent.

The review presented in the report on the microbiology of bodily discharges outlines biological populations normal to feces, urine, perspiration and sebum. There is substantial evidence that few of the purifying organisms associated with aerobic waste treatment are found in those discharges. The treatment biota must therefore be external to man and available only through the route of "contamination."

In a closed system ecology there is no opportunity for comparable "contamination" to seed the waste. It follows that a capsule biological treatment system must be functioning when it leaves earth, or be placed in operation at some

subsequent time by artificially introducing a purifying biota seed to a treatment process. Such seed can be obtained from active sludges of an earth process by lyophilization. Mixed cultures should be carried in frozen state until used. Possible needs for replacement of microorganisms in the event of a temporary treatment failure, and the possible need for constant replenishment of seed organisms requires careful consideration. In the event that a workable biological system was developed, both replacement and replenishment would probably be required. Mixed culture seeding would obviate the necessity of tedious isolations of pure culture and as a practicality eliminate both the chance of erroneous choice and the rather hopeless state of dependence on one or even a few selected organisms for survival.

It is practically certain that any reserve biota used for the treatment process will have to be carried in preserved form to replace viable cultures aboard the space vehicle. Most of these organisms are not found in bodily wastes at discharge, but are introduced as "contaminants" subsequent to discharge.

Cubage, weight, dependability, and control are important considerations in determining the feasibility of using biological waste treatment methods. Limitations imposed by non gravity and reduced pressures complicate control, make dependability questionable, and impose the need for closed systems that operate independently of cabin conditions.

Tentative estimates of cubage projected from present knowledge handicap biological processes at the outset. Until research opens an avenue of rapid biological treatment capable of handling concentrated wastes, the volume of either aerobic or anaerobic systems must be predicated on diluted wastes possibly in the range of 60 to 100 parts diluent to 1 part bodily waste. The wash waters available for dilution are strong wastes and even these may require dilution and recirculation to bring the end product to a degree of stabilization that might suffice as a usable medium for algal growth, cabin cleansing, and other non potable water uses. None of the biological processes are capable of providing potable water directly. The liquid fraction taken from such treatment must be further treated to remove nitrite, ammonia, and trace organics.

Microorganisms would require inactivation to prevent explosive growth in the water storage units such as has been

experienced in space cabin simulators. Use of chemical inactivators adds to the burden of cubage and weight but must be faced as a practicality.

While in flight status, limited numbers of people could be provided with minimum water needs by more direct, more positive, and more dependable processes such as controlled distillation. It has been established that most if not all of the human water requirement can be met by methods utilizing that principle and taking urine, personal hygiene washings, clothes washings, and cabin washings as source liquid. It has not been established that these methods would be applicable to larger crews requiring more water and producing more wastes at more permanent outposts. It is conjectured that biological processes may have applications at such outposts. Those stations would possibly have fewer limitations on cubage and weight and could afford the penalties of volume required for biological treatment and storage of replacement water. Wider latitude on multiple water use, growth of algae, sludge purification, and recovery of food adjuvants is also conceivable. Under those circumstances biological waste treatment may be considered more feasible and may even be necessary as waste volume exceeds the limits of more direct treatment and recovery processes.

Under space capsule conditions presently conceived, there are more dependable ways of treating and recovering liquid fractions of waste from urine, cabin waste, and wash waters for limited numbers of personnel than the biological system if reuse of the liquid is the major consideration. However, for bodily wastes of greater numbers of personnel living at some form of semi-permanent installation, exploration of biological processes should continue.

For such installations there is potential value in intensive development of modifications of both aerobic and anaerobic treatment to obtain maximum benefit from each.

REFERENCES

1. Gaume, J. G., "Plants as a Means of Balancing a Closed Ecological System", J. of Astronautics, 4, 72 (1957).
2. Bassham, J. A., "Use of Controlled Photosynthesis for Maintenance of a Gaseous Environment", Report UCRL 2707, University of California Radiation Laboratory, Berkeley, Calif. (1954).
3. Gustavson, J., "Synthetic Atmosphere for Space-Ships", Astronautics, 2, 48 (1957).
4. Konecni, E., "Human Factors and Space Cabins", Astronautics, 3, 42 (1958).
5. Fenno, R. M., "Man's Milieu in Space: Summary of Physiological Requirements of Man in Sealed Cabin", J. Aviation Medicine, 25, 6, 612 (1954).
6. Myers, J., "Basic Remarks on the Use of Plants as Biological Gas Exchangers in a Closed System", J. Aviation Medicine, 25, 407 (1954).
7. Milner, H. W., "The Chemical Composition of Algae", Algal Culture from Laboratory to Pilot Plant, p. 285, Publication 600, Carnegie Institution of Washington, Washington, D. C. (1953).
8. Fisher, A. W. and Burlew, J. S., "Nutritional Value of Microscopic Algae", Algal Culture from Laboratory to Pilot Plant, p. 303, Publication 600, Carnegie Institution of Washington, Washington, D. C. (1953).
9. Golueke, C. G., Oswald, W. J., and McGauhey, P. H., "The Biological Control of Enclosed Environments", Sew. & Indus. Wastes, 31, 10, 1125 (1959).
10. Golueke, C. G., "The Use of Photosynthesis in the Control of Enclosed Environments", Am. J. Pub. Hlth., 52, 2, 258 (1962).

11. Myers, J., "Space Logistics. II - Biosynthetic Gas Exchangers", Lectures in Aerospace Medicine, School of Aviation Medicine, USAF Aerospace Medical Center (ATC), Brooks AFB, Texas (January 1960).
12. Bowman, R. O. and Thomae, F. W., "Long Term Non-Toxic Support of Animal Life with Algae", Science, 134, 55 (1961).
13. Bates, J. H., "Recent Aspects on the Development of a Closed Ecologic System", Aerospace Medicine, 32, 12 (1962).
14. Tamiya, H., "Growing Chlorella for Food and Feed", World Symposium on Applied Solar Energy, Phoenix, Ariz. (November 1955).
15. Hundley, J. M., Ing, R. B., and Krauss, R. W., "Algae as a Source of Lysine and Threonine in Supplementing Wheat and Bread Diets", Science, 124, 536 (1956). Also: Nutrition Reviews, 15, 87 (March 1957)
16. Enebo, L., "On the Supply of Oxygen and Food During Long-Lasting Space Journeys", Astronautik, 2, 2, 103 (1960). (Stockholm).
17. Gradwohl, R. B. H., "Feces", Chapter VIII, Clinical Laboratory Methods and Diagnosis, Vol. 2, p. 1261, C. V. Mosby Co., St. Louis (1956).
18. Sargent, F. and Johnson, R. E., "The Effect of Diet on Renal Function in Healthy Men", J. Am. Clin. Nutr., 4, 466 (1956).
19. Fruton, J. S. and Simmonds, S., General Biochemistry, John Wiley & Sons, New York (1953).
20. Berman, M. L., Quantitative Estimation of Urinary Metabolite Adrenaline and Noradrenaline as Possible Indication of Tolerance to Gravitational Stress, WADC Tech. Note 59-376 (November 1959).

21. Ingram, W. T., The Engineering Biotechnology of Handling Wastes Resulting from a Closed Ecological System, AFOSR Report No. TR 58-148, ASTIA Document No. AD 162 277 (February 1958).
22. Taylor, A. A., Finklestein, B., and Hayes, R. E., Food for Space Travel. An examination of Current Capabilities and Future Needs, ARDC Technical Report No. 60-8, Andrews AFB (July 1960).
23. Phillips, J. N., "Biological Systems in Space Vehicles", Lectures in Aerospace Medicine, School of Aviation Medicine, USAF Aerospace Medical Center (ATC), Brooks AFB, Texas (January 1960).
24. Welch, B. E., "Logistics of Photosynthesis", Symposium on Medical and Biological Aspects of the Energies of Space, Columbia University Press, New York (1961).
25. Clamann, H. G., "Some Metabolic Problems of Space Flight", Fed. Proc., 18, 1249 (1959).
26. McLester, J. S. and Darby, W. J., Nutrition and Diet in Health and Disease, 6th Edition, W. B. Saunders Co., Philadelphia (1952).
27. Miller, R. J., Bergeim, O., Rehfuss, M. E., and Hawk, P. B., "Gastric Responses of Food. The Psychic Secretion of Gastric Juice in Normal Men", Am. J. Physiol., 52, 1 (1920).
28. Atwater, W. O., Principles of Nutrition and Nutritional Values of Food, Farmers Bull, 142, p. 24, U. S. Dept. of Agriculture (1902).
29. Goldblith, S. A. and Wick, E. L., Analysis of Human Fecal Components and Study of Methods for Their Recovery in Space Systems, ASD Technical Report 61-419 (August 1961).

30. Hawk, P. B. and Bergeim, O., Practical Physiological Chemistry, 11th Edition, P. Blakiston's Son & Co., Inc., Philadelphia (1937).
31. Robinson, S. and Robinson, A. H., "Chemical Composition of Sweat", Physiol. Rev., 34, 2, 202 (1954).
32. James, A. T., "Occurrence of Unusual Fatty Acids in Fecal Lipids from Human Beings with Normal and Abnormal Fat Absorption", Biochem. J., 78, 333 (1961).
33. Fantus, B., Wozasek, O., and Steigman, F., "Studies on Colon Irritation. Examination of Feces", Am. J. Digest. Dis., 8, 296 (1941).
34. Boerner, F. and Sunderman, F. W., "Feces", Chapter 29, Normal Values in Clinical Medicine, W. B. Saunders & Co., Philadelphia (1949).
35. _____, Present Knowledge in Nutrition, The Nutrition Foundation, 2nd Edition (1956).
36. White, A., Handler, P., Smith, E. L., and Stetten, Jr., D., Principles of Biochemistry, McGraw-Hill Book Co., Inc., New York (1959).
37. Wollaeger, E. E., Comfort, M. W., Weir, J. F., and Osterberg, A. E., "The Total Solids, Fat and Nitrogen in the Feces: 1. A Study of Normal Persons and of Patients with Duodenal Ulcer on a Test Diet Containing Large Amounts of Fat", Gastroenterology, 6, 2, 83 (1946).
38. Albritton, E. C., Standard Values in Nutrition and Metabolism, WADC Technical Report 52-301 (December 1953).
39. Wallman, H. and Barnett, S. M., Water Recovery Systems (Multi-Variable), WADC Technical Report 60-243 (March 1960).

40. Spector, W. S., Handbook of Biological Data, WADC Technical Report 56-273, ASTIA Document No. AD 110 501 (October 1956).
41. Consolazio, C. F., Johnson, R. E., and Marek, K. E., Metabolic Methods, C. F. Mosby Co., New York (1951).
42. Ingram, W. T., Newman, B., Palevsky, G., and Slote, L., "Exploratory Research on the Theoretical Considerations of Waste Water Cycles in a Closed Ecological System", Advances in Astronautical Sciences, Vol. 4, p. 444, Plenum Press, Inc., New York (1959). Also: Proceedings of the Fifth Annual Meeting of the American Astronautical Society (December 1958).
43. Coughlin, G. J., "Detergents in Sewage", presented at the Annual Meeting of the AAS & GP, New York (1956).
44. Sawyer, C. N., "Effects of Synthetic Detergents on Sewage Treatment Process", Sew. & Ind. Wastes, 30, 6, 757 (1958).
45. Schmidt, J. O., "Synthetic Detergents in Water and Sewage", Public Works (April 1957).
46. Isaac, P. C. G., "Synthetic Detergents: Their Effect on Sewage Treatment and Water Supply", Water & Sanit. Engr., 3, 413 (1953).
47. Haney, P. D., Chairman, "Character and Effects of Synthetic Detergents", AWWA Task Force Report E 5.15, JAWWA, 46, 8, 751 (1951).
48. Ingram, W. T., Recovery of Usable Water from Wastes of a Closed Ecological System, Prepared by Research Division, New York University College of Engineering, New York, for School of Aerospace Medicine, Brooks AFB, Texas, under Contract AF 41(657)-366 (February 1962).

49. Personal Communication, Winthrop Laboratories, 1450 Broadway, New York City (November 1961).
50. Wilson, G. S. and Miles, A. A., Topley and Wilson's Principles of Bacteriology and Immunity, Vol. II, 3rd Edition, The Williams & Wilkens Co., Baltimore (1946).
51. Evans, C. A., Smith, W. M., Johnson, E. A., and Gilbert, E. R., "Bacterial Flora of the Normal Human Skin", J. Invest. Dermatol., 15, 305 (1950).
52. Lovell, D. L., "Skin Bacteria: Their Location with Reference to Skin Sterilization", Surg. Gynecol. Obstet., 80, 174 (1945).
53. Price, P. B., "The Bacteriology of Normal Skin; A New Quantitative Test Applied to the Study of Bacterial Flora and the Disinfectant Action of Mechanical Cleansing", J. Infect. Dis., 63, 301 (1938).
54. Kraus, F. W. and Gaston, C., "Individual Constancy of Numbers Among the Oral Flora", J. Bact., 71, 703 (1956).
55. Williams, N. B., Eickenberg, H., and Florey, B. M., "A Preliminary Report on the Quantitative Inter-relationship of Microorganisms Cultivated from Human Saliva", J. Dent. Res., 32, 691 (1953).
56. Richardson, R. L. and Jones, M., "A Bacteriologic Census of Human Saliva", J. Dent. Res., 37, 697 (1958).
57. Thomson, D. and Thomson, R., "The Common Cold. V. Researches on the Bacterial Flora found in the Healthy Nose", Ann. Pickett-Thompson Res. Lab., 8, 30 (1932).
58. Watson, E. D., Hoffman, N. J., Simmers, R. W., and Roseburg, T., "Aerobic and Anaerobic Bacterial Counts of Nasal Washings: Presence of Organisms Resembling *Corynebacterium Acnes*", J. Bact., 83, 144 (1962).

59. Johannson, K. R. and Sarles, W. B., "Some Considerations of the Biological Importance of Intestinal Micro-organisms", Bact. Reviews, 13, 1, 25 (1949).
60. Zubrzycki, L. and Spaulding, E. H., "Studies on the Stability of the Normal Human Fecal Flora", J. Bact., 83, 5, 968 (1962).
61. Ingram, W. T. and Diachishin, A., "Effect of Cabin Cruiser Waste Discharge on a Small Harbor", Proceedings, A. S. C. E., Separate 455 (June 1954).
62. Ingram, W. T., "Advances in Design Trickling Filtration", p. 33, Advances in Sewage Treatment Design, Proceedings of a Symposium held at Manhattan College, New York on May 15, 1961. Published by the Sanitary Engineering Division, Metropolitan Section, A. S. C. E.
63. _____, Standard Methods for the Examination of Water and Waste Water, APHA, AWWA, WPCF, 11th Edition. Published by American Public Health Assn., New York (1960).
64. Theriault, E. J., "The Oxygen Demand of Polluted Waters", Public Health Bulletin No. 173 (1927).
65. Orford, H. T. and Ingram, W. T., "Deoxygenation of Sewage: I. Critical Review of Monomolecular Formula. II. The Logarithmic Formula as Applied to Sewage", Sew. & Indus. Wastes, 25, 4, 419 & 424 (April 1953).
66. Eckenfelder, W. W. and O'Connor, D. J., Biological Waste Treatment, Pergamon Press, Inc., New York (1961).
67. Simpson, J. R., "Some Aspects of the Biochemistry of Aerobic Organic Waste Treatment", p. 1, Waste Treatment, edited by P. C. G. Isaac, Pergamon Press, Inc., New York (1960).
68. Gale, E. F., The Chemical Activities of Bacteria, 2nd Edition, University Tutorial Press, London (1952).

69. Lamanna, C. and Mallette, M. F., Basic Bacteriology, 2nd Edition, The Williams & Wilkins Co., Baltimore (1959).
70. Buchanan, R. E. and Fulmer, E. I., Physiology and Biochemistry of Bacteria, Vol. 1, Williams and Wilkins Co., Baltimore (1928).
71. Knaysi, G., "Chemistry of the Bacterial Cell", Chapter 1, Bacterial Physiology, edited by C. H. Werkman and P. W. Wilson, Academic Press, Inc., New York (1951).
72. McKinney, R. E., Microbiology for Sanitary Engineers, McGraw-Hill Book Co., New York (1962).
73. Hopkins, E. W., Peterson, W. H., and Fred, E. B., "The Composition of the Cells of Certain Bacteria with Special Reference to Their Carbon and Their Nitrogen Content", J. Biol. Chem., 85, 21 (1929).
74. Belozersky, A. N., "On the Nucleoproteins and Polynucleotides of Certain Bacteria", Cold Spring Harbor Symposia, Quant. Biol., 12, 1 (1947).
75. Freeland, J. C. and Gale, E. F., "The Amino Acid Composition of Certain Bacteria and Yeasts", Biochem. J., 41, 135 (1947).
76. Stokes, J. L. and Gunness, M., "The Amino Acid Composition of Microorganisms", J. Bact., 51, 570 (1946).
77. McCarty, P. L. and McKinney, R. E., "Volatile Acid Toxicity in Anaerobic Digestion", J. WPCF, 33, 223 (1961).
78. Cantarow, A. and Schepartz, B., Biochemistry, W. B. Saunders Co., Philadelphia (1954).
79. Seneca, H., Peer, P., and Nally, R., "Microbial Urease", Nature, 193, 1106 (1962).

80. Snell, J. R., "Anaerobic Digestion: III. Anaerobic Digestion of Undiluted Human Excreta", Sew. Wks. J., 15, 679 (1943).
81. McCarty, P. L. and McKinney, R. E., "Salt Toxicity in Anaerobic Digestion", J. WPCF, 33, 399 (1961).
82. Hanke, M. T. and Koessler, K. K., "Studies on Proteinogenous Amines: XX. On the Presence of Histamine in the Mammalian Organism", J. of Biol. Chem., 59, 879 (1924).
83. Hacker, L. R., Neuman, A. L., and Johnson, B. J., "Feed from Sewage. III. Dried Activated Sewage Sludge as Nitrogen Sources for Sheep", J. Animal Science, 16, 125 (1957).
84. Hawkes, H. A., "Ecology of Activated Sludge and Bacteria Beds", Waste Treatment, edited by P. C. G. Isaac, Pergamon Press, Inc., New York (1960).
85. Committee on Refuse Disposal, APWA, "Municipal Refuse Disposal", APWA Research Project 104, Public Administration Service, Chicago (1961).
86. McKinney, R. E. and Harwood, M. P., "Fundamental Approach to the Activated Sludge Process. 1. Floc Producing Bacteria", Sew. & Indus. Wastes, 24, 117 (1952).
87. Butterfield, C. T., "Studies of Sewage Purification. II. A Zooglea-Forming Bacterium Isolated from Activated Sludge", Public Health Reports, 50, 671 (1935).
88. Wooldridge, W. R. and Standfast, A. F. B., "The Biochemical Oxygen Demand of Sewage", Biochem. J., 27, 183 (1933).

89. Oswald, W. J., Golueke, C. G., Gee, H. K., and Cooper, R. C., Microbiological Waste Conversion in Control of Isolated Environments, First Annual Report, Geophysics Research Directorate, A. F. Cambridge Research Labs., AF Res. Div. Contract AF 19(604)-6637 (February 1961).
90. Pipes, W. O., "Waste-Recovery Process for a Closed Ecological System", Publication No. 898, Natl. Academy of Sciences, Natl. Res. Coun. (1961).
91. Chapman, D. D., "Incorporation of Waste Disposal Processes into the Closed Ecological System", Proc. of Inst. Environ. Sci., p. 283 (1960).
92. Bogen, R. H., Chapman, D. D., and Ericsson, L. H., "Aerobic Biological Degradation of Human Waste in Closed Systems", Adv. Assoc. Sci., 6, 390 (1961).
93. Sawyer, C. N., "An Evaluation of High-Rate Digestion", Water & Sew. Wks., 105, 255 (1958).
94. Mueller, L. E., Hindin, E., Lunsfords, J. V., and Dunstan, G. H., "Some Characteristics of Anaerobic Sludge Digestion. I. Effect of Loading", Sew. & Ind. Wastes, 31, 669 (1959).
95. Golueke, C. G., "Temperature Effects on Anaerobic Digestion of Raw Sewage Sludge", Sew. & Ind. Wastes, 30, 1225 (1958).
96. Pote, H. L., Gafford, R. D., and Richardson, D. E., "Anaerobic Waste Processing for Closed Ecological Systems", Advances in Astronautical Sciences, Vol. 6, p. 399 (1961).
97. Hurwitz, E., "The Use of Activated Sludge as an Adjuvant to Animal Feeds", Proc. of the 12th Industrial Waste Conference, Purdue University, p. 395 (1957).

98. Rose, W. C., Wixon, R. L., and Lockhart, H. B., "The Amino Acid Requirements of Man. XV. The Valine Requirements; Summary and Final Observations", J. Biol. Chem., 217, 987 (1955).
99. Buswell, A. M., "Fermentations in Waste Treatments", Chapter 14, Industrial Fermentations, Vol. II, edited by L. A. Kofler and R. J. Hickey, Chemical Publishing Co., New York (1954).
100. Phelps, E., Public Health Engineering, Vol. I, John Wiley & Sons, New York (1949).
101. Bloodgood, D. E., "Sludge Digestion", (Series 2--Part 8), Water & Sew. Wks., 104, 1, 38 (1958).
102. Society of American Bacteriologists, Manual of Microbiological Methods, McGraw-Hill Book Co., Inc., New York (1957).
103. Breed, R. S., Murray, E. D. G., and Smith, N. R., Bergey's Manual of Determinative Bacteriology, 7th Edition, The William & Wilkens Co., Baltimore (1957).
104. Allen, M. B., "The Thermophilic Aerobic Sporeforming Bacteria", Bact. Revs., 17, 125 (1953).
105. Smith, N. R., Gordon, R. E., and Clark, F. E., "Aerobic Sporeforming Bacteria", Agriculture Monograph No. 16, U. S. Dept. of Agriculture (1952).
106. Knight, B. C. G. and Proom, H., "A Comparative Survey of the Nutrition and Physiology of Mesophilic Species of the Genus Bacillus", J. Gen. Mic., 4, 508 (1950).

107. Gordon, R. E. and Smith, N. R., "Aerobic Sporeforming Bacteria Capable of Growth at High Temperatures", J. Bact., 58, 327 (1949).
108. Dole, S. H., Design Criteria for Rotating Space Vehicles, RM 2668, The Rand Corp., Santa Monica, Calif. (October 1960).
109. Adamson, A. W., Physical Chemistry of Surfaces, Interscience Publishers, Inc., New York (1960).
110. Ingram, W. T., "An Investigation of the Mechanisms of Controlled Filtration", Sew. & Ind. Wastes, 31, 10 1147 (October 1959).