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Environmental Significance of Helminth Parasites and Their Removal in Wastewater Treatment Processes

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Abstract

Epidemiological surveys have reported important risks regarding intestinal parasites because of their widespread distribution (Crompton 1989), their high survival rate in the environment (Shuval *et al.* 1988) and their low infective dose (Kowal 1985 Gunnerson *et al.* 1985). The presence of large number of helminth eggs in various effluents used for agricultural purposes has been reported by several studies (Barbier *et al.* 1990 Schwartzbrod *et al.* 1986). Occurrence of helminthes, a human intestinal parasite, in sewage is of considerable environmental significance. In the past, the different helminth parasites were distributed only in some pockets on earth. But the migration of people and enormous production of sewage with population explosion has resulted in widespread occurrence of these parasites. These parasites are transmitted through their resistant life stages such as eggs and larvae and are responsible for infection of intestine, illness, pneumonia, cough, eosinophilia, blood stained sputum, bowel obstruction, infection of liver, gall bladder and appendix, vitamin deficiency and also occasional death in both children and adults. Contaminated articles like vegetables, hands, utensils and dust help in distribution and transmission of parasites. Sewage treatment plant workers are also exposed to the infection by helminth parasites.

Strict standards for the number of eggs are laid down by W.H.O. for the reuse of treated wastewater. Various wastewater treatments processed differ in their efficiency in the removal of highly resistant helminth eggs. In sewage treatment, most of the helminth eggs are concentrated in the sludge, which require further treatment such as composting, aerobic digestion and anaerobic digestion. The information on all these aspects are reviewed in this paper, including the approaches proposed by different workers to make the treatment more effective to make sewage and sludge suitable for safe reuse and the suitable monitoring methods developed for the enumeration of helminth parasites in wastewater and treated effluents and sludge.

Key words: Helminthes parasites, Life cycle, Clinical aspects, Sewage treatments, Parasite removal

Introduction

Numerous different parasites are of common occurrence in sewage in different life stages of their life cycle. These are viruses, bacteria, protozoa and worms. Worms are of three types-nematode,cestode and trematode. However, the common nematode (helminthes) parasites i.e. roundworm, hookworm, tapeworm and pinworm along with reference to some other worms are dealt with in this document.

Occurrence of helminth parasites of human origin in wastewater and their transmission during recycling of the treated wastewater are the greatest public health problems in sanitary engineering. Man is the positive host for the adult helminth parasites e.g. *Ascaris*, hookworm and tapeworm etc. The eggs of these parasitic worms and their dormant stages are discharged in large numbers in the faeces of infected people and then pass to the sewage treatment works. Contamination of crops irrigated by wastewater effluent leads to transmission of infection. Of course, intermediate hosts are also there who carry the infection further. Fishes and aquatic edible species are the carriers of nematodes when sewage is used in aquaculture ponds. The most important practical problem lies in the foolproof treatment technology, which can guaranty a safer user of effluent and sludge for the agriculture and pisciculture by employing cheaper and acceptable solution to the problem. So it is obvious that eradication of these eggs/ova from sewage effluent and sludge should be the prime motto, so that hazards from the use of sludge and sewage for agricultural and pisciculture use of effluent will be removed.

The stages of intestinal parasites found in sewage effluent and sludge are either the helminth eggs or cysts in case of protozoan. Eggs and cysts are the dormant resting stages and are highly resistant to treatments and drying, than the full-grown adults.

Helminth parasites are of concern due to their ability to resist the conditions existing during wastewater treatment. Because of their small dimensions and the density nearly equal to that of water, they are only partially removed in sedimentation processes. However, the sewage treatment processes e.g. oxidation ponds are largely quiescent. Nevertheless, they appear in greatly concentrated numbers in primary sludge compared with sewage and treated effluent. But still the risk prevails in the agricultural use of effluent and more so with the sludge. Hence sewage treatment and the ensuing sludge will have to be tackled equally on war footing so as to rid them of all possible pathogenic parasites.

Most of the parasites, but by no means all, are removed by settlements in the primary or secondary stages of treatment technology, the survival and distribution of eggs can be ascertained by studying the viability of these eggs, which have undergone treatment. Viability of helminth eggs assumed an important parameter in public health aspects of reuse of sewage effluents. All the above aspects are discussed in detail in this review.

Common Helminthes of Human Nature

Biological characteristics and environmental significance of three major helminthes, which are commonly prevalent in human and a few others, are discussed here. They are hookworms like*Ancylostoma duodenale, Ancylostoma* spp., *Necator americanus* and round worms like *Ascaris lumbricoides* and others like *Taenia saginata, Cysticerxcus bovis* and *Schistosoma mansoni* etc.

Occurrence and Distribution

Historically, human hookworm infections were probably confined to the Eastern Hemisphere of the earth.*Necator americanus* occurring south of 20^0 North latitude and *Ancylostoma duodenalee* north of 20^0 North latitude. In the past 500 years, population migrations, mostly notably those involving Spanish and Portuguese colonization in the New world and Southern Africa led to the introduction of *Ancylostoma duodenale* in these areas as well as the introduction of *Necator americanus* to Portugal. At the same time the slave trade from Africa to North and South America and the Caribbean islands led to the widespread distribution of *Necator americanus* in the Western Hemisphere. *Ascaris* worms are very common and occur worldwide and particularly in warm climates in rural community. It is one of the most prevalent human helminthes and it infects 700 - 1000 million people. Children are the most vulnerable for these infections.

Biological Characteristics

A. duodenale and *N. americans* are dioecious, sexually dimorphic, round worms belonging to the phylum Nematoda, order Strongylida, superfamily Ancylostomatoidea. The adult worms are small and off white or rusty in colour. *A. duodenale* is somewhat larger than *N. americanus*, the males being 5-10 mm long and the females 10-18 mm long depending on the species. The eggs of *A. duodenale* measures 56-60 µm by 36-40 µm and those of N. *americanus* are 64-76 µm by 36-40 µm in size.

Ancylostoma ceylanicum, a hookworm of dogs, cats and other animals, can infect man and develop into the adult stage. It has been reported to be of some importance in India, Surinam, West Indies (Indonesia) and elsewhere (Banwell and Sctiad 1978). *Ancylostoma braziliense* and *Ancylostoma caninum*, the cat and dog hookworm, rarely develop to the adult stage in man but their larvae can cause a creeping dermatitis called larvamigrans.

Ascaris lumbricoides, a nematode, is the common roundworm of man. Females are 200 - 400 mm in length, whereas males are 150 - 300 mm. The fertile eggs are ovoid and measures 45-70 µm by 33-50 µm. Man is the host for *Ascaris lumbricoides*. Pigs and dogs may disperse the undeveloped eggs of*Ascaris lumbricoides* by eating them in human faeces and excreting them later at another place

Health Risk of Handling Sewage and Night Soil

Human excreta and the domestic sewage are the principal vehicles for the transmission and the spreading of a wide range of communicable diseases (Table 1). The severity of some of the diseases are such that whole of the community gets wiped out. In rural and semi-urban communities where poverty, malnutrition and poor hygienic condition prevails, the helminthic diseases are of very common occurrence in children, mostly and also elders are no exception. Besides helminthic parasites, dreaded diseases like diarrhea, endemic malaria and cholera are the other main causes of death among the children and infants in developing countries.

Table 1. Some Diseases and Causative Organisms of Concern in HandlingNightsoil and Sewage Sludges

Category	Disease	Organisms (where identified)
Viral	Infective hepatitis	
	Gastroenteritis	Adenovirus
	Respiratory illness	Reovirus
	Poliomyelitis	Enterovirus (poliovirus)
Bacterial	Typhoid fever	**Salmonella typhosa**
	Salmonellosis	*Salmonella* sp.
	Bacillary dysentery /(shigellosis)	*Shigella* sp.
	Cholera	**Vibrio cholera**
	Tuberculosis	**Mycobacterium tuberculosis**
Protozoan	Amoebiasis (amoebic dysentery)	**Entamoeba histolytica**
Helminthic	Roundworm	**Ascaris lumbricoides**
	Pin worm	**Oxyaris vermicularis**
	Whip worm	***Trichuris trichura***
	Tape worm	***Taenia saginata***
	Hook worm	**Ancylostoma duodenale**
		Necator americanus

These and many others diseases start their origin from an infected person to a new victim through excreta or sewage. Therefore, the collection, transport, treatment, handling and ultimate disposal of human excreta and the domestic sewage are of utmost importance in the protection of the health of whole human community throughout the universe. It is particularly very important in places like south-east Asia (Taiwan, Indonesia and Malaysia), China and African countries where the human excreta is used with/without proper treatment for fertilization of the fish ponds, agriculture and also for biogas production which are cheaper than alternative methods of disposal. Hence, in developing countries and under developed countries such reuse of excreta and domestic sewage are a welcome proposal as it supports their economic activity and food production.

Khuder *et al.* 1998 observed that wastewater treatment workers (WWTW) are potentially exposed to a variety of infectious agents and toxic materials. The WWTW exhibited a significantly higher percentage of gastroenteritis, gastrointestinal symptoms (specifically abdominal pains) and headache.

Clinical Symptoms

Ancylostomiasis and Ascariasis are the infections of small intestine caused by hookworms and*Ascaris* respectively. Ascariasis is the infection of particular importance to those engaged in sanitation programs. Ancylostomiasis is frequently symptomless but it does produce illness and constitutes a public health problem. The most important features are anaemia and resulting weakness, debility and other consequences. Gastrointestinal pain, transient, cutaneous and pulmonary symptoms andodema may also be experienced. Hookworm is seldom recorded as a direct cause of death. Some grossly anemic individuals die of heart failure. Thedisease is undoubtedly a common contributory cause of death when other normally nonfatal infections attack a severelyanemic and debilitated person. Ascariasis is extremely common in most part of the world because eggs of the *Ascaris* worms are very persistent in environment and difficult to be eliminated by conventional sewage or night soil treatment processes. About 85% of Ascariasis infections are without any outward visiblesymptoms, but the presence of even few worms is potentially dangerous. The earliest symptoms are the pneumonia with severe cough, fever, eosinophilia and sometimes blood stained sputum. Infection generally occurs in small children and also in adults. In case of heavy Ascariasis infection, adult worms are passed in the faeces or by mouth. Serious complications like bowel obstruction or death due to the migration of the adult worm to the liver, gall bladder or appendix and rarely due to perforation of the intestine are also observed. Ascariasis leads to deficiency in vitamin A and C in children.

Transmission and Larval Development

Thin shelled ovoid unsegmented eggs are discharged by the adult female worms into the lumen of the small intestine. A full grown larger and stouter female of *Ancylostoma duodenale* produces approximately 3 times more number of eggs per day (10^4 to $2 X 10^4$) than *Necator americanus* ($5 X 10^3$ to 10^4). The eggs develop rapidly in the gut and are usually at the four or eight cells stage, when excreted along withfaeces. If faeces are deposited in a suitable environment, the eggs hatch in 24-48 hours to give rise to rhabdiform first stage larvae. Optimum conditions for hatching and subsequent larval development are- protection from strong sunlight; soil environment of the right particle size, denseness and structure i.e. ideally a light sandy loam; adequate but not excessive moisture, and both desiccation and water logging are rapidly lethal to hatched hookworm larvae. A temperature between 28^0 C-32^0 C for *Necator americanus* and between 20^0 C-27^0 C for *Ancylostoma duodenale* is ideal. Below these temperature ranges, larval development slows down and is completely arrested below 10°C and above 40 °C.

Adequate quantity of decomposing organic material and microorganisms in the soil provide food supply for the developing larvae. If conditions are satisfactory, the larvae undergo two molts outside the human body on the third and fifth day of their free living existence, giving rise to third stage filariform larvae which become infective to man about 6 days after hatching from the eggs. These larvae survive normally for 3-6 weeks and have a maximum life span of 15 weeks, and larvae have vertical range of migration of about one metre in suitable soils but their lateral movement is restricted to about 0.3 meters and usually much less.

Mode of Infection

Infection of man occurs most commonly when the third stage larvae penetrate the skin usually between the toes or on feet and ankles. In the case of *Ancylostoma duodonale*, the third stage larvae can also infect man when they are ingested with unwashed raw vegetables, contaminated by the soil where they have grown.

After penetration of the skin, larvae of both hookworm species enter small veins and lymphatic vessels and are carried to the heart and then to the lungs. *N. americanus* undergoes a period of development in the lungs then moves to bronchi and trachaea, and is swallowed and reaches small intestine in 24-48 hours after the skin penetration. Two further molts occur, one at about first day and second at 13^{th} day after reaching the small intestine. *Ancylostoma duodenale* under goes the whole of its development in the small intestine regardless of whether it enters the human body by the oral or cutaneous route. Sexually mature fertilized females of both species begin egglaying between 4 and 8 weeks after infection.

In case of *Ascaris*, the females lay upto 2, 00,000 eggs per day. The unsegmented fertilized eggs are passed in the faeces. About 15 percent of the excreted eggs are infertile and these are longer and narrower than the fertile eggs. The first stage larva in the egg must molt to produce second stage larva before the egg is infective under ideal conditions of moisture and temperature (22 °C-33 °C). A minimum of 10-15 days are requiredfor about 75 percent of freshly passed eggs to become infective. Infective eggs can survive for long periods. Survival of infective *Ascaris* eggs in soil has been reported for a period of 7 years.

Ascaris infection spreads through contaminated vegetables, infected hands, utensils,dust etc. When the infective *Ascaris* eggs are ingested through these agents, the larvae hatch in the duodenum and are carried by the blood vessels to liver, heart and lungs. They develop into adults in about 60-75 days and then live upto 1.5 years.

The Incubation Period

Incubation period in case of hookworms (the interval between infectionand the development of symptoms and signs of illness) varies from a few weeks to several years depending on numberof worms that parasitize an individual, his daily iron intake, body iron stores, blood losses etc.

In case of *Ascaris,* the incubation period varies from a few days to several months. The development of clinical illness depends upon the number of worms in the body and the state of health of the host. In case of many light infections in healthy people, no evidence of illness ever appears.

Prevalence of Infection

Hookworm and Ascaris infections are extremely common and occur in many countries. Perhaps over 700 million people are infected worldwide. Table 2 shows the prevalence ofhookworm infections in fifteen countries. There are many hundred of reports on *Ascaris* prevalence in various communities. Persons having acute ascariasis infection excrete huge number of eggs upto 2,00,000 per female worm per day and hence causes considerable potential transmission. Individual egg outputs of upto about 3, 00,000 per gram of faeces are reported. Assuming 100 gram of faeces per day per capita, this suggests atleast 150 mature female worms in the intestine.

Table 2. Prevalence of Hookworm Infection in Fifteen Countries

Sr. no.	Country	Location	Age (years)	Prevalence (percent)	Age of max. prevalence (years)
1	Bangladesh	Rural	All ages	29	ND
2	Columbia	Urban	All ages	35	10 – 14
3	Egypt	Rural	All ages	28	20 – 30
4	Gambia	Rural	All ages	23	40
		Rural	1 - 5	12	ND
5	Gautemala	Urban Poor	1 – 5	4	ND
		Urban wealthy	– 5	0	ND
6	Haiti	Rural	All ages	24	15 – 19
7	India	Urban	All ages	8	ND
		Rural	1 – 11	68	ND
		Rural	All ages	44 – 71	ND
8	Iran	Rural	All ages	25	11-15
		Urban	All ages	8	6 – 10
9	Ivory Coast	Rural	7 – 14	73	ND
10	Malaysia	Rural	6 – 12	43	ND
		Urban poor	4 – 6	5	13 – 15
		Urban wealthy	4 – 6	0	ND
11	Papua New Guinea	Rural	All ages	68	20 – 29
12	South Korea	Urban	All ages	14	30 – 39
		Rural	All ages	19	30 – 39
13	Taiwan	Rural	All ages	52	Over 60
14	Thailand	Rural	All ages	61	ND
15	Zambia	Rural	All ages	49	6 - 10

ND : data not available

Life Span and Survival in the Environment

Eggs will continue to be excreted, as long as there are adult worms in the small intestine of host human body. Adult hookworms may live forupto 7 years in the case of *Ancylostama duodenale* and 15 years in *Necator americanus* (Miller 1979). These are exceptional life span however 80% of worms survive for less than three years. Full-grown adult worms of *Ascaris* generally live for less than 10 months with maximum life span of upto 1.5 years.

Hookworm eggs and larvae in nature have been found mainly in soilat place where people defecate or where excreta are applied on fields. Farm workers working on fields and sanitary workers, getthe percutaneous infection. Vegetables grown on sewage farms get contaminated withthese parasitic eggs and become a potential health hazard. Hookworm eggs' survival in environment is comparativelyshorter than *Ascaris* eggs, which are much sturdier in nature and therefore provide a better indicator organism for environmental helminthology.

Hookworm eggs will tend to settle in water and eventually die in the bottom sediments. Their survival in seawater has been reported as less than 5 hours, compared to over 30 hours for *Ascaris* eggs under the same experimental conditions (Livingston 1978). Helminth parasites are found in surface waters, night soil, sewage, vegetables grown in sewage or nightsoil applied farms, soils etc.

Recovery and Viability of Helminthes Eggs

Effects of wastewater treatment technology upon the survival and distribution of eggs can be ascertained by studying the viability of these eggs, which have undergone treatment. The fertilized eggs of *Ascaris* are released from the female worm, undergo cell divisions and develop into infective larval stage in which embryonic motile form can be seen inside the shell. Incubation of treated eggs in acidified water for three weeks is often used as the basis of assaying viability of *Ascaris* eggs (Pike *et al.* 1983). Care must be taken in interpreting the results of the experiments to determine the viability of parasitic eggs.

As the number of helminth eggs in effluent of wastewater treatment plants is normally very low (Lakshminarayana and Abdulappa 1972, Mara and Silva 1986, Schwartzbrod *et al.* 1987), any method used must have a high recovery rate and/or use a suitable sample volume to allow detection to the guideline level; additionally it must also be suitable for routine use in only basically equipped laboratories.

Ayers *et al.* 1991 compared four methods for egg recovery on effluents of various qualities and their speed, ease and cost were assessed. The recovery of eggs was found to be consistently higher using the method recommended by World Health Organization (Bailenger Method) but only when 10 L samples, rather than 1 L sample, were processed.

Stien and Schwartzbrod 1988 proposed an experimental method which associates both the detection of very low numbers of *Ascaris* eggs and their viability determination from samples of wastewater after n-butanol treatment, which precipitates fertile eggs. In this study, 86% of the fertile eggs separated by n-butanol was viable and 90% of the non-fertile ones were non-viable. The detection of the low levels of eggs required a minimal sample volume; further study shows that 25 L was the best sample size for this method. The recoveries obtained with this method compared quantitatively with those reported by Arthur *et al.* 1981 (39% recovery), Reimers *et al.* 1981 (62% recovery) and Teichman 1986 (33-50% recovery). This method has the advantage of supplying information from water samples. Classical methods use toxic reagents that exclude any possibility of estimating viability.

Gaspard *et al.* 1996 developed a method to estimate viability of helminth eggs to evaluate the parasitic risks. The extraction of these eggs was performed with a diphasic treatment (DS 0.01% + ethylacetate) coupled with a filtration on 500 µm and 100 µm sieves followed by concentration using two flotation with NaCl (d-1.19). For the culture, 3 parameters tested showed a faster egg development at 30^0 C in deionized water with continuous aeration, whereas organic compounds reduce this development. This culture was performed during 13, 10 and 8 days respectively to obtain *Ascaris suum, Toxacara cannis* and *Capillaria* sp. larva and 16 days for *Trichuris vulpis*, which presented a slower development. The quantification was realized by microscopic examination (100X) after sonication and sodium hypochloride treatment (0.5^0 chl). the eggs reaching the larva stage were considered as viable and the percent of viability was expressed by the ratio: nematode eggs at the larva stage/total of nematode eggs counted.

Standards for Reuse of Wastewater Effluent and Sludge

The Engelberg Report 1985 published under the auspices of several international organizations (U.N.D.P., W.H.O., W.B.), reevaluates sanitary risks posed by the agricultural use of treated wastewater and proposed new recommendations. The World Health Guidelines for the microbiological quality of treated wastewater used for crop irrigation require an arithmetic mean of 1 intestinal nematode egg per litre (IRCWD 1985, Prost 1988, W.H.O. 1989). Using epidemiological evidence and a risk assessment model, the authors specifically show the importance of strict parasitological criteria for such reuse, recommending a limit of less than one viable intestinal nematode egg per litre (Cross and Strauss 1985, Blum and Feachem 1985, Mara and Cairmeross 1987, Hespanhol and Prost 1994). However, there is no standard limitation regarding the use of sludge for agricultural purposes. According to W.H.O. Guidelines (Engelberg 1985) and to the EPA Part 503 biosolid rule (Cross and Strauss 1985), only the viability of parasitic eggs is important from public health viewpoint.

Elimination/Survival of Parasites in Wastewater Treatment

Sewage Treatment Processes

Sewage treatment works are usually designed to remove or oxidize as much decomposable matter as possible so that the resulting effluent will not deplete the dissolved oxygen in the receiving water. So different sewage treatment processes stress more on the removal of organic pollutants than the microorganisms. In the process, the final effluent may satisfy the recommended standard for general pollutants but may contain large numbers of disease producing pathogenic organisms. Helminth parasites are of concern due to their ability to resist the conditions existing during wastewater treatment.

Study of the effect of various sewage treatment processes on ova and cysts of intestinal parasites were carried out way back in 1932. Since 1932, when Hirst 1932 undertook a study of hookworms in sewage, numerous workers have evaluated treatment plants in relation to elimination of helminth parasitic eggs. First report on the parasitic helminth eggs was reported from Russia by Vasilkova 1936 and in Brazil by Amaral and Leal 1940. Later Wright *et al.* 1942 reported effects of different sewage treatment processes on the ova and cysts of intestinal parasites. They reported that ova of parasitic helminths, particularly those of *Ascaris lumbricoides* may remain viable after passing through various treatment stages of sedimentation, digestion and drying. Total of 75 samples of sludge was examined from the seventeen Army Camps with parasitic helminth ova in 27 or 36% of the samples. Results are indicated in the Tables 3 and 4.

Mostly these studies reported have used eggs freshly dissected from proglottids. However, although proglottids may shed some of their eggs whilst still within the human alimentary canal, eggs are still found within proglottids recovered from faeces. Additionally the treatment of taeniasis usually involves the chemical purging of the worm from the gut. In these circumstances whole tapeworms containing viable eggs will find their way into sewage treatment plants. The proglottids may afford some protection to the eggs when on fields (Jepsen and Roth 1952) equally, eggs may be similarly protected by the proglottids during the sewage treatment process.

Cram 1943 studied the survival rate of helminth ova under different conditions of sewage treatment processes with inoculam of known qualities of helminth eggs and he reported their survival rates in detail. He further reported that primary settling did not remove hookworm and *Ascaris* (Table 5). Studies also revealed that helminth ova may pass through trickling filters and that they would survive activated sludge treatment irrespective of the efficiency of the filter.

Table 3. Presence of Helminthes Ova in 75 Samples of Sewage Sludge

Sr. No.	Source of samples	No. of samples examined	No. of samples positive	Helminthes ova encountered: Ascaris	*Ascaris* & *Trichuris*	*Ascaris, Trichuris* & *Hymenolepis*
1	Raw sewage	2	1	1	--	--
2	Primary settling tank	14	3	2	1	--
3	Secondary settling tank	9	1	-	1	--
4	Activated sludge return	4	1	1	--	--
5	Primary digester	7	3	2	1	--
6	Secondary digester	9	5	4	1	--
7	Digester	7	1	1	--	--
8	Dry bed	17	12	10	1	1
9	Imhoff tank	5	0	--	--	--
10	Wet well sump	1	0	--	--	--
	Total	75	27	21	5	1

-- : absent

Table 4. Positive Findings of Helminthes Ova in Various Types of Sewage Sludge from Different Sewage Treatment Processes

Sr. No.	Origin of sludge sample	*Ascaris*	*Trichuris*	*Hymenolepis*	Hook worm	Length of time in drying bed
1	Raw sewage	+				2 samples & 13, 62 days respectively
	Activated Sludge Return	+				
	Digester	+				
	Drying Bed	+				
2	Secondary Digester	+				
3	Primary Settling Tank	+				
	Drying bed	+	+			4 days
	Drying Bed	+				6 weeks
4	Primary Settling Tank	+	+		?	
	Primary Settling Tank	+				
	Secondary Settling Tank	+	+		?	
	Primary Digester	+				
	Primary Digester	+	+			
	Secondary digester	+	+			
	Drying Bed	+				4 to 6 weeks
5	Secondary Digester (Two samples)	+				
	Drying Bed	+				3 samples 2, 2, 22 day 4 hrs.
	Drying Bed	+				
6	Secondary Digester	+				
7	Primary Digester	+	+	+		3 samples 1 sample 55 days, others unknown
	Drying Bed	+				

The complete treatment of sewage (primary & secondary treatment without chlorination) if properly carried out, would appear to provide a high degree of removal of helminth eggs. In contrast, primary treatment alone allows a large percentage of eggs to pass through. Tertiary treatment of secondary effluent by filtration, land treatment or lagooning will also remove the remaining hookworm eggs. Final effluent chlorination does not have much effect on hookworm and *Ascaris* eggs (Cram 1943).

Table 5. Comparative Counts of Helminthes Ova during Primary Settling

Inoculum - no. of ova of *Ascaris lumbricoides* - 1, 500,000				Inoculum - no. of ova Hookworms – 50,000 *Toxascaris* – 35,000				
	Count (no. of ova)				Count (no. of ova)			
Level Time	Top	¼ down	Bottom	Level Time	Top Hook-worms	1/3 down Hook-worms	Bottom Hook-worms	*Toxas-caris*
At start	300	208	220	10 min	30		252	918
15 min	10	0	1261	20 min	27		280	540
30 min	0	0	0	30 min	51		396	210
1 hr	0	0	5040	45 min	8			
1 ½ hr	0	0	406	1hr	0		25	
1 ¾ hr	0	0	48	1 1/4hr	2			
2 hr	0	0	24	1 ½ hr	3			
2 ¼ hr	0	0	104	2 hr	0		5	
2 ½ hr	0	0	0	2 ½ hr	3		3	
2 ¾ hr	0	0	22	3 hr	0		0	

Large number of hookworm, *Ascaris* eggs enter the sewage treatment plants in endemic areas. The fate of hookworm and *Ascaris* eggs during sewage treatment is similar. The major difference being that hookworm eggs are less dense and that they may hatch. Specific gravity of hookworm is 1.055 and that of *Ascaris* is 1.11. Hence hookworm eggs have a lower settling velocity and thus are less prone to removal by sedimentation process (Cram 1943). Sedimentation is the main mechanism of removal of helminth eggs during sewage treatment and therefore the reported removals for hookworm eggs are typically a little lower than those of *Ascaris* eggs.

Unlike Ascaris eggs, hookworm eggs may hatch during sewage treatment. Cram 1943 observed the hatching of hookworm eggs on trickling filter stone, in activated sludge tanks and in drying sludge. Larvae will tend to stay in the liquid fraction and are carried along in the effluent. But generally the eggs are concentrated in the sludge. The larvae may survive in sewage treatment process for upto 5 days, which is quite sufficient for them to be discharged in the final effluent. Removal hookworm eggs by sewage treatment is generally quoted as percentage reduction in concentration between influent and effluent. These obscure the fact that most eggs not found in effluent have not been destroyed but are merely concentrated in the sludge. Conventional sewage treatment plants have short detention times and operate at an ambient temperature and therefore do not destroy hookworm eggs. Their role is to transfer eggs from liquid fraction (the effluent) to the solid fraction (the sludge).

Newton *et al* 1949 studied the effects of various pilot plant treatment processes on the eggs of *T. saginata*. The results indicated that sedimentation at moderate overflow rates of 1 to 2 hours would remove the majority of the eggs but significant percentage of eggs still would appear in the effluent. In two experiments, 30% and 38% of the eggs passed through a trickling filter operated at slow rates. Activated sludge treatment of sewage showed little effect on the eggs of helminthic parasites except a marginal reduction in numbers in the effluent, indicating that there was no noticeable effect upon the eggs after five months of operation.

In sand filtration process at a rate of one mgd, complete (100 percent) removal of helminth eggs were achieved in three out of four experiments and a removal efficiency of 99.6% were observed in the fourth experiment. This is a positive indication that sand filtration technique would be an effective method for the removal of *Taenia saginata* eggs from primary effluents. Thus sewage treatment processes ordinarily employed with the exception of sand filtration probably are not efficient in the removal of helminth ova (Newton *et al.* 1949). Silverman and Griffiths 1955 have critically reviewed the different sewage disposal methods with special reference to epizoology and *Cysticercus bovis*.

Bhaskaran *et al.* 1956 carried out studies on the effects of sewage treatment processes on the survival of intestinal parasites. They have reported that while septic tanks removed about 70 percent of *Ascaris* and hookworm eggs, the trickling filter and activated sludge plant removed 81 to 100% and over90% respectively (Table 6). Dozanska and Ivanczuk 1958 have reported influence of different sewage treatment methods on the destruction of worm eggs

Table 6. Removal of Helminthes by Conventional Treatment Process

Sr. No.	Treatment process	Hours of settling	Percentage removal	
			Hookworm	Ascaris
1	Sedimentation	1.5	46	67
		2.0	75	75
2	Trickling filter	--	100	100
3	Activated sludge			
	Unit I	--	81	--
	Unit II	--	96	--

Kabler 1959 has detailed the removal of pathogenic organisms by sewage treatment processes. He has discussed different treatment processes like trickling filter, activated sludge, anaerobic digestion, chlorination and sedimentation ponds (Table 7). Trickling filter was effective against most of the parasites; activated sludge was effective only against *Ascaris*, hookworm and *Trichuris* while anaerobic digestion destroyed the eggs of tapeworm, ova, *E. histolytica*, cysts, *Schistosoma japonivum*, *Ascaris* and *A. lumbricoides*. Chlorination was not effective.

Table 7. Percentage of Organisms Removal by Different Methods

Organisms studied	Trickling filter	Activated sludge	Anaerobic digestion	Chlorina-tion	Stabilization pond	Remark
Tapeworm ova	18-26	--	97	No effect	--	Air drying for 2 yrs removed
E. histolytica cyst	88-99	No reduction	Removed	--	--	--
Ascaris lumbricoides	70-76	Does not affect viability	Not effective	--	--	Viability not reduced after 3 months. Viability after 64 days at 20^{0}C
Ancylostoma canninum (dog hookworm)	70-76	Do	Not effective	--	--	Viability not reduced after 3 months. Viability after 64 days at 20^{0}C
Toxascaris leonina ova (dog ascaris)	70-76	Do	Not effective	--	--	Viability not reduced after 3 months. Viability after 64 days at 20^{0}C
Tapeworm ova	--	Not removed	--	--	--	--
Schistosoma japonicum	Reduced	Excellent hatching medium	90 in 25 to 30 days	--	--	--
S. japonicum ova Miracidia	--	--	--	Killed	--	30 min residual of 3.9 – 11 mg/l
S. mansoni Miracidia	--	--	--	--	--	30 min residual of 0.2 to 4 mg/l
A. lumbricoides	--	--	Reduced	--	--	Removed by I hr settling
T. saginata	62-70	Little effect	Very slow	--	--	Normal eggs recovered after 6 months digestion at 75^{0}C – 85^{0}C
Ascaris	99.8	93-98	45	--	--	In 105 days at 80^{0}F to 92^{0}F
Hookworm	100	81.5-96	--	--	--	--
Trichuris	--	91.8-100	--	--	--	--

Studies were carried out in Puerto Rico to compare the efficiency of three types of sewage treatment processes in removing eggs of *Schistosoma mansoni and Ascaris lumbricoides* (Rowan 1964). The results revealed that primary sedimentation and decantation methods removed 35 - 75% of the *Ascaris* eggs and 83% of *Schistosoma* eggs, whereas secondary treatment by biological filtration or activated sludge process removed 94.7- 99.8% and 97.0-100% of the *Ascaris* eggs respectively and 99.7% of *Schistosoma* eggs. Waste stabilization ponds were also used for helminth removal and were able to eliminate hookworm eggs completely and reliably. Eggs of hookworm hatch in aerobic waste stabilization ponds and larvae were observed in the effluent (Lakshminarayana *et al.* 1969).

Panicker and Krishnamoorthy 1978 reported hookworm and *Ascaris* removal by a variety of sewage treatment plants in India. Removal rates of hookworms were consistently less than those of *Ascaris* and this was almost certainly due to the lower specific gravity of hookworm eggs and the resultant poorer removal by sedimentation (Table 8). Arundel and Adolph 1980 are of the opinion that modern sewage treatment methods like activated sludge and trickling filter do not remove the eggs of *Taenia saginata* from the final effluent. While helminth eggs because of their size and density are removed by sedimentation during primary treatment of wastewater and are concentrated in sludge (Dean and Lund 1981). Lagooning is slightly less efficient than either of digestion systems, but offers an inexpensive solution to the solution to the problem of sewage disinfection of parasitic eggs. Zhao XiHui 1985 has discussed the survival of parasitic pathogens in biogas fermentation process. At a retention time of 30 days, when the digested slurry is in a static state, about 95% of parasitic ova are distributed in the sediments and in the scum. The Table 9 gives details of the survival time of pathogens in biogas digester.

Table 8. Reduction of Helminthes Ova by Sewage Treatment Process in India

Sr. No.	Process		Helminthes			
		Hook-worm	*Ascaris*	*Hymenolepis*	*Trichuris*	*Taenia*
1	Two hour sedimentation	80	96	90	90	75
2	Complete activated sludge plant	85	98	95	100	ND
3	Two complete trickling filter plants	82	95	80	93	ND
4	Pilot scale biodisc plant with one hour of secondary sedimentation	50	79	60	60	ND
5	Pilot scale oxidation ditch with secondary sedimentation	81	94	89	100	100
6	Pilot plant aerated lagoon without secondary sedimentation	70	92	78	100	100
7	Four waste stabilization systems 1	93	100	100	100	100
	2	88	100	100	100	100
	3	100	100	100	100	100
	4	100	100	100	100	100

Table 9. Survival Time of Pathogens in Biogas Digester

Pathogens	Thermophilic fermentation 50-55^{0}C fatality		Mesophilic fermentation 35-37^{0}C fatality		Ambient fermentation 8-25^{0}C fatality	
	Days	Rate (%)	Days	Rate (%)	Days	Rate (%)
Sallmonella	1-2	100	7	100	44	100
Shigella	1	100	5	100	30	100
Poliovirus			9	100		
Colities	2	10^{-1}-10^{-2}	21	10^{-4}	40-60	10^{-5}-10^{-4}
Schistoma ova	Several hours	100	7	100	7-22	100
Hookworm ova	1	100	10	100	30	90
Ascaris ova	2	100	36	98.8	100	53

Impact of wastewater treatment processes has been reported by Schwartzbrod *et al.* 1989. Different processes like activated sludge, lagoon treatment and sand filtration have been investigated for the removal of helminthes (Tables 10 and 11). Activated sludge treatment was carried out by primary decantation, followed by the biological treatment with activated sludge. After anaerobic digestion (15 to 20 days at 35^{0}C) and conditioning with lime and ferric chloride and then the sludge is partially dehydrated. Lagoon treatment was carried out in two stabilization basins in series, retention time of 5 days in each basin (total retention of 10 days) with a flowof 6 litres/day. The number of eggs recovered from the samples varied from 160 to 340 eggs/100 g wet matter depending upon the sample type. Trematode eggs completely disappeared while nematode and cestode eggs were always found. Activated sludge treatment seemed to have an effect particularly on the cestode eggs, which wee found only in low numbers. It is concluded that among the three treatments studied, lagoon treatment was most efficient in eliminating helminthes eggs from treated wastewater. Not a single helminthes egg was found in the effluent from the stabilization basins. Two treatments, activated sludge and sand filtration, reduced the number of helminthes eggs by 77.7% and 98.8% respectively in their final effluent. Most of the eggs were found in the sludge and sediments from 160 to 340 eggs in

100 g wet matter.

Table 10. Number of Helminthes Eggs in Wastewater (Raw and Treated)

Treatment process	Activated sludge Entry	Activated sludge Exit	Lagoon treatment Entry	Lagoon treatment Exit	Sand filtration Entry	Sand filtration Exit
Number of analyses	15	15	23	23	6	6
Number of helminthes eggs	9	2	18	0	840	40
TYPES OF EGGS RECOVERED						
Nematode	7	1	9	0	670	7
Cestode	2	1	9	0	140	3
Trematode	0	0	0	0	30	0
Percent reduction in parasitic load	77.7		100		98.8	

Nematode - *Ascaris, Toxascaris, Trichuris*
Cestode - *Hymenolepis, Taenia,*
Trematode - *Dirocoelium*
Helminthes eggs were found in all sludge and sediment samples

Table 11. Number of Helminthes Eggs in Sludge and Sediment

Sample types	Sludge sample at the treatment plant exit (effluent)	Sediment sample at the lagoon basin	Sludge sample after sand filtration
Number of analyses	15	23	6
Average number of helminthes eggs/100 g of wet matter	160	215	340
Types of eggs recovered			
Nematode	156	114	170
Cestode	4	101	170
Trematode	0	0	0

Finally it has been concluded that although all the three types of wastewater treatments investigated are effective in elimination of helminthes eggs, the lagoon treatment is by far the most efficient, even with very short retention time. This simple treatment procedure ensures optimal removal of parasites allowing the eventual reuse of treated effluent for agricultural purposes. Nevertheless, the contaminated sediment poses a problem, which this treatment method does not address.

Literature shows that the number of eggs recovered from wastewater varies considerably from 4 eggs/L Panicker and Krishnamoorthy 1978) to 16,000 eggs/L (Mara and Silva 1978) for ex. 62 eggs/L (Forester and Engelbrecht 1974), 360 eggs/L (Bradely and Hadiday 1981), 10 eggs/L (Kabrick and Jewell 1982) and 1440 eggs/100g (Arther *et al.* 1981).

Gaspard *et al.* 1997 observed the parasites in 99 samples including urban sludges (89), lagoon sediments (3), and composts (7). The results expressed per 100g of dry matter showed a high proportion (47%) of samples presenting concentrations lower than 60 eggs. Thirty eight percent of the samplers were with higher concentrations ranging from 60 to 240 eggs; only 15% of the analysis results indicated concentrations higher than 240 eggs, with a maximum of 898 eggs. In lagooning sediments, the concentration observed ranged from 56 to 569 eggs and the analysis of compost samples yielded average concentration of 40.8 eggs. When whole study is taken into account nematode eggs (*Toxacara, Ascaris, Capillaria, Trichuris, Ascaridia, Enterobins*) are mostly represented with 93.2%

whereas cestode eggs (*Taenia, Hymenolepis*) are detected only in a proportion of 6.8%. The study of lime treatment impact on 10 treatment plants showed a decrease in helminthes eggs concentration in seven samples and no difference for the three others. For the egg viability, sludge from all types of treatments (mesophilic, anaerobic and aerobic digestion, composting, liming) contained viable eggs in concentrations higher than or equal to 10 eggs per 100 g of dry matter.

Aulicino *et al.* 1998 reported the presence of helminthes eggs only form 3 out of 27 total samples. Jimenez and Chavez 1998 described the application of advanced primary treatment system (APT) to remove helminthes eggs and faecal coliforms from domestic wastewater in order to obtain an effluent suitable for agricultural reuse. The system comprises a coagulation-flocculation unit with a high rate sedimentation tank and a sludge blanket (Densadeg) along with a system of sand filtration and chlorine disinfection. It was found thatDensadeg reduced the helminthes eggs (HE) from 22.6 HE L^{-1} to 1.2 HE L^{-1} with hydraulic load of 1440 $M^3.M^{-2}.d^{-1}$ (60 mh^{-1}) in the sedimentation tank. It is necessary to filter the effluent and apply 17 mg L^{-1} of chlorine with a contact time of 1 hour.

Treatment of Parasite Laden Sludge

Little work has been done on the sludge associated organisms, but available information indicates that there are numerous different parasites in many stages of their life cycle found in sewage sludges. Sewage treatment processes which include period of sedimentation prior to biological treatment are likely to produce sludges in which the eggs of parasities have been concentrated. When this sludge is applied on pastures, these parasitic eggs remain dormant for considerable length of time. As favourable conditions return they become health hazards. To reduce the health risks to animals and humans, treatment of sludge prior to their disposal on land is desirable. Many different treatment systems are effective in reducing parasitic viability to some extent.

Golueke and Gotaas 1954 have evaluated health aspects of aerobic composting in detail. To reduce health risk of helminth parasites to animals, treatment of sludges from sewage treatment processes is subjected to further treatment by :

(1) Composting
(2) Aerobic digestion, and
(3) Anaerobic digestion.

Aerobic/anaerobic digestion may be followed by lagooning. Sludge digestion will reduce number of parasitic helminth eggs. Of course, the destruction depends on the temperature. Little information exists on the efficacy of composting or liquid sludge storage (lagooning) in reducing the viability of tapeworm eggs.

Composting

The most practical method of parasites elimination from sewage sludges is suggested as composting where higher temperatures needed to destroy pathogens are achieved. In aerobic composting, microorganisms utilize oxygen and feed upon the organic matter, in this process a great deal of energy is evolved in the form of heat. When composting is done by pilling the wastes the inner temperature may go as high as 75°C during fermentation. Destruction of parasitic helminthes during composting solely depends on temperature (Gotas 1956, Forestner 1971, Their *et al.* 1978). Biological antagonism in composting probably may also effect the destruction of pathogenic organisms and parasites (Wiley 1962).

Composting of night soil or sludge with city garbage and woodchips or other suitable carbonaceous bulking material is highly effective in removal of hookworm eggs and larvae. Studies on hookworm elimination by composting have been reported from China (Oldt, 1926, Hou *et al.* 1959), Srilanka (Nicholls and Gunawardana 1939) and the USSR (Gudzhabidze and Lyubchenko 1959). *Ascaris* being very sturdy, it is preferable to monitor *Ascaris* eggs in the final compost.

Bhoyar 1990 has reported a detail study on the fate of intestinal parasites during aerobic and anaerobic composting. Both laboratory and field surveys were carried out and the results obtained are shown in Table 12. He studied windrow method of aerobic composting. Samples from both surface and central core revealed good destruction of parasites (Table 13).

Anaerobic composting is generally carried out in pits. It is reported that the viability of ova is more in anaerobic composting than in aerobic composting. The work on anaerobic composting by Bhoyar 1990 indicates that even after 120 days of composting, the helminthes ova present in the sludge were viable. Only after 180 days of composting, they become non-viable (Table 14).

Table 12. Aerobic Composting at Different Temperatures and Effect on Ova Viability

Composting time	Composting temperature, ^{0}C					
	30		40		50	
	Ascaris	*Trichuris*	*Ascaris*	*Trichuris*	*Ascaris*	*Trichuris*
0	60.17	54.29	31.81	28.64	42.2	38.0
10	57.56	53.70	5.36	*	*	*
20	56.31	51.64	*	*	*	*
30	56.12	49.99	*	*	*	*
60	53.64	49.99	*	*	*	*

All values are expressed as percent viability
* Nil viability

Table 13. Parasitological Analysis of Field Composting

Composting time in days	Temperature Surface	Temperature Centre	*Ascaris* ova (% viability) Surface	*Ascaris* ova (% viability) Center
0	69.0	72.66	70	--
7	58.0	62.00	55	0
14	48.5	60.00	19	0
21	44.5	49.50	5	0
28	30.0	35.50	0	0
35	30.0	30.00	0	0
50	28.0	28.00	0	0
60	28.0	28.00	0	0

Ambient temperature: 28^{0}C – 30^{0}C
Viability of *Ascaris* at the start of experiment was 78%

Table 14. Aerobic Composting (Pit Composting)

Composting time (days)	Temperature (^{0}C)	Viable ova (%)
0	28	40
1	30	
2	32	
3	35	
4	35	
5	40	
30	30	Ova viable
90	30	Ova viable
120	30	Ova viable
180	30	Ova non viable

Aerobic Sludge Digestion

Cram 1943 reported that *Ascaris* and hookworm ova survive the digestion and subsequent drying of sludge. Silverman and Griffiths 1955 recommended that sludge might be allowed to dry for one year to kill *T. saginata* eggs. Reports are there that heating of sludge to 103 °C for 3 minutes destroyed 100% of *Ascaris* and hookworm eggs. Kabrick and Jewell 1982 reported that the removal of various pathogens in thermophilic aerobic digestion was superior to anaerobic digestion. Carrington 1980, however, suggested that cold storage of wet sludge has little effect on the viability of eggs.

The effects of mesophilic anaerobic or aerobic sludge digestion on survival of eggs from roundworm *Ascaris suum, Toxacara canis, Trichuris vulpis* and *Trichuris snis* and from rat tapeworm *Hymenolepis dimenuta* were reported (Black *et al.* 1982). In this study destruction of eggs throughout a 15-day treatment period as well as their viability after reisolation was analysed. Studies were carried out on laboratory model digesters at 15 days HRT. *Ascaris & Trichuris* eggs were destroyed in aerobic digester as 38% and 11% respectively.

There have been reports on the effects of sewage sludge stabilization processes on parasite egg survival (Black *et al.* 1982, O' Donnel *et al.* 1984). These studies were largely concentrated upon parasitic nematodes and have been provided clear relationship between eggs, survival and treatment, especially the importance of the temperature of storage and digestion.

Storey 1987 reported the survival of tapeworm eggs, free and enclosed in proglottids during the treatment of simulated sewage sludge. In this study, the effect of various digestion processes and wet sludge storage has been tried. It has also been reported that on storing the liquid sludge at different temperatures of 4°C, ambient and 35 °C, the eggs were still viable in the proglottids upto 43%, 18% and 4% respectively at the end of the experiment. Destruction of eggs in digesters was correlated with the state of eggs before subjection to the treatment processes i.e. some *Ascaris* and *Trichuris* eggs were already embroyonated in the host intestinal contents or faeces and hence passed their most resistant stage. The viability of *Ascaris* and *Toxacara* eggs that survived the digestion processes were greater in anaerobically treated than in aerobically treated material. Eggs from *Hymenolepis dimunuta* were non-viable before use in the experiments. However, their removal was more in aerobic digestion than in anaerobic digestion. Storey 1987 reported a 100% removal of parasitic helminth eggs at 55°C in aerobic digestion system.

Anaerobic Sludge Digestion

In the treatment of wastewater for hygienic disposal, a portion of the solids, which is removed and require further processing, is highly organic and biodegradable in nature. The segregation of suchsolids along with other solids produced in the treatment is subjected to separate fermentation step in an anaerobic digester for further stabilization. This process is termed as "Anaerobic sludge digestion"

As early as the eighteenth century, the formation ofmethane from anaerobic decomposition of organic sludges was known. However, it was just one century ago (1881) when anaerobic treatment was reported to be a useful method for reducing the solids bulk and putriscible nature of suspended organic material removed from municipal wastewater. Since then the applications of anaerobic treatment have grown steadily, as has knowledge about the chemistry and microbiology involved.

Since the seventies, more attention has been paid to pollution control and human environment. In every country, nightsoil and sewage disposal is being tackled as high-level priority problem to improve sanitary situation and its multifunctional benefits. Sludge digestion is a very common treatment technology used for the sewage sludge, excreta and cattle dung. In recent times, sludge digestion by biodigesters is being advocated in general for organic sludge disposal. In India, Khadi Village Industries Commission has taken up this problem and is helping rural as well as urban population in erecting biogas digesters.

Of course, sludge digestion provides valuable methane as fuel butat the same time partially or improper digestion of sludge leads to spreading of many diseases including helminthic parasite infection. These health hazards may be assessed on the basis of available information on the occurrence and survival of pathogenic organisms in raw sewage digesters and in sludge digesters and in sludge used on the agricultural fields. The hazard in the use of sewage sludge comes from contact of the sludge with the edible portion of the crops and vegetables, which are eaten raw. Inspite of the survival of some pathogens and parasites, the literature documents no disease outbreaks associated with the use of digested sewage sludges or nightsoil and animal wastes in crop production.

The inhabitants of most rural communities or villages in developing countries are probably already exposed to the enteric diseases endemic to their area. The introduction of anaerobic sludge digestion for sewage sludge, night soil, organic wastes and agricultural residues, therefore, should not create any new or additional health hazard, on the contrary it should reduce the present health hazards significantly. The literature available on survival of pathogenic microorganisms in the anaerobic digestion process has a wide range of values. Survival data for some of the more important enteric pathogens are shown in Table 15 (Methane Fermentation). These examples, together with other data available demonstrate the importance of the anaerobic sludge digestion process in the treatment of sewage sludge and nightsoil. With few exceptions, pathogenic enteric microorganisms are effectively killed off if the digestion time is not significantly shorter than 14 days at a temperature not significantly lower than 35°C.

Table 15. Dieoff of Enteric Microorganisms in Public Health Significance during Anaerobic Digestion

Organisms	Tempera-ture (°C)	Residence time (days)	Dieoff (%)
Poliovirus	15	2	98.5
Salmonella sp.	22-37	6-20	82-96
Salmonella typhosa	22037	6	99
Mycobacterium tuberculosis	30	Not reported	100
Ascaris	29	15	90
Parasitic cysts	30	10	100

a : time indicated is time of digestion
b : does include ascaris

Ascaris eggs are the major exceptions to the pathogenic microorganisms effectively killed during anaerobic digestion while other encysted helminths are completely destroyed. *Ascaris* cysts are able to survive even after 14 days of anaerobic fermentation at 35 °C. Another study reported that thermophilic digestion destroyed the Ascaris *eggs* completely. However, sludge from mesophilic digestion required additional storage and drying for about 6 months to destroy the *Ascaris* eggs completely (Wright *et al.* 1942). Nevertheless, since the die off rate is expected to be sufficiently high say 90%, anaerobic digestion of organic material for biogas production provides a public health benefit beyond that of any other treatment likely to be in use in rural areas of developing countries.

Hookworm eggs did not develop in digesting sludge but that they could survive for upto 64 days at 20 °C and 41 days at 30 °C (Cram 1943). He reported that during anaerobic digestion even though the development of eggs were stopped, few eggs got embryonated probably because they were brought to the top layer by gas bubbles. Digestion for three months hardly affected the viability of eggs whereas digestion for six months destroyed about 10% of the viable eggs. He also reported that eggs of *Ascaris* and hookworm were found to be extremely resistant to digestion and viable even after digestion of the sludge for a period of one year.

Newton *et al.* 1949 reported a 50% mortality of *T. saginata* eggs after 60 days digestion at 24-29.5 °C and 85- 90% after 200 days, It was assumed without much experimental study that digestion of raw sludge and subsequent drying of the digested sewage sludge could destroy all viable helminthes ova present in the raw sludge. But later studies revealed that large percentage of viable ova escape digestion process as well as sludge drying. It was observed by Hoggs 1950 that dehydration even at moisture content as low as 5.5% did not destroy the viable eggs completely.

Petrik 1954 reports that sludge digestion at temperatures below 40 °C does not eliminate hookworm eggs. Silverman and Guiver 1960 achieved a 100% kill in 5 days at 35°C but only 40-50% kill after 20 days at 4 °C whereas Menchel 1963 found that *T. saginata* eggs survived aerobic digestion at 26 - 28° C for only 56 days.

The hookworm infections are very common to many tropical and semitropicaldeveloping countries. Studies on the survival of *Ancylostoma duodenalee* in septic tanks suggested that a few viable hookworm eggs were recovered in the septic tank effluents, although high removal rates were achieved (90%) (Majumder et al. 1960). Since biogas plants will similarly carry out process, the risk of indiscriminate dissemination ofeggs of parasitic organism is minimized. Storage and drying after the digestion period will further minimize and perhaps eliminate the risk of parasitic eggs spreading in rural environment of developing countries.

Reyes *et al.* 1963 conducted aerobic and anaerobic digestion of nightsoil and observed that at low temperatures of 25 °C to 35 °C of digestion, eggs were found viable and even developed when transferred tofavourable conditions in both cases. When the temperature was maintained at or over 38 °C for anaerobic digestion and 45^0 C for aerobic digestion, it was observed that nightsoil could be well digested and eggs were destroyed within 20 days in the aerobic digestors and within 30 days in the anaerobic digesters. Forstner 1970 reported that *T. saginata* eggs survived upto 26 days digestion at 30 °C. Elevated temperature of >50°C in the digesters has been reported to result in rapid death of helminth eggs (Silverman 1955, Silverman andGuvier 1960, Smith *et al.* 1975, Owen and Crewe 1982).

McGarry and Stainforth 1978 reported studies in China on a biogas plant which may be likened to septic tank with a long retention time. The influent contained hookworm eggs in 87% of samples with an average concentration of 840 eggs per litre, the effluent contains hookworm eggs in 23% samples with an average concentration of 4 eggs per litre.

Anaerobic digestion will destroy the eggs of *Taenia saginata* with continuous digestion being more effective than batch processing. This may be because of the continuous feed, which ensures a renewable carbon source for microbes. Continuous digesters also have a greater proportion of active digesting sludge and consequently a higher enzymatic activity than batch digesters. Eggs survived for longer periods in aerobic than in anaerobic digestion. This conflicts with the findings of Leftwich *et al.* 1981 on the survival of *Ascaris* and *Toxacara* at 35^0 C in aerobic and anaerobic digester. All the sludge digestion processes generally will reduce the number of viable helminth eggs, their efficiency being clearly dependent upon the length of treatment and exposure temperature with apparently higher death rate above 35^0C.

Anaerobic sludge digestion has been reported to have destroyed only 23% of *Ascaris* eggs, whereas *Trichuris* eggs in anaerobic digesters and *Toxocara* eggs in either anaerobic or aerobic digestion were not destroyed (Black *et al.,* 1982). Pike *et al.* 1983 found similar death rates at 4^0 C and 39^0 C, however these experiments were performed in saline medium where microbial activity would be expected to be low. Pike*et al.* (1983, 1988) have carried out detailed experiments to study the inactivation of ova of the parasites *T. saginata, Ascaris suum* during heated anaerobic digestion (Table 16). They reported that mesophilic anaerobic digestion at 35^0 C has shown to reduce viability of *A. suum* ova recovered by only 35-50%, but after thermophilic digestion at 49^0 C viability was reduced by atleast 99%. However, atleast 50 to 75% of the ova added could not be recovered after digestion and may have autolysed. To determine the death rate for these ova, at the temperatures of 50 °C to 60 °C and subsequent mesophilic digestion, needs further studies.

A 0.4 m model steel biodigester was used for studying the survival of *Ascaris* ova and other entero pathogens. A system of three electric heat tubes, mercury thermostats and transistor relays were used to control the temperature of the digesters by Hubei Sanitation and Anti epidemic Station (Shanta 1989). The results reported are given in Table 17.

Shanta 1989 has reported a detailed study on the fate of *Ancylostoma duodenale* and *Ascaris lumbricoides* during mesophilic and thermophilic anaerobic digestion. Work on both laboratory and pilot plant scale studies at mesophilic range at different HRTs and organic loadings have been reported (Tables 18 and 19). A single laboratory digester was operated for a period of one month at a constant temperature of 50 °C and was inoculated with known numbers of viable eggs of *Ascaris* and hookworm eggs. After every 5 minutes, the samples were withdrawn and studied for the survival of eggs till no viable eggs were found. On the basis of available information, it seems clear that using the sludge from heated digesters and thermophilic digesters as fertilizer will not pose any health risk, as complete elimination of helminth parasites can be achieved in the anaerobic thermophilic process. The results of thermophilic laboratory digesters (Table 20) revealed a 100% removal of *Ancylostoma duodenale* after 15 minutes and 100 % removal of *Ascaris lumbricoides* after 45 minutes (Shanta 1989).

Anaerobic digestion of sewage sludge alongwith refuse and the fate of *Ascaris* and *Trichuris* has been reported by Bhoyar 1990. He reported that within 60 days period of digestion, reduction in viability of *Ascaris* ova was 48.15%. Whereas in batch-fed digesters, where only sludge was used, 43.79% reduction in *Ascaris* viability was noted. In case of *Trichuris*, 43.79% reduction in *Ascaris* viability during batch digestion of sludge & reuse and 48.35% reduction with only sludge was reported (Table 21). A slightly modified table of the results reported by Bhoyar on the continuous fed digester is shown in Table 22.

Table 17. The Survival Rate of *Ascaris* Ova in Biodigesters

Temp. (^{0}C)		Days										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**10**	**12**
51±1	N	800	800	800	800	600	333					
	R	76.1	0.5	0	0	0	0					
48±1	N	800	800	578	800	600	218	800				
	R	90.8	79.3	1.3	0	0	0	0				
43±1	N	800	--	835	--	400	--	800	800	800	800	
	R	82.6	--	96.8	--	88.0	--	86.6	90.7	58.5	58.2	

N : Number of ova examined R : Survival rate (%)

Table 18. Percent Reduction of Helminth Ova in Lab.Nightsoil Digester

Detent-ion time (days)	Influent No. of ova/lit		Effluent No. of ova/lit		% Reduction	
	Ascaris	Hook-worm	*Ascaris*	Hook-worm	*Ascaris*	Hookworm
20	4000	58200	2080	21360	48	63.2
25	11400	18200	38324	1693	66.5	90.6
30	94110	21820	28248	1528	70	93

Table 19. Removal of Helminthes Ova in 18 m^3 Capacity Pilot Digester

Loading (Kg VS/m3.d)	% Removal	
	Hookworm	*Ascaris*
1.99	66±23.18	37.0±22.61
1.60	70±12.11	52.1±16.51
1.79	65.3	35.3±22.7

Table 20. Survival of Helminthes Ova at Thermophilic Lab. Digesters

Time (minutes)	% Removal	
	Ascaris	Hookworm
5	25	80
10	45	86
15	58	100
20	79	
25	83	
30	96	
35	100	
40	100	
45	100	

Table 21: *Ascaris* and *Trichuris* Ova Viability During Anaerobic Digestion (Batch Experiment)

Temperature (^{0}C)	Parasite	Period of digestion (days)				
		0	15	30	45	60
30^{0}CReuse+ sludge	**Ascaris**	66.66	57.91	55.06	27.30	18.51
	Trichuris	58.33	50.00	46.4	19.76	14.54
30^{0}C sludge	*Ascaris*	60.51	52.77	43.71	31.66	15.49
	Trichuris	58.94	51.79	40.44	32.75	10.59
40^{0}C sludge	*Ascaris*	31.88	0.0	0.0	0.0	0.0
	Trichuris	28.91	0.0	0.0	0.0	0.0
50^{0}C sludge	*Ascaris*	62.05	0.0	0.0	0.0	0.0
	Trichuris	46.42	0.0	0.0	0.0	0.0

Table 22: Ova Viability in Continuous Fed Anaerobic Digester

Detention time		Ova concentration / litre Influent		Effluent		Ova concentration in bottom sediment
		Ova concen-tration.	% viability	Ova concen-tration	% viability	
Mesophilic 16 days	17	400546	46-50	540	42.85	
	18	--	--	560	40.32	
	19			480	38.24	
	20			320	45.45	100-1509 (28% viable)
	21			480	41.30	
	22			580	40.00	
	23			240	42.50	
25 days	26			685	30.12	
	27			274	35.36	
	28			548	30.00	
	29			137	45.00	90-150 (30% viable)
	30			137	42.65	
	31			274	41.32	
	32			548	40.54	
Thermop-hilic 50^{0}C 16 days	17			60	5.00	
	18			66	8.25	
	19			90	10.00	
	20			105	15.00	150-300 (5-10%viable)
	21			110	15.5	
	22			95	20.0	
	23			105	20.25	

Conclusions

Occurrence of common nematode parasites of human intestine in sewage and sludge from wastewater treatment plant is extremely significant from public health point of view. As the dormant stages of these parasites and eggs are resistant, the conventional methods needs to be modified to increase their efficiency, not only in accumulating them in sludges of wastewater treatment plant but to kill them effectively by further treatment of sludge. Advanced primary treatment system has been advocated by some to kill the helminth parasites from wastewater. Similar such studies are required to be carried out to eradicate the public health problems. Aerobic/anaerobic digesters may also be used for the same purpose. Presently, the effluent standard with respect to presence of helminthes for agricultural reuse of wastewater is available. However, the standard for reuse of digested sludge as fertilizer is not available. Efforts are needed in the direction in evolving standard for the treated sludge so as to ensure safe reuse of digested sludge as fertilizer for enhancing the production of food crops.

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