London School of Hygiene & Tropical Medicine

Sanitation Ventures Literature Review: on-site sanitation waste characteristics

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1. Composition of Faecal Matter.

1.1 Characteristics and composition of human faeces.

There is relatively little information available in the scientific literature concerning the composition of human faeces. Normal human stools consist of roughly 70-80% water[1] and around 20-30% solid matter[2], though the water content of faeces is dependent on dietary intake and digestive function [3-5]. The majority (84%) of the solid matter in faeces is organic in nature [6], and is mainly composed of bacteria (≈55%) and residual dietary fibre (17%)[7]. If bacteria are 80% water, 55% of the dry weight becomes an even larger proportion of the wet stools, about 75%[7]. Faecal bacteria composition had been studied previously by other groups[8] who reported a figure of 30% for the bacterial component of faecal solids. However in these earlier studies the quantification was done with less precise techniques and the bacterial component was probably underestimated.

Faeces consist not only of residual food but also of more complex substances derived from distinct endogenous sources: secretions of the stomach, pancreas, liver and intestine make important contributions. Therefore bile acids, small amounts of pancreatic juice, hydrobilirubin, stercobilin, lecithin, cholesterol and some decomposed bile salts may all be found in association with dead epithelial cells from the mucous membrane[9].

Modelling studies carried out by Zavala[6] to characterise faeces and to describe their biodegradability showed that in terms of chemical oxygen demand (COD, ie organic matter) 80% of human faeces are made up of slowly biodegradable organic matter and the other 20% is biologically inert material. Some definitions of parameters widely used to characterise faecal matter are given in the box below:

Total solids (TS): *material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids include:* ***"total suspended solids (TSS)"*** *(the portion of total solids retained by a filter, and* ***"total dissolved solids (TDS)"*** *(the portion that passes through the filter. "****Volatile Solids (VS)"****Is a measure of the organic matter present, as determined by the loss of weight on ignition at 550^{0}C. The remaining material which is not volatilised by this procedure constitutes the inorganic matter in the sample.*
Chemical oxygen demand (COD): *is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. COD_{total} include: $COD_{soluble}$ and $COD_{particulate}$.*

The composition of human excreta shows a wide range of variability from person to person and from country to country. Table 1 presents a summary of different parameters characterizing human faeces and urine. These data will serve as reference for the chemical assays of pit latrine contents planned to be undertaken by this project.

Table 1. Composition and characterization of human faeces and urine[10-11].

Parameter	Faeces	Urine
Quantity(wet) per person per day(g)	70-520	1000-1500
Quantity(dry solids) per person per day(g)	30-70	50-70
Moisture content (%/gwet sample)	66-85 %	93-99 %
Total solids (%/gwet sample)	14-22%	1.3-4%
Volatile solids (%/gdry sample)	79-84%	0.4%
COD_{total} (g/l)	46.2-78.3	12.8-
$COD_{soluble}$ (g/l)	-	11.3
$COD_{particulate}$ (g/l)	-	1.5
Nitrogen ($gpe^{-1}day^{-1}$)	5.0-7.0	15-19
Total Phosphorus ($gpe^{-1}day^{-1}$)	0.7-2.5	1.1-2.2
pH	-	7.1-9
Protein (g)	4-12	0.3
Total lipids (g)	4-6	-
Polysaccharides (g)	4-10	0.7

1.2 Microbial composition of faeces.

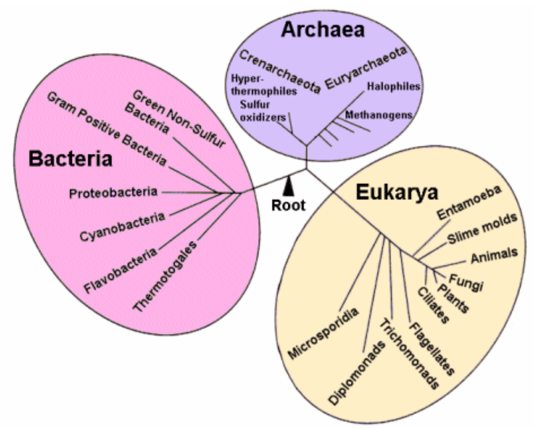
Bacteria make up a large proportion of the mass and volume of faeces. The intestinal tract is a natural methanogenic environment, where microorganisms can grow with a few simple substrates, such as formate, methanol and acetate, and they therefore depend on other microorganisms that degrade more complex organic compounds for substrate supply[12]. Microbes inhabiting the distal gut synthesize essential amino acids and vitamins and are involved in processing components of otherwise indigestible parts of our diet, such as plant polysaccharides[13]. The human gut is enriched for genes involved in starch, sucrose, glucose, galactose, fructose, arabinose, mannose and xylose metabolism[14].

> **Taxonomic Ranks**
> *In biological classification, rank is the level (the relative position) in a taxonomic hierarchy. There are 8 main taxonomic ranks: domain, kingdom, phylum, class, order, family, genus and species. The most basic rank is that of species, the next most important is genus, and then family.*

Several hundred different bacterial species are known to colonize the gut, with species belonging to the genera *Bacteroides*, *Eubacterium*, *Clostridium* and *Bifidobacterium* being among the dominant members of the faecal microflora[15-18]. The two most abundant bacterial phyla that colonize the large intestine are the gram-negative *Bacteroidetes* and the *low %G+C Firmicutes*. The most abundant *Firmicutes* are members of the *Clostridial clusters IV and XIVa* with lower abundance of the *cluster IX group*. In total, these groups may comprise up to 60% of the colonic microbiota (Table 2)[19]. *Firmicutes* are related to known butyrate-producing bacteria, and there are expected to have a prominent representation in healthy subjects as they have an important role in the maintenance and protection of the normal colonic epithelium[20].

Anaerobic bacteria outnumber aerobic bacteria by a factor of 100-1,000[21]. The genera *Bacteroides, Bifidobacterium, Eubacterium, Clostridium, Peptococcus, Peptostreptococcus* and *Ruminococcus* are more predominant in human beings[22-23], whilst the aerobes (facultative anaerobes) such as *Escherichia, Enterobacter, Enterococcus, Klebsiella, Lactobacillus,* and *Proteus* are among the subdominant genera in animals?[21]. Molecular procedures have shown that aerobes, including *E. coli, Enterococci,* and *Lactobacilli*, achieve very high densities and metabolic activity in the human caecum, since 50% of total bacteria ribosomal RNA in caecal contents correspond to these species[24]. By contrast, these species account for only 7% of bacteria ribosomal RNA in faecal samples[24].

In addition, members of the archaea family are also present, predominantly *Methanobrevibacter Smithii*[14, 25]. These belong to a different evolutionary branch of bacteria and constitute a separate domain. There appears to be much less archaeal diversity in the distal gut and faecal microbiota than bacterial diversity, which is remarkable.



Bacteria and archaea live in syntrophic communities in this anaerobic environment and this means that they need to combine their metabolic capabilities to catabolise a substrate that cannot be catabolised by either one of them alone. This results in the bacteria and archaea forming compact aggregates, and these communities exist in conditions that are close to thermodynamic equilibrium[12].

Table 2. Abundance of main colonic bacterial groups in adult human faecal samples and fermentation profiles. (Table taken from Louis et al)[19]

Bacterial group	Abundance*	Main acidic fermentation products from hexoses†
Low G+C-content gram positives		
Clostridial clusters XIVa+b	10·8–29	
Roseburia/Eubacterium rectale group	2·3–8·8	Butyrate, formate, L-lactate
Eubacterium hallii	0·6–3·8	Butyrate, formate, acetate
Ruminococcus obeum	2·5	Acetate
Lachnospira spp.	3·6	Formate, acetate, lactate, succinate
Clostridial cluster IV	25·2	
Faecalibacterium prausnitzii	3·8–15·4	Butyrate, formate, D-lactate
Ruminococcus bromii, Ruminococcus flavefaciens	1·7–10·3	Acetate, formate, lactate, succinate
Clostridial cluster IX	7·1	Propionate, various minor acids
Clostridial cluster XVI		
Eubacterium cylindroides	0·4–1·4	Butyrate, acetate, lactate, succinate, formate
Lactobacillus/Enterococcus	0·01–1·8	Lactate, acetate
High G+C-content gram positives		
Bifidobacterium spp.	2·5–4·9	L-Lactate, acetate, formate
Atopobium spp.	2·1–11·9	Acetate, formate, lactate
Gram negatives		
Bacteroides-Prevotella group	8·5–27·7	Acetate, propionate, succinate
Proteobacteria	0·1–0·2	Lactate, acetate, succinate, formate

*Percent of total bacteria (mean values of several volunteers) based on fluorescent *in situ* hybridization (FISH) data from several publications as reported by Flint (2006).

†Some groups are heterogenous and/or under-researched; therefore reported acids are just indicative of cultured representatives.

1.3 Effect of diet on faecal composition

Changes in diet result in both quantitative and qualitative changes in the supply of substrates to the large intestinal microbiota and therefore are reflected in the faeces as a result of the ease and extent of microbial conversion. Diet, especially fibre content, can also affect the transit time through the gut which will have a major effect on the final faecal composition.

Many secondary plant metabolites ingested , such as polyphenolic substances, may also reach the large intestine and are subject to bacterial transformations[19]. A proportion of undigested food can be found in faeces when ingested in great quantities, such as: elastin, tendons, uncooked starch, various phosphates and salts of the alkaline earths, and neutral fats[9].

The presence in the diet of cellulose, a key component of plant cell walls and hence dietary fibre, has a major influence on the composition of faeces. Unless the food contains cellulose, there is little or no food residue, and therefore no protein, soluble carbohydrate, nuclein, nor connective tissue found in faeces. On a concentrated cellulose-free diet, 70 % of the faeces consist of water and the other 30 % of fatty acid soaps, lecithin, neutral fat, mucin and nucleoprotein, but no protein nor starch. The ash is chiefly calcium phosphate[9].

By adding cellulose to the diet, there is not only food-residue, but because of this, an increase in other constituents of the faeces. There is a greater secretion of succus entericus (intestinal juice) because of increased peristalsis due to irritation by the cellulose and the bacterial decomposition of the carbohydrates.

Proteins are generally well absorbed when taken in the form of meat, eggs and milk, with a loss generally ranging from 5-7 % in a diet of 100 grams of protein, or from 0.8 to 1.2 grams of nitrogen. In contrast , a much larger proportion of the protein of vegetable origin remains undigested as a result of its insoluble cellulose envelope (the plant cell wall), and hence cannot be absorbed. Voit has shown that as much as 42% of the nitrogen present in a vegetarian diet was evacuated in faeces[9].

Dietary components found in faeces are those that have escaped digestion and absorption during transit through the gastrointestinal tract. Both endogenous and microbial enzymes are involved in the digestive processes, and those materials found difficult to degrade, include: resistant starch, plant cell wall material and oligosaccharides[15]. Insoluble plant cell wall material reaching the human colon consists of cellulose, hemicellulose, pectins and lignin. These complex polymers together form the plant cell wall, and are degraded by a battery of microbial hydolases, esterases and lyases. Starch degrading enzymes are grouped into different families depending on their structure and included α-amylases, type I pullulanases and amylopullalanases[26]. Fructo-oligosaccharides (FOS) are degraded by different bacterial β-fructofuranosidases[19]. A synergistic activity of several enzymes such as endo-1,4- β-xylanase, β-xylosidase, α-glucoronidase, α-L-arabinofuranosidase and acetylesterase is essential for the complete breakdown of branched xylans (components of hemicellulose)[27].

Microbial degradation in the large intestine, particularly of lignin and cellulose, is incomplete because of extensive cross-linking and their physical structure, and thus particles of plant fibre persist through to the distal bowel and are commonly found in faeces[19].

Diet can alter the microflora in the gut and thus have an impact on faeces. For example, inulin and fructo-oligosaccharides stimulate the growth of *Bifidobacteria* and *Lactobacilli*[19] and there is evidence from in vivo studies with prebiotics that changes in the supply of non-digestible carbohydrate can lead to shifts in the species composition of the colonic bacterial community, as monitored by faecal sampling[28].

Different substrates can also give rise to different products as a result of fermentation via different metabolic routes[29]. Short-chain fatty acids (SCFA), largely derived from microbial fermentation, may exceed 100 mmol l^{-1} in human faecal samples. The three major short-chain fatty acids which have been detected are acetate, propionate and butyrate[30].

In the last few years, increasingly sensitive methods of bacterial quantification have led to new insights into the composition of the human gut microbiota. However, many species remain uncultured and some remain undetected with the techniques currently used. The microbial ecosystem is highly complex and science is in the early stages of understanding the effect of diet on the composition and activity of the gut microbiota. It has been suggested that adaptations to varying substrates and environmental conditions might result in more prominent changes of activity rather than of bacterial populations and molecular techniques targeting RNA rather than DNA are now emerging to address this[19].

1.4 Influence of age on faecal composition.

The bacterial composition of human faeces can vary greatly with factors such as age and disease.

In some groups of bacteria, species diversity was found to change with age despite the overall numbers of organisms being similar at genus level. Species such as *Bifidobacteria*, which are regarded as being protective, are thought to decline in numbers, whereas *Clostridia* and *Enterobacterial* populations, which are viewed as being detrimental to health, increase[31].

Diarrhoea is a common cause of mortality and morbidity in developing countries and the second most common cause of under-five mortality worldwide. There are many pathogens associated with infectious diarrhoea, which include: many viruses, bacteria, protozoa and helminths. For most individuals, diarrhoea means an increased frequency or decreased consistency of bowel movements. This will result in loss of fluids, which may lead to dehydration and electrolyte imbalances.

In a study carried out by Krogius-Kurikka[32] of the microbial community changes between patients with diarrhoea-predominant irritable bowel syndrome (IBS) and healthy individuals, it was shown that microbial communities of IBS patients were enriched in *Proteobacteria* and *Firmicutes*, but reduced in the number of *Actinobacteria* and *Bacterroidetes* compared with control.

2. Composition of Urine

2.1 Characteristics and composition of human faeces.

Urine is a dilute aqueous solution of metabolic wastes such as urea, salts, and organic compounds. In total the dissolved material amounts to about 5% by weight. Fluid and materials being filtered by the kidneys, destined to become urine, come from the blood or interstitial fluid.

Urine is sterile until it reaches the urethra where the epithelial cells lining the urethra are colonized by facultative aerobic Gram negative rods and cocci[33]http://en.wikipedia.org/wiki/Urine - cite_note-0. Subsequent to elimination from the body, urine can acquire strong odours due to bacterial action. Most noticeably, ammonia is produced by breakdown of urea. Some diseases alter the quantity and composition of the urine, such as sugar as a consequence of diabetes.

Urine contains a range of substances that vary with diet. These can include proteins, hormones, and a wide range of metabolites. The composition of urine is described in Table 1.

3. Daily Excretion of Urine and Faeces

The amount of faeces and urine excreted daily by individuals varies considerably depending on water consumption, climate, diet and occupation. The only way to obtain an accurate determination of the amount at a particular location is direct measurement[34]. Only a limited number of studies have tried to quantify the amount of faeces produced and quantities may vary between cultures and diet (Table 3).

Table 3 Quantity of wet faeces excreted by adults (in grams per person per day).

Place	Quantity	Reference
China (men)	209	Scott (1952)
India	255	Macdonald (1952)
India	311	Tandon & Tandon (1975)
Peru (rural Indians)	325	Crofts (1975)
Uganda (villagers)	470	Burkitt et al. (1974)
Malaysia (rural)	477	Balasegaram & Burkitt (1976)
Kenya	520	Cranston & Burkitt (1975)

Even in comparatively homogeneous groups there may be a wide variation in the amount of excreta produced per person. For example, Egbunwe[35] reported a range of 500-900 g of faeces per person per day in eastern Nigeria. Generally, active adults eating a high-fibre diet and living in a rural area produce more faeces than children or elderly people living in urban areas eating a low-fibre diet. Both Shaw[36] and Pradt[36] suggested that the total amount of excreta (urine plus faeces) is about 1 litre/person/day. The amount of urine is greatly dependent on temperature and humidity, commonly ranging from 0.6 to 1.1 litres per person per day[34].

In the absence of local information the following figures are suggested as reasonable averages[34]:
- high-protein diet in a temperate climate: faeces 120 g, urine 1.2 liter/person/day.
- vegetarian diet in a tropical climate: faeces 400 g, urine 1.liter/person/day.

4. On-site sanitation

Excreta management should comprise four stages:
-Collection of excreta
-Transportation of excreta to a suitable location
-Storage and/or treatment of excreta.
-Reusing and/or returning excreta to the environment.

For a large percentage of the world's population, the only real sanitation option available is 'on-site sanitation'; an approach where all four of the above stages take place within the boundaries of the household. It differs from sewer based systems, commonly used in developed countries in that excreta is not transported 'off-site' for treatment and disposal.

4.1 Types

Pit latrine or traditional latrine
It is the most common of all the on-site latrine designs, and consists of a pit, either lined or unlined depending on the soil conditions which is dug to a depth of around 3 metres. At its simplest, the pit is covered with large logs which act as a means of support for smaller logs/branches in order to form a squatting platform. Access to the pit for faeces is via a small drop hole. The platform is finished with a layer of mud to form a smooth level surface. A superstructure is added for privacy and can be constructed from a wide range of locally available materials.

Ventilated improved pit latrines (VIP) or Blair latrine

A pit (either lined or unlined depending on soil conditions) is dug to a depth of around 2.5 metres and covered with a concrete slab (flat or domed). The slab, which has been finished to a smooth surface to allow easy cleaning, has foot rests to prevent foot fouling, a hole to take a ventilation pipe, and has a drop hole to allow faeces to enter the pit. The slab is positioned so as to cover the whole of the pit and removes the need for providing supporting beams. A ventilation pipe is positioned on the slab to take the foul smell away from the pit and to vent it to the external air above the superstructure roof line. A fly screen is added to the top of the ventilation pipe to prevent the access and egress of flies. Designers usually specify a block or brick superstructure, a good quality zinc or concrete roof, no windows and a tight fitting door in order to keep the inside of the superstructure dark. This is done in the belief that flies hatching in the pit will fly to the sunlight shining through the ventilation pipe and not fly through the drop hole into the dark superstructure.

Twin pit pour flush latrine

Used in communities using water for anal cleansing. A simple pour flush squatting platform (porcelain or cement) is set into a brick and concrete base and the outlet is piped to a junction box. From this box the excreta and flush waste are directed to one of two shallow pits (more pits are possible) for storage and maturing. The alternating pit principle is used i.e. one filling whilst the other is maturing, when the second is full, empty the first and use again. The pits are usually brick lined and covered with a wooden or concrete slat to prevent people falling into them. A superstructure is added for privacy and can be constructed from a wide range of locally available materials.

Arborloo

This is the simplest type of non-urine diverting eco sanitation based latrine and the one that involves the least amount of behaviour change from the conventional pit latrine. A shallow pit (0.75m is recommended) is dug and a slab and easily movable superstructure placed on top. The family uses the latrine, adding a mixture of soil and ash after each use, until it is three quarters full (usually between four and nine months). After this the slab and the superstructure are moved to another pit. A layer of soil is added to the full pit and a sapling planted into the soil. The tree grows and utilises the compost to produce fruit.

Aqua-privy

A simple type of latrine in which the excreta falls directly though a submerged pipe into a watertight settling chamber below the floor and from which the effluent overflows to a soak-away or drain.

Cess pool (pit)

A leak-proof covered holding tank for receiving untreated sewage. There is no seepage to the soil so cess pools need to be frequently emptied.

Composting latrine

The range of non-urine diverting ecological latrines (skyloo, Fossa Alterna, Arborloo) to which a soil and ash mix is added. As the temperature within the vault rarely rises to composting temperatures, a true composting process does not occur.

Fossa Alterna

This is a twin pit system based on the principles of non-urine diverting ecological sanitation. Two shallow pits are dug and used like the twin pit latrine i.e. one filling whilst the other is maturing, when the second is full, empty the first and use again. A dry mixture of soil and ash is added after each use which helps to prevent odour and keeps the contents damp and aerobic and opposed to saturated, smelly and anaerobic. A thin layer of soil is placed on the full pit making it ideal for plants whilst the 'manuring' process takes place. The watering of these plants helps the composting process. When the second pit is full the contents of the first are dug into the garden or farm to increase soil fertility.

Off-set pit latrine

A pit latrine where the pit is positioned partially or wholly away from the latrine superstructure.

Septic tank

An underground tank where all the water-borne waste from a house is deposited and decomposed by bacteria. Solids and dead bacteria settle to the bottom as sludge while the liquid portion flows into the ground through a 'soak away' comprising of either a network of underground pipes or a stone filled pit that allows the effluent to percolate into the soil. The sludge needs to be removed periodically in order for the tank to function properly.

Skyloo

An above the ground twin vault urine diverting eco-sanitation based latrine. Two vaults are built above ground level and a slab and superstructure built on top. The faeces drop through a squat hole into one of the vaults with the urine being diverted to a separate receptacle or soak away. The twin pit system is used i.e. one filling whilst the other is maturing, when the second is full, empty the first and use again. A dry mixture of soil and ash (or just ash) is added to the faeces after each use which prevents odour, keeps contents damp and aerobic. The vault contents should only be emptied when they are deemed to be relatively free from pathogens, normally following a period of 12 months. After emptying they are dug into the ground to increase soil fertility. In areas of rock or high water table the skyloo is the ecological sanitation based solution.

Solar drying eco-sanitation latrine

These are twin vault based latrines whose design and working principles are based on urine diverting eco sanitation. The main embellishment is that the vault has been extended at the rear of the latrine and covered with a metal plate. This is usually painted black and oriented to directly face the sun. The arrangement has the effect of increasing the temperature of the vault contents and complimenting the desiccating action of the ash in reducing the pathogen load in the faeces.

Urine diverting eco-sanitation latrine

These can either be twin pit or twin vault based latrines and all separate the urine from the faeces straight after it leaves the body. They use the rotation principles of alternating pit latrines. The urine is either stored in an air tight receptacle or run to ground through a soak away. Wood ash is added to the faeces which have the effect of lowering the moisture content to such an extent that it stops smell and destroys pathogens. The pits or vaults have a large enough volume to store 12 months' worth of a family's faeces and are covered with a slab which has been finished to a smooth surface to allow easy cleaning, has foot rests to prevent foot fouling, and a specially designed drop hole (or pedestal) to separate the urine and faeces. A superstructure is added for privacy and can be constructed from a wide range of locally available materials.

Water Closet

It can be used in households with a connection to a septic tank and preferably one connected to a piped water supply. The latrine is usually a pedestal, although squat versions are available and are made from porcelain. Both paper and water wash anal cleaning is possible. The faeces and urine are initially deposed in an S-trap at the base of the pedestal until the user activates a lever which releases a large volume of water stored in a tank (cistern) situated at the rear of the pedestal. The momentum created by the water flushes the faeces and urine into a drain and eventually carries it to the treatment site. The flush water also washes over the surface of the pedestal bowl giving it a clean appearance for the next user. Smell and flies are prevented from entering the superstructure by the S-trap which creates an effective temporary seal to the drain. This seal is so effective that the water closet can be placed indoors without causing any smell nuisances to the occupants.

4.2 Composition of pit latrines

Pit latrines will contain faeces, urine, anal cleansing material and/or anal cleansing water[37]. Anal cleansing materials vary widely around the world, from those requiring little or no storage space, such as water, to those having a greater volume than the excrete, such as corn cobs, cement bags or stones[34]. The diversity of this material will influence the decomposition process occurring in the pit latrines. Apart from that, latrines can be used as a place for household waste disposal, and sometimes are found to contain different non-degradable material (plastics, bottles and blankets)[37]. Grey water from sinks, washing dishes, showering etc. is often added by householders into pits[38].

As any given latrine may be used by all the members of a family (who probably share the same diet) or different families (with possibly different diets) a large heterogeneity in contents within and between different latrines may be expected. The individual variation in faecal composition will also contribute to the complex heterogeneity found within and between latrines.

Ideally, the rate at which the pit contents break down through biological activity should be similar to the rate of filling, thus providing a long service life for the pit. In practice, it is often observed that pits fill rapidly, particularly if a significant portion of the material added to the pit is non-degradable[39].

One solutions that is available in some countries to extend the life of a filling or full pit is the application of commercial pit latrines additives[40]. These additives are promoted as being able to reduce the sludge accumulation rate in pit latrines, and also to reduce potential problems of flies and odours[39]. While anecdotal evidence suggests that they may well be effective, independent scientific evidence of their efficacy is scarce[37]. Most of the commercial additives contain bacteria believed to be effective in increasing degradation of organic matter [41]. There is evidence that chemical additives such as kerosene, old engine oil, and salt are used by householders to reduce the volume of material in their pits[42].

4.3 Decomposition processes in pit latrines

Very little research has been performed regarding decomposition processes in pit latrines, and there are very few publications dealing with this topic directly. Nwaneri et al. analysed samples of fresh faecal material and samples from range of pit latrines from different location for different organic and inorganic characteristics: COD, moisture content, total solids and inorganic solids. All these tests were use to give an approximation of biodegradability characteristics of the material found in the different layers of the pit latrines[11].

They found that COD values for the faecal material present in the first layer were significantly lower than those measured for fresh faeces. They also found a decreasing trend of COD values from the top to the bottom layers of the latrine. The same trend was observed with organic solids and moisture contents, implying that degradation of organic material had occurred with time resulting in more stabilized material being located in the lower layers of the pit.

Although mean total COD and organic solids values decreased while progressing from surface layer to the bottom layer, there was a large variability of values between the different pits. This fact was also observed for moisture contents values between different latrines. In some of them they found an inverse trend, ie. there was an increase in moisture content values as they moved from the top to the bottom layers.

Some authors believe that anaerobic digestion is the main degradation process which occurs in pit latrines[10]. However Buckley & Foxon[37] proposed that a significant amount of aerobic degradation occurs in material while it resides on the surface of the pit. During aerobic degradation readily degradable material is converted to CO_2 and new biomass. Under anaerobic conditions, slowly degradable material is converted to CO_2, CH_4, soluble by-products and a small amount of new anaerobic biomass[37, 43].

On the basis of measurements of characteristics of pit latrine contents, and observations from the many samples handled during their studies Buckley & Foxon presented a general theory to describe the fate of organic material that enters a pit latrine[37]:
(i) all readily biodegradable material originating from faeces is aerobically degraded by naturally occurring micro-organisms within a very short time of arriving on the surface of the pit. This can only occur on the very top of the pit contents since oxygen is very quickly depleted below the first few millimetres of pit contents[44]
(ii) a significant portion of the remaining biodegradable material is aerobically degraded before being covered over by new pit contents
(iii) the remaining biodegradable material, including organic residual from dead
cells from micro-organisms and from the original faeces are slowly converted to (intermediate products, including soluble organic compounds, especially organic acids, and

end products including CO_2, CH_4, water, non-biodegradable organic material, $NH4^+$, phosphates and a small amount of new anaerobic micro-organisms) soluble products, methane gas and carbon dioxide in the buried layers of the pit contents (the fraction of the original organic material that is converted by this path is not large) by the presence of appropriate anaerobic micro-organisms
(iv) the material that remains at the bottom of the pit latrine or after a long residence time in the pit is largely non-degradable.

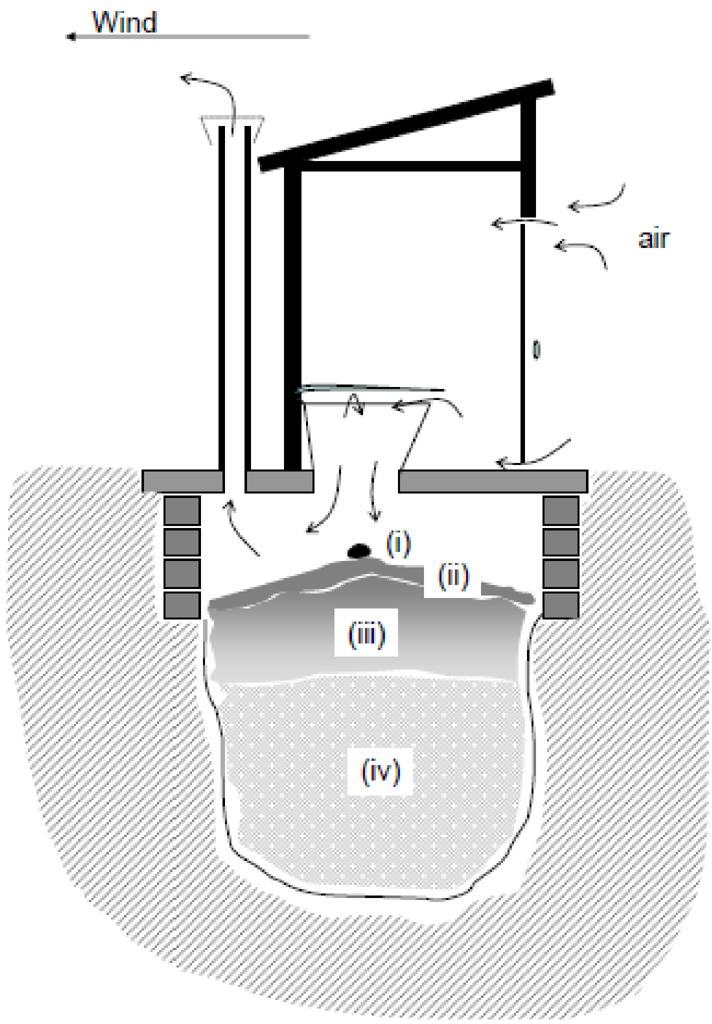


Figure 1 Basic structure of a VIP(taken from Buckley et al)[37].

While this is a useful model further studies are needed to clarify some questions that it raises. In particular, given the low relative abundance of aerobic organisms in fresh faeces, it is not clear whether they could be fully responsible for the rapid degradation observed at the surface of the latrine. Indeed, the aerobic conditions would also suppress the anaerobic microorganisms which predominate. Thus it is not clear what systems are mediating this rapid early breakdown. It is possible that extracellular hydrolases already released or originating from dying cells could play a role. In addition, other organisms, both microbes and higher organisms such as protozoa and metazoa, could migrate in from the surrounding soil. Much more detailed diagnostic studies, as planned in the present project, are thus needed to characterise the processes and test this theory.

Pit latrines receive waste cumulatively and this means that they act as a batch-fed systems with a slow accumulation of solids in the pit. With respect to the solids Chaggu proposed that pits could be called “Accumulation systems” and therefore they share many common characteristic with anaerobic reactors or digesters[10]. If this was the case, an efficient pit should end up with stable sludge material, at the time when it is full and have approximately zero organic fatty acid at the time of emptying[10].

Regarding the functioning of pit latrines, we believe the anaerobic process is likely to be very important particularly for the more slowly biodegradable material. Therefore in the next section we will carry out a more exhaustive review about anaerobic digestion to help us to understand the main processes occurring inside pit latrines and to identify the factors influencing the degradation of the contents.

4.4. Anaerobic digestion

Anaerobic digestion involves the degradation and stabilization of organic materials under anaerobic conditions by microorganisms and leads to the formation of biogas (a mixture of carbon dioxide and methane, a renewable energy source) and microbial biomass[45]. In theory, anaerobic digestion technology is a cost-effective biological means for the removal of organic pollutants in waste and wastewater. The technology has two significant advantages over aerobic biological treatment. Firstly, it is more cost-effective because aeration in not required and a small amount of excess sludge is produced. In aerobic treatment the microorganisms use oxygen in the air to metabolise a portion of the organic waste to carbon dioxide and water. They obtain energy from this oxidation, thus their growth is rapid and a large proportion of the organic waste is converted to new cells, which are not actually stabilised but simply bio-transformed. In contrast the anaerobic conversion to methane gas provides relatively little energy to the microorganisms, resulting in a slow growth rate and only a small portion of the waste being converted to new biomass. Secondly, anaerobic digestion generally produces gaseous methane as an energy resource[46-47].

Anaerobic digestion is a complex process that involves a number of strongly interacting groups of microorganisms (figure 2).

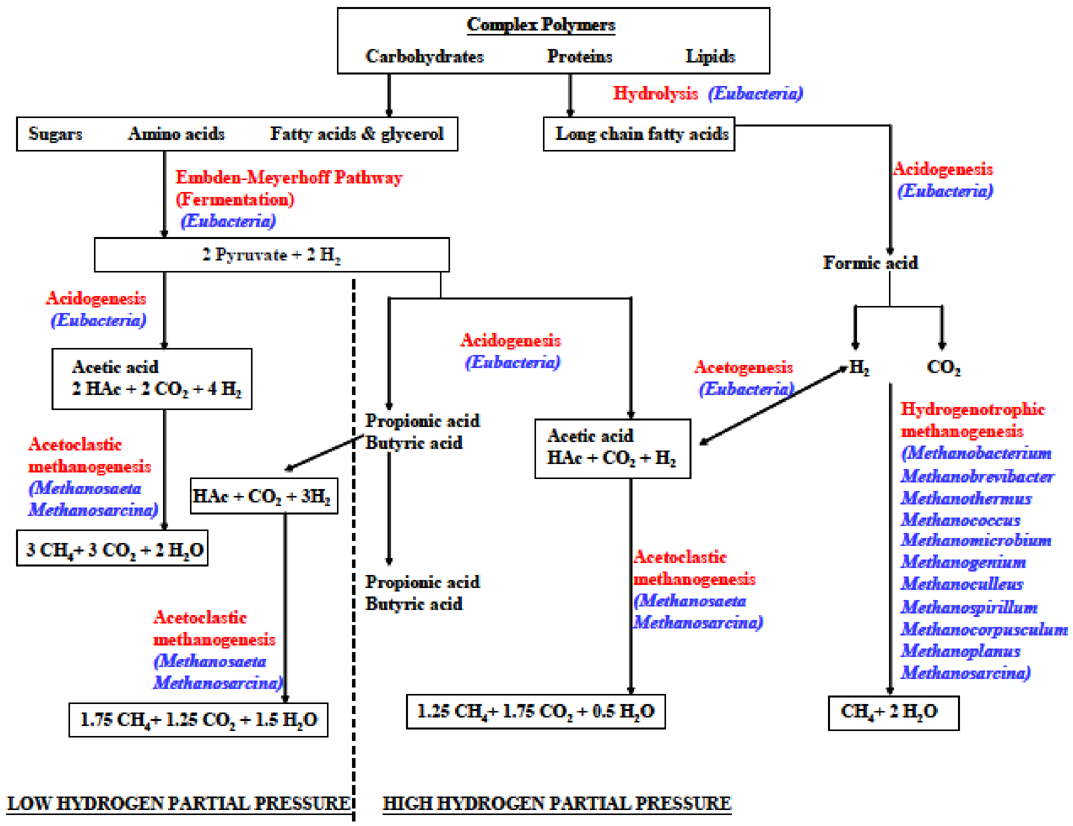


Figure 2 **Schematic representation of anaerobic process (figure from J. Bell)[48].**

There are four key biological and chemical stages of anaerobic digestion:

1. Hydrolysis
2. Acidogenesis
3. Acetogenesis
4. Methanogenesis

In the first stage complex long-chain macromolecules (lipids, carbohydrates and proteins) are hydrolysed to short-chain compounds (fatty acids and glycerol, sugars, and amino acids, respectively). This **hydrolysis** is catalysed by enzymes from hydrolytic bacteria (cellulase, protease and lipase)[49-50]. Hydrolysis of these high molecular weight polymeric components can be a slow process and therefore a rate-limiting step in fermentation, particularly if the influent contains particulate or large complex molecules in large quantities. This process is generally carried out by the bacteria belonging to the groups *Cytophaga-Firmicutes* and *Low G+C bacteria (LGC)*[51]. Acetate and hydrogen produced in the first stages can be used directly by methanogens. Other molecules such as volatile fatty acids (VFA's) with a chain length greater than acetate must first be catabolised into compounds that can be directly utilised by methanogens.

In the **acidogenesis** step, the soluble substrates produced in step 1 are degraded by fermentative acidogenic bacteria to form organic acids (eg formic and lactic), alcohols, ketones volatile fatty acids (VFAs), carbon dioxide (CO2) and hydrogen (H2)[49-50]. Major acid-forming bacteria are *Bacillus, Clostridium, Peptostreptococcus, Micrococcus* and

Pseudomonas. Other bacteria involved in this step belong to the groups *Bacteroides, LGC*, ð-*Proteobacterian* and *α-proteobacteria*[51-52].

The third stage of anaerobic digestion is **acetogenesis**. Here simple molecules created through the acidogenesis phase (propionate, VFA's and certain aromatic compounds) are further digested by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen. A minor group of hydrogen-consuming acetogens reduce CO2, CO and methoxyl-groups of aromatic compounds to acetate and sometimes butyrate[50]. Common bacteria associated with this process include *Acetobacter* and *Syntrophomonas* and other bacteria belonging to the High G+C group[51, 53].

The terminal stage of anaerobic digestion is **methanogenesis.** Here archaeal methanogens utilise the intermediate products of the preceding stages and convert them into methane, carbon dioxide and water. It is these components that makes up the majority of the biogas emitted from the system. Methanogenesis is sensitive to both high and low pH and occurs between pH 6.5 and pH 8. The remaining, non-digestible material which the microbes cannot utilise, along with any dead bacterial, forms the digestate.

Phylogenetically, the methanogenic archaea are diverse and are classified into five well-established orders. Representatives of the orders *Methanobacteriales, Methanomicrobiales* and *Methanosarcinale*s are common in anaerobic environments, where they serve as partners for anaerobic bacteria[12]. The microorganisms that form methane are physiologically specialized. They can grow only with a few simple substrates, such as H_2 and CO_2, formate, methanol and acetate, and they therefore depend on the other microorganisms that degrade more complex organic compounds for their substrate supply.

It is generally accepted that two-thirds or more of the methane produced in an anaerobic bioreactor is derived from acetate. Of the many methanogenic genera, only two, *Methanosaeta* and *Methanosarcina* are known to grow by an acetoclastic reaction, producing methane from acetate. Methanosarcina can form also methane from hydrogen and carbon dioxide (hydrogenothrophs), from methanol and methylamines (methylotrophs), and from acetate (acetoclasts)[54].

4.5 Factors affecting decomposition process in pit latrines

4.5.1 Temperature

There is no literature available regarding temperature values inside pit latrines. It is well known that temperature is one of the key factors influencing the overall digestion of waste in anaerobic reactors[10]. It is one of the most important factors affecting microbial growth and biological reactions. Temperature influences the rates of enzymatically catalyzed reactions and affects also the rate of diffusion of substrate to the cell[55]. Most of the microorganisms exhibit a relatively narrow temperature range over which they can be active[56]. Within that range, most reaction rate coefficients increase as the temperature is increased, but then eventually decrease as the heat begins to inactivate cellular enzymes. Zavala studied the effect of temperature on aerobic biodegradation of faeces in the bio-toilet system[56]. He showed that the optimum temperature for improving faeces biodegradability is within the thermophilic range, nearly 60°C. But at 70°C the activity of biomass was very low due to the diminishing enzymatic activity that microorganisms show at this high temperature.

In anaerobic reactors it has been shown that compared with mesophilic digestion (10-35°C), thermophilic anaerobic digestion (55-65°C) has additional benefits including a high degree of waste stabilization, and destruction of viral and bacterial pathogens[57].

Temperature also affects the concentration of different inhibitory substances found in the anaerobic process[58]. This will be developed in more detail in the inhibitory substances section below.

4.5.2 pH

pH stability is of great importance to the anaerobic process. It is reported that for appropriate microbiology activity pH should be within a range of 6.5-8[44]. If the pH falls below or close to 6.0, the sensitive methanogenic bacteria are inhibited and start to die [49, 59]. In anaerobic digesters a process imbalance may lead to a decrease of the steady-state rate of methane gas production and accumulation of organic acids (produced during acidogenesis)[60]. This provokes a decrease in the pH of the system, causing the inhibition of methanogenesis and cessation of the degradation process[43].

Thus in many systems, adjustment of the pH buffering capacity through addition of an external source of alkalinity such as lime dosing may result in improved process stability and increased rates of anaerobic stabilisation[61]. Courderc performed a study in which they investigated the effect of increased alkalinity on the rate of anaerobic digestion in samples taken from latrines and placed in modified serum bottles. They measured gas production rates from material taken from different depths in the pits. They concluded that poor gas production rates were observed from the material collected at the lower part of one latrine. However when they analysed the effects of additional alkalinity using material from the top layers they did not observed any change in gas production[38].

It has been reported that among societies that use human excreta from latrines as an agricultural fertilizer, the use of ash or lime (calcium oxide) is a common habit. Especially in eastern Asia (China and Vietnam)[62] and central and South American countries (Panama, Bolivia, Peru)[63-64].

pH plays also an important role in determining the concentration of inhibitory substances which can cause imbalance in the anaerobic process[58].

4.5.3 Moisture

Martin et al[65] showed that a minimum moisture content is necessary to facilitate mass transfer by promoting the diffusion of substrates to, and waste products away from, microorganisms, leading to a better biodegradation rate. Bhagwan[44] suggested that if a system is too dry there will be problems due to viscosity and osmotic pressure limitations, and if there is an excess of water in a draining environment, it would allow soluble substrates to leach form the pit, possibly slowing down the biological process. Courderc also studied the effect of moisture content on the anaerobic biodegradation of pit latrine sludge as was described in the last section. He observed some correlation between moisture content and gas production rate. Increasing the moisture content from 76% to 91% of anaerobically incubated pit latrine samples increased the rate of anaerobic gas production by between 0.006 and 0.02 mL gas/g total solids/day per 1% increase in moisture content[38].

4.5.4 Characteristics of the surrounding soil

Geological characteristics of the surrounding soil where the pit latrines are place can have an important influence in the processes happening inside the pit[44]. These include:

-Type of soil/rock: The porosity of the soil will determine the leaching and draining process that will occur in the pit. This will affect the liquid water level and moisture contents, as well as potentially pH. It will also influence diffusion of soluble components in or out of the pit, and hence microbial metabolism.
-Water table: Height of the water table will also influence levels of soluble components in the pit. Flooding of pit-latrines is a common phenomenon in situations of high water table conditions and during the rainy season. This is a major problem that has been described in different settings[10]. Flooding could also change microbial composition either directly through losses or indirectly through altering the pit environment.

Soil type may also affect decomposition through the alteration of the ecosystem in the pit. Soil microflora and microfauna (higher organisms such as protozoa, metazoa and worms) may move into the pit from the surrounding soil and contribute to decomposition of organic material.

4.5.5 Pit latrine dimensions.

The design of the pit latrine will probably constitute a key factor for the life span of pit latrines but there is little or no data in the literature on this point. One of the most important components to consider is the sizing of the pit[44], which will be based on the volume of faecal waste that accumulates per person per year (r=0.05m^3/person/year), the number of users (P) and the design life of the pit (n=usually 10 years). Pit working volume is equal to rPn(m^3). Depth and side wall surface area will influence other factors such as pH, moisture content and temperature which are known to affect degradation processes.

The depth of the pit to some extent affects the plan shape. Deep pits (deeper than about 1.5m) are usually circular, whereas shallow pits are commonly square or rectangular. As the pit gets deeper the load applied to any pit lining by the ground increases. At shallow depths, normal pit linings (concrete, brick masonry, etc.) are usually strong enough to support the soil without a detailed design. Also square or rectangular linings are easier to construct. At greater depths, the circular shape is structurally more stable and able to carry additional loading. Commonly, pits are 1.0-1.5 m wide or in diameter, since this is a convenient size for a person to work inside during excavation[34].

The pit latrine substructure and superstructure must be constructed properly to prevent collapse, control flies and odours, and facilitate emptying if this will be required[39].

4.5.6 Complexity of macromolecules

When considering solid wastes, hydrolysis of complex polymeric substances such as cellulose constitutes the rate-limiting step[66]. Mata-Alvarez et al. describe many studies exploring the different possibilities to increase hydrolysis of complex polymeric substances in order to enhance anaerobic process[67]. Different pre-treatment methods (biological, mechanical or physico-chemical) were tried in order to improve performance of solid waste digesters. Hasegawa and Katsura (1999)[68] reported a 50% improvements in methane yields when sewage sludge was solubilised under slightly thermophilic aerobic conditions prior to anaerobic digestion. They suggest that thermophilic aerobic bacteria secrete external enzymes which dissolve sludge more actively than commercial proteinases. Two other groups have used addition of complexes of enzymes to improve the efficiency of anaerobic sewage sludge digestion[69-70]. They added to thickened municipal primary sludge a mixture of peptidases, carbohydrolases and lipases (from 0% to 10% on total solids) which significantly improved hydrolysis at 39 °C and 51 °C.

Size reduction of particles with the resulting increase in the specific surface area available to the medium is another mechanical treatment that has been suggested. Engelhart et al[71] studied the effects of mechanical disintegration (by a high-pressure homogenizer) on anaerobic biodegradability of sewage sludge, and obtained a 25% increase in volatile solids reduction.

Whereas mechanical and chemical pre-treatment methods are well studied and commonly used in practice to increase methane yields, the prospects for a biological pre-treatment for anaerobic digestion processes are less clear[27, 72]. Enzymatic pre-treatment is quite expensive and demands strict control of reaction conditions[73], but the use of vital micro-organisms is probably more dynamic and efficient due to their ability to regenerate and produce diverse enzymes in response to a given substrate[27]. Bagi et al[74] inoculated biogas reactors with external hydrogen-producing bacteria with cellulolytic activity (eg. *Caldicellulosyruptor saccharolyticus*) and they obtained an increase of biogas formation of about 60-70%. The expected hydrolytic species resulting from the cultivation on xylan as monosubstrate belong to the genera *Clostridium, Bacteroides and Pseudomonas sp*.[75].

4.5.7 Oxygen

Oxygen is extremely toxic to the obligate anaerobic methanogens and these bacteria are inhibited by even small concentrations[49, 59]. It is possible that such levels could be attained within pit material by diffusion of oxygen from the surface, particularly in wet pit conditions.

4.5.8 Inhibitory substances

In anaerobic digestion, the acid-forming and the methane-forming microorganisms differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions[76]. Failure to maintain the balance between these two groups of microorganisms is the primary cause of reactor instability[77]. Inhibitory substances are the main cause of anaerobic reactors failure and they are mainly found in wastewaters and sludge[58].These include:

-Ammonia: This is produced by the biological degradation of nitrogenous matter, mostly in the form of proteins and urea[78].The two principal forms of inorganic ammonia nitrogen in aqueous solution are ammonium ion (NH4+) and free ammonia (NH3). Free ammonia has been suggested to be the main cause of inhibition since it is freely membrane-permeable[60, 79] and may diffuse passively into the cell, causing proton imbalance, and/or potassium deficiency[80-81].

Among the four types of anaerobic microorganisms, the methanogens are the least tolerant and the most likely to cease growth due to ammonia inhibition[82]. However there is no agreement in the literature about the sensitivity of acetoclastic and hydrogenotrophic methanogens.

It is generally believed that ammonia concentrations below 200 mg/L are beneficial to the anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms[83]. There is a large variation in the inhibitory total ammonia nitrogen concentrations reported in the literature ranging from 1.7 to 14g/L[60, 79, 81-82, 84-85]. This difference can be attributed to the differences in substrates and inocula, environmental conditions (pH, temperature) and acclimatisation periods[79, 86-88].

An increase in pH would result in increased toxicity[89] because of the shift to a higher free ammonia to ionized ammonia ratio. This increase often results in volatile fatty acid accumulation which leads to a decrease in pH and thereby declining concentration of free ammonia. The interaction between free ammonia, VFA and pH may lead to an "inhibited steady state" which will result in a lower methane production[90-91]. Chen showed different studies where controlling pH conditions could results in better performance in the anaerobic reactors. Slightly reduction of pH, from 8 to 7.4 or 7.4 to 7.0, can relieve ammonia-induced inhibition[58].

An increase of temperature in general has a positive effect on the metabolic rate of the mircroorganisms but also results in a higher concentration of free ammonia[58]. There are contradictory studies regarding the effect of temperature on bacteria and their tolerance to free ammonia[58].

Slow acclimatisation of methanogens to ammonia by exposing them to slowly increasing concentration was shown to improve digestion[58].

-Sulphide: Sulphate is a common component of many industrial wastewaters[92]. In anaerobic reactors, sulphate is reduced to sulphide by sulphate-reducing bacteria (SRB). Two stages of inhibition exist as a result of sulphate reduction: the first one is due to competition for common organic and inorganic substrate from SRB, which suppresses methane production[93], and secondary inhibition results from the toxicity of sulphide to various bacterial groups[94-97].

-Metal ions (Na, K, Mg, Ca and Al): High salt levels cause bacterial cells to dehydrate due to osmotic pressure[79, 98]. Metal cations including sodium, potassium, calcium, and magnesium are present in the influent of anaerobic digesters as they may be released by the breakdown of organic material (such as biomass), or added as pH adjustment chemicals[99]. They are required for microbial growth, but while moderate concentrations stimulate microbial growth, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity[100].

-Organics: A wide range of organic chemicals can inhibit anaerobic processes. They are poorly soluble in water and they can be adsorbed to the surfaces of sludge solids. The accumulation of apolar pollutants in bacterial membranes causes the membrane to swell and leak, disrupting ion gradients and eventually causing cell lysis[101-102]. Examples of such agents are: surfactants, detergents, long chain fatty acids alcohols, ketones, acrylates[103-106].

4.5.9 Higher organisms in pit latrines

In conventional sewage treatment it is well know that solids reduction involves both microorganisms and higher organisms such as protozoa and metazoa which can feed on the microbial biomass without affecting the decomposition of substrate[107]. Table 4 presents data on expected biomass reduction by various predators.

Table 4. **Various predators used for excess biomass reduction.(Table taken from Ramakrishna DM[108]).**

Predator	Prey	Expected biomass reduction
Tetrahymena pyriformis[109-110]	Ciliated Pseudomonas fluorenses	12-43%
Protozoa and Metazoa[111-112]	Bacteria, organic matter	60-80%
Oligochaete worm[113]	Bacteria,organic matter	25-50%
Bdelloid Rotifers[114]	*	10-25%

*They graze on suspended solids, including bacteria,, and they also facilitate flocculation, contributing to the overall reduction in suspended solids

To our knowledge the presence of such microfauna has not been studied in pit latrines. However, one predator, the larvae of the black soldier fly (*Hennetia Illucens*) which are voracious feeders of organic material, has been observed in pit latrines[115] (Irish, 2010, personal communication). A recent study of feeding rates[116] suggested that black soldier fly larvae would be capable of consuming up to 130 mg of human faeces per larva per day. In other words it would require around 10,000 larvae to process the faeces from one person per day.

4.5.10 Microflora present in pit latrines.

The human intestine and pit latrines both are environments where a large variety of microorganisms coexist. Compared with the human intestine, however, pit latrines are uncontrolled systems with very variable environmental conditions. As we have seen decomposition will involve communities of organisms working together. Maintaining the spatial and functional integrity of these communities will be a major challenge in a simple pit latrine, given the fluctuations in the environment .

5. Conclusions and Implications

This literature review was undertaken to ensure that we were fully aware of previous work and to help formulate our approach to the cross-sectional and longitudinal studies in terms of key parameters to explore and developing a working hypothesis. We believe we have fully covered the relevant literature in this review.

Our key insights from this literature can be summarised as follows:

- The digestion process occurring in pit latrines is very complex and involves a whole range of different organisms which are very sensitive to their environment.

- Pit latrines are essentially uncontrolled environments so it is no surprise that in most cases decomposition is sub optimal and they fill up too fast.

- In principle therefore if we could identify what is slowing or blocking the process the solution could be quite simple – eg changing the pH.

- Pit latrines contents are highly variable due to diet, habits and surrounding soil type.

These insights suggest that once we have better data on pit environments and key parameters it may be possible to design interventions which could be added to pits to stimulate endogenous metabolism and decomposition.

Based on this analysis we have formulated the following working hypothesis:

Pit latrines are inefficient in digesting organic matter because neither aerobic nor anaerobic processes can work effectively for long enough, due to inappropriate and uncontrolled environments, and both stall, resulting in slow/incomplete breakdown.

To test this hypothesis, we plan to use the following key parameters in our cross sectional and longitudinal studies of pit latrines:

- Indicators of aerobic and anaerobic digestion – Chemical oxygen demand, Volatile fatty acid levels, ammonia concentration, methanogenesis and biodegradability (Wageningen University).

- Potential regulating /inhibiting factors – pH, temperature, moisture, redox (oxygen).

- Microbial communities and diversity- 454 sequencing technique (Sanger Institute).

- Higher organisms studies – (tbd, light microscopy).

- Substrate availability (biochemistry studies).

- Measures of cell function (eg hydrolytic enzymes, RNA expression).

Table and figure list

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