

***SMALL SCALE WASTE MANAGEMENT PROJECT***

**Use of Bacteria in Assessing Waste Treatment  
and Soil Disposal Systems**

by

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## USE OF BACTERIA IN ASSESSING WASTE TREATMENT AND SOIL DISPOSAL SYSTEMS

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The bacterial flora of sewage is distinctive and for that reason can be a useful parameter for following the treatment and safe disposal of sewage. In fresh raw sewage the kinds and numbers of bacteria are predominantly those of intestinal origin. In all warm-blooded animals, man included, the intestinal flora is fairly stable, although a great variety of bacteria are ingested with daily food. The gut offers an excellent environment for bacteria such as the coliform group, fecal streptococci, lactic acid bacteria, anaerobes (both spore forming and non-spore forming), and numerous other minor groups including the pathogens, if the host is currently infected. Counts of these important groups can be made by proper bacteriological methods and, indeed, the Standards for coliform, fecal coliform, and fecal streptococci are well known examples of their application.

As the fresh sewage passes on through treatment, conditions change and new types of bacteria grow and compete with the intestinal bacteria. The flora of sewage thus is a very complex but relatively balanced mixture, capable of biodegradation of innumerable chemical compounds of sewage. Thus the sewage bacteria are useful and, in fact, essential to the treatment.

### Anaerobic treatment

The septic tank is anaerobic with the majority of the functioning bacteria being facultative, capable of growth with or without free oxygen. In the septic tank, without free oxygen, they use the combined oxygen of their organic and inorganic substrates. Their primary function in the septic tank is hydrolysis of complex compounds such as proteins, carbohydrates, and fats. Table 1 gives values for some chemical components of raw wastewater, expressed as the average of composite samples taken from three households prior to any type of treatment; average values from five septic tank effluents are also given. The relative percentage of nitrogen as ammonia-N in wastewater (22%) as compared to that in septic tank effluent (70%) indicates considerable deamination in the septic tank. Similarly, protein hydrolysis is indicated by the relative percentages of organic nitrogen and phosphorus (74% nitrogen as organic-N and 55% phosphorus as organic-P in raw wastewater as compared to 29% organic-N and 21% organic-P in the septic tank effluent).

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Approximately 80% suspended solids reduction can be attributed to hydrolysis by bacteria and the settling of solids. The subsequent use of the products of hydrolysis for growth and respiration (energy) accounts in part for BOD removal (343 mg/l in the raw and 158 mg/l in septic tank effluent) leaving a relatively high BOD still to be degraded aerobically in later treatment.

Counts of coliform bacteria, fecal streptococci, and pathogens (*Pseudomonas aeruginosa*, staphylococci, salmonellae, and enteric viruses) are still high and therefore septic tank effluents are unsafe for release without danger to public health. Table 2 presents bacterial counts on septic tank effluents, pH and temperature conditions to be expected in septic tanks. Along with high coliform, fecal streptococci and *Ps. aeruginosa* counts salmonellae have been detected in 59% of 17 different septic tank pumpout sludges, clearly showing that septic tank effluent should not be released to the surface of soil where natural waters may become contaminated or where other public health problems may arise. It can be released subsurface in soil as in the conventional absorption field where further purification by soil adsorption, and aerobic degradation will take place.

#### Aerobic Treatment

Aerobic treatment of sewage offers a more advanced degree of biodegradation, i.e. gives average BOD reductions to 36-55 mg/l in mechanical aeration unit effluents and to 10-36 mg/l in sand filter effluents; oxidation of malodorous products of proteolysis; eventual oxidation of ammonia to nitrate; of hydrogen sulfide to sulfate; and of volatile fatty acids to carbon dioxide and water. These products of total oxidation are indicative of a stabilized final effluent with little or no oxygen demand at the disposal site, either soil or water. This is accomplished when, under aerobic conditions, metabolic activities of facultative organisms in the sewage change from anaerobic to aerobic or when a more aerobic population gains ascendancy by growth. There are a number of choices as to aerobic treatment for small systems waste management, and bacteriological data are helpful in assessing the stage of oxidative biodegradation in each case.

Soil, as in absorption fields, mound systems or sand filters, provides strong adsorptive removal of bacteria including pathogens, and aerobic conditions for oxidative stabilization of waste water. In the absorption field the sewage bacteria merge with soil bacteria. The transition from high to low numbers of coliform and streptococcal indicator bacteria and total bacteria occurs in a relatively short distance (1-2 ft) from the base of the trench. Table 3a gives representative data for such a system. The transition zone is also notable for the predominance of rapidly growing heterotrophic soil bacteria such as actinomycetes, sporulating bacilli, and high numbers of pigmented bacteria including melanin producers. In a well functioning absorption field the indicator and pathogenic bacteria must not be allowed to surface in ponding water or to descend to contaminate the ground water. In this respect absorption fields are very efficient in purifying septic tank effluent.

In a mound system treated effluent is applied to an absorption bed or trench constructed on two feet of sand fill over the natural top soil. It offers another form of aerobic treatment similar to that of the absorption field in sandy soil. Table 3b provides results of analyses on liquids collected after percolation through such a mound.

Table 1 Some chemical and physical parameters of raw wastewater and septic tank effluent

Parameter (mg/l)	Raw wastewater		Septic tank effluent	
	Mean	Relative	Mean	Relative
BOD <sub>5</sub> , unfiltered	343		158	
BOD <sub>5</sub> , filtered	212		120	
Suspended solids total	259		50.8	
volatile	203	78	35.5	70
Ammonia-N	8.8	22	38.7	70
Organic N	30.3	74	16.0	29
NO <sub>2</sub> + NO <sub>3</sub> -N	1.8	4	0.6	1
Ortho-P	9.4	45	11.5	79
Organic-P	11.3	55	3.1	21

Table 2 Data for bacteria, pH and temperature on effluents from five septic tanks

Bacteria <sup>1</sup>	Mean	95% confidence interval of mean	Range
Fecal streptococci per 100 ml	3,800(97) <sup>2</sup>	2000- 7200	< 100- 1,00,000
Fecal coliforms per 100 ml	420,000(94)	290,000- 620,000	500- 18,000,000
Total coliforms per 100 ml	3,400,000(91)	2,600,000- 4,400,000	150,000- 40,000,000
<u>Ps. aeruginosa</u> per 100 ml	10,000(13)	1,900- 54,000	210- 350,000
Total bacteria x 10 <sup>5</sup> per ml	34 (88)	25-48	0.3-2300
pH	7.3 (58)	7.2-7.4	6.4-8.0
Temperature in tank, °C	17 (13)	15-19	12-23

<sup>1</sup> log normalized data

<sup>2</sup> number of samples used in calculation

Table 3a Bacteriological data on samples from a typical absorption field in sandy soil

Sample	Bacteria/ 100 mls or per 100 g of soil			
	Fecal streptococci	Fecal coliforms	Total coliforms	Total bacteria x 10 <sup>6</sup>
Septic tank effluent	160,000	1,900,000	5,700,000	3
Soil of clogged zone at base of trench	54,000	4,000,000	23,000,000	4400
Soil 30 cm below base of trench	< 200	< 200	< 600	3.7
Soil 30 cm lateral to trench, midpoint level	< 200	< 200	< 600	0.6
Soil 30 cm lateral to trench, 8 cm below base	< 200	17,000	23,000	6.7
Soil 30 cm lateral to trench, 38 cm below base	< 200	700	1,800	2.8

Low numbers of indicator bacteria were found to pass through the two feet of fill; 22, 33 and 6% of these samples were negative for fecal streptococci, fecal coliforms and total coliforms respectively. However, the detection of *Ps. aeruginosa* in two samples confirms the presence of pathogens possibly indicated by the coliform and streptococci. The additional layer of natural soil below the mound is necessary for further purification.

Aerobic purification of treated wastewater can also be accomplished through sand filtration. The sand filter in many respects is similar to an absorption field in sand or to a mound system. It differs in that hydraulic loading rates are generally much higher and the filtration medium is coarser. Table 3c indicates a significant removal of the sewage indicator bacteria. A greater efficiency of absorption fields and mounds vs. sand filters is probably related to loading rates and media size. From a bacteriological standpoint, sand filtration of septic tank effluent gives greater relative removal than filtration of mechanical aeration unit effluent. The preliminary evidence of the presence of *Ps. aeruginosa* as well as of the indicator bacteria implies that further purification by disinfection is warranted, especially if the effluent is to be surface discharged.

Mechanical aeration units provide highly stabilized effluents (as was indicated previously) after hydraulic detention times of 1 to 3 days. The intestinal bacteria (indicators and pathogens) are present in the mixed liquor of the aeration tank in numbers in the order of those in septic tank effluent (see Table 4). Clarification of the mixed liquor effects a 1 to 2 log reduction in the numbers of indicator bacteria and *Ps. aeruginosa*, these bacteria being retained in the sludge. However, significant high numbers remain in the clarified effluent, and further purification is needed.

Table 3c Representative bacteriological data on effluent from sand filters loaded with effluent from septic tank or mechanical aeration unit

Bacteria <sup>1</sup>	Filter loaded with septic tank effluent		Filter loaded with mechanical aeration unit effluent	
	Influent	Effluent	Influent	Effluent
	Mean 95% confidence interval of mean		Mean 95% confidence interval of mean	
Fecal streptococci per 100 mls	2,100 (20) <sup>2</sup> 660 - 6,500	94 (16) 40 - 230	2,600 (29) 1,500 - 4,400	730 (26) 380 - 1,400
Fecal coliforms per 100 mls	230,000 (19) 43,000 - 1,200,000	4,200 (16) 1,000 - 17,000	14,000 (29) 9,600 - 22,000	3,100 (24) 1,800 - 5,200
Total coliforms per 100 mls	2,400,000 (20) 1,500,000 - 4,000,000	13,000 (14) 3,100 - 53,000	51,000 (29) 33,000 - 86,000	14,000 (21) 8,200 - 23,000
<i>Ps. aeruginosa</i> per 100 mls			2,200 (6) 2,200 - 52	520 (4) 520 - 52
Total bacteria $\times 10^4$ per ml	470 (18) 240 - 940	40 (15) 22 - 72	31 (29) 21 - 44	11 (25) 7 - 16
Filter characteristics				
loading (gal/ft <sup>2</sup> /day)	16 - 32			4 - 6
area (ft <sup>2</sup> )	27.5			26.9
depth (ft)	2			2
Sand characteristics				
effective size (mm)	0.45			0.19
uniformity coefficient	3.0			3.3

<sup>1</sup> log normalized data

<sup>2</sup> number of samples used in calculation

Table 3b Bacteriological data from a septic tank-mound system

Bacteria <sup>1</sup>	Septic tank effluent	Liquid collected at mound base	
	Mean; 95% interval	Mean positive samples	95% interval % negative <sup>2</sup>
Fecal streptococci per 100 mls	2000(27) <sup>3</sup> 700-5900	45	11 - 180 22% (23)
Fecal coliforms per 100 mls	270,000 (28) 170,000-450,000	390	66-1500 33% (21)
Total coliforms per 100 mls	2,800,000 (28) 1,700,000- 4,700,000	2500	590-11,000 6% (18)
<i>Ps. aeruginosa</i> per 100 mls	92,000 (2)	19	0% (2)
Total bacteria x 10 <sup>4</sup> per ml	910 (26) 490- 1700	6	2.2-.17 6% (19)

<sup>1</sup>log normalized data, 95% interval = confidence level of mean

<sup>2</sup>negative sample = less than 1 bacteria/100 mls

<sup>3</sup>number of samples used in calculation

*Pseudomonas aeruginosa* is found in higher numbers in aerated mixed liquor than in septic tank effluent. This bacterium is aerobic and biochemically very versatile. Its high survival and potential growth in aerated units is indicated. Pumpings from aeration units should be handled with care and application to soil may be inadvisable unless safety can be assured by disinfection.

#### Pathogens in Sewage

It is a generalization of medical bacteriology that man is subject to a number of enteric diseases during which pathogens are shed in the feces in tremendous numbers. Most patients cease to shed after recovery but some continue as carriers, even though apparently well. In a single family there may or may not be pathogens, depending upon family history of enteric disease, but it should be emphasized that disease is potential at any time.

*Salmonellae* cause a number of forms of salmonellosis, the most familiar being food poisoning and typhoid fever. Only 2 out of 55 samples of family sewage were positive at 3.4 and  $\geq 20/100$  mls; the samples were from septic tank effluent and aeration tank mixed liquor. Although incidence and MPN numbers are very small, the potential for salmonellae in family sewage exists.



Table 4 Bacteriological data from three mechanical aeration units

Bacteria <sup>1</sup>	Aeration mixed liquor	Clarified effluent
	Mean 95% confidence interval of mean	Mean 95% confidence interval of mean
Fecal streptococci per 100 mls	$\frac{120,000 (81)^2}{82,000-170,000}$	$\frac{3300 (70)}{2300-4800}$
Fecal coliforms per 100 mls	$\frac{250,000 (80)}{190,000-340,000}$	$\frac{11,000 (67)}{7400-15,000}$
Total coliforms per 100 mls	$\frac{2,100,000 (81)}{1,600,000-2,700,000}$	$\frac{110,000 (74)}{71,000-150,000}$
<u>Ps. aeruginosa</u> per 100 mls	$\frac{59,000 (6)}{8200-420,000}$	$\frac{2500 (7)}{240-26,000}$
Total bacteria $\times 10^5$ per ml	$\frac{160 (79)}{130 - 210}$	$\frac{18 (80)}{7.4 - 28}$

<sup>1</sup>log normalized data

<sup>2</sup>number of samples used in calculation

Pseudomonas aeruginosa is considered an opportunistic pathogen, a common cause of severe ear infection and some enteric disorders of infants. It is said to be shed by approximately 15% of all persons. In initial work Ps. aeruginosa was found in sewage from all ten sites tested; some counts have been given in the above tables.

Staphylococcus aureus is a causative agent of food poisoning, skin lesions and wound infections. Approximately 18% of adults (4% of children under two years) shed this organism in their feces. Initial work has shown it present in 3 of 9 families, in numbers ranging 10 to 4000/100 ml of their sewage.

The demonstrated incidence of pathogens in family sewage supports the concern for handling of sewage in unsewered areas.

