Literature review on levels of pathogens and their abatement in sludges, soil and treated biowaste

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General introduction

This report is one of the five Project Horizontal desk study reports that attempt to assess hygienic parameters (WP 3), which may be needed to assure the sanitation of sludges, soils, soil improvers, growing media and biowastes. The four desk studies 3A, 3B, 4 and 5 reports highlight draft potential methods for the hygienic parameters likely to be included in future sludge and biowaste Directives, the Desk study 6 is a literature review :

Desk study report 3A "Feasibility of horizontal standards for *Escherichia coli* and *Salmonella* in sludges, soils, soil improvers, growing media, and biowastes" deals with *Escherichia coli* and *Salmonella* spp.,

Desk study report 3B "Rapid Methods for detection of *E. coli* (including *E. coli* O157) and *Salmonella* in sludges, soils, soil improvers, growing media, and biowastes", deals with rapid methods available for *E. coli* (including *E. coli* O157) and *Salmonella*,

Desk study 4 "Feasibility of Horizontal standard methods for detection of *Clostridium perfringens* and *Enterococci* in sludges, soils, soil improvers, growing media, and biowastes" deals with *Clostridium perfringens* and *Enterococci*,

Desk study 5 "Feasibility of horizontal standards for the enumeration of viable helminth ova in sludges, soils, soil improvers, growing media, and biowastes", deals with viable helminth ova,

Desk study 6 "Literature review on levels of pathogens and their abatement in sludges, soils, and treated biowastes" deals with the occurrence of pathogens and their abatement.

It is not only necessary to make methods available to determine specific micro-organisms, but also to provide a detailed protocol for sampling heterogeneous matrices such as sludges, soils, soil improvers, growing media, and biowastes to obtain fit for purpose results. Results are needed for validating plant performance (percentage pathogen reduction) and end product specification in terms of hygienic microbiological parameters (e.g. EU 2000). This will include co- and pre-normative research, including consideration of carrying out method validation for complementary bacterial indicators (e.g. *Enterococci* and *Clostridium perfringens*), and viable helminth ova (cestodes and nematodes). For parameters likely to be included in future Directives (i.e. *E. coli* and *Salmonella* spp.), the selected methods will be assessed in large Europe-wide interlaboratory trials involving many European countries. For other parameters, there is a need to develop preliminary standards in order to carry out the relevant research. In the sludge and Biowaste draft directives (EU 2000 and EU 2001), *E. coli* and S*almonella* are specifically mentioned. This leads to the logical choice to start the work on these organisms as one of the parameters in phase 1 of project Horizontal. For the other parameters, Project Horizontal desk studies 4 and 5 of WP3 to prepare draft potential protocols for CEN and ISO discussion are also being prepared.

In this literature review on levels of pathogens and their abatement in sludges, soils and treated biowastes, after an overview of the production of sludge and biowaste in Europe and the regulation, we have tried to determine the main Pathogens found in Sludge, Biowaste and soil. The different strategies for checking the quality of the final product before land spreading or demonstrating the pathogen removal efficiency of a process are presented and then the literature on the influences of different treatment processes on the abatement of micro-organisms is summarised.

1 Sludge and Biowaste return to soil

1.1. Definition

The CEN (European Standardisation Committee) defines the sludge as a "mixture of water and solids separated from various types of water as a result of natural or artificial processes". The generic sludge term, generally used for untreated sludge (raw sludge), include sewage sludge (sludge from urban waste water), septic tank sludge and industrial sludge.

Biodegradable waste means any waste that is capable of undergoing anaerobic or aerobic decomposition (EU, 2001). In this definition, the term "waste" includes any substance or object which is covered by the Directive 75/442/EEC .

1.2. Production in Europe

1.2.1. Biowaste

As noted by the Commission (COM, 2003), total waste generation in the EU is about 1.3 billion tonnes per year (excluding agricultural waste). This means that total waste, which includes municipal solid waste, industrial waste, etc, amounts in the EU to approximately 3.5 tonnes per capita and year. There are three main sources of biodegradable wastes that can be identified:

- agriculture and forestry – crop residues, vine shoots, slurries and manure, spent mushroom compost, pruning wastes, forests maintenance wastes, avoid-fires maintenance wastes, barks and wood chips from sawmills and wood industry etc;
- municipalities – food waste, green waste, septic tank sludge, sewage sludge;
- industries – food processing wastes and sludges, paper mill sludges, wool wastes, leather wastes etc.

In 1998 almost 180 million tonnes of municipal solid waste were produced in the Community (eurostat 2000). Depending on local conditions, food and drink habits, climate and degree of

industrialisation, gardening possibilities, between 30 and 50% of municipal solid waste consists of biodegradable waste. In the EU the total amount of biowaste and green waste that have been collected separately from the residual municipal waste can be expected to be of the order of 15 million tonnes. The actual potential is estimated to be 55 million tonnes, with a theoretical compost production of up to 20-25 million tonnes of compost from source separated wastes.

A wide range of wastes and by-products of industrial processes are produced in the EU. The total is estimated to be more than 107 million tonnes.

It is very difficult to estimate the production of slurries and manure in the EU, as only a few Member States have published data. One can nevertheless affirm that much more than 900 million tonnes are produced, as data from important countries are missing. Slurries and manure are probably applied in agriculture in their entirety.

Other agricultural waste to be considered is crop residues. For the main crop types, one can estimate that the crop residues produced in the EU are about 415 million tonnes per year. In general, crop residues are returned to soil, with the possible exception of cereals residues like wheat straw, oat straw, barley straw, rye straw. For instance, it is estimated that only 40% of these crop residues are brought back to the soil, the reminder being exported as animal litter, used as bio-fuel or burnt on site.

Other fruit and vegetable residues are generated on production area (such as fields, orchads or greenhouses) or result from market withdrawals (during prices supports). The total amounts of these residues is very difficult to estimate, due to variability from one year to another (changing climate conditions, word trade…). These residues are for example tomatoes haulms and leaves, cucumber haulms, withdrawals fruits and vegetable, sorting fruits residues. These residues are not always applied to agriculture lands. In France, their total amount is roughly estimated to be $3 \cdot 10^6$ t/year.

1.2.2. Sewage Sludge

The production of sewage sludge from urban waste water treatment plants in Europe is about 7.5 millions of dry solids tons in one year (AGHTM, 2002). The waste water sludge production is also described by the ratio kg of dry matter/year/inhabitant. In Europe, the average is about 20 kg of dry matter/year/inhabitant, with extreme values of 2,5 for Portugal and 37 kg of dry matter/year/inhabitant for Denmark (Figure 1). This ratio analysis traduces the difference of collective cleaning development between north and south European countries. It also appears, that a more important agricultural land spreading pressure (quantity of sludge to spread expressed in kg of dry matter / ha / year) exists in countries where a more important fraction of effluents are treated (ADEME, 1999a).

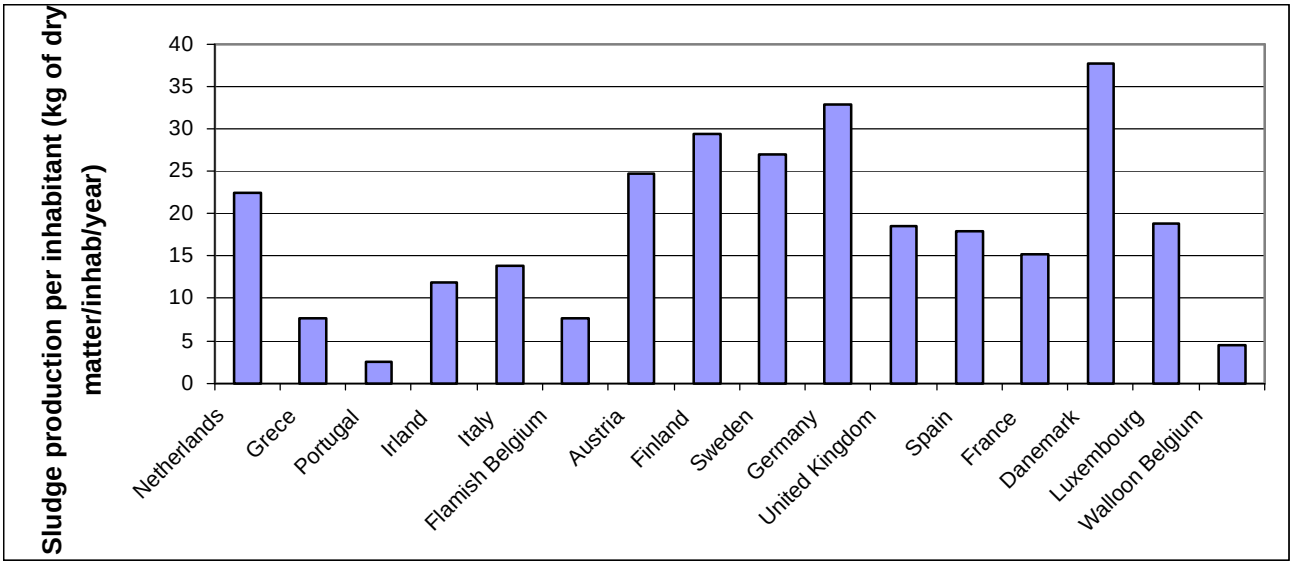

Figure 1: Ratios of sludge production per inhabitant and per year (kg of dry matter/inhab./year): (From ADEME, 1999a)

According to the commission, an increase of about 40% of sludge production is foreseen by the year 2005. It is crucial that the reuse of sludge on agricultural outlet will be put under stress by this increase in quantities. It is crucial that the reuse of sludge on agricultural soils is not unnecessarily hampered.

1.3. Recycling modes

The success of waste recycling differs widely between Member States. The mean recycling rate of domestic waste for the fifteen Member States is 15% (in Member States it varies between 0 and 44%). The mean recycling rate of hazardous waste (from eleven Member States) is about 19% and on other waste 60%. Traditionally, sludge and biowaste are landfilled, incinerated or landspread.

1.3.1. Return to soil

1.3.1.1. Agriculture

Recycling composted sludge and biodegradable waste in agriculture is considered as a way of maintaining or restoring the quality of soils, because of the fertilising or improving properties of the organic matter contained in these materials. Indeed, sewage sludge is primarily a supplier of nutrients (nitrogen, phosphorus and, to a lesser extent, potassium and sulphur), while compost is also a provider of well-stabilised organic matter with soil improving properties, due to its capacity to contribute to the formation of humus, which eventually intervenes to determine the soil characteristics (e.g. water retention capacity, physical stability, reduced erodibility). This has a special relevance in Southern and

Central Europe, where it is a valuable instrument for fighting against soil organic matter depletion and, thus, also desertification and soil erosion.

The proportion of sludge production spreading on agricultural lands in European Union and in Switzerland is about 40 %. This average value does not reveal the wide disparity between European countries, and between different regions in these countries. The share of agricultural land spreading is between 55 and 70 % in France, Denmark and Luxembourg, and this percentage is significantly higher in Flemish Belgium, where 90 % of the waste water sludge are spread on land. In contrast, the share of agricultural land spreading is lower than 20 % in Italy and Walloon Belgium.

Although reliable statistic are lacking, it is estimated that sludge represents about 1% (in weight) of the waste that is brought to land. Industrial wastes account for another 2%, while animal manure and slurries make up the difference (97%) (Gendebien *et al.,* 2001).

In the case of biowaste, despite the fact that the estimation of the production of slurry and manure in the EU is very difficult, it is accepted that they are probably applied in agriculture in their entirety. In general, crop residues are returned to soil, with the possible exception of cereals residues for which only 40% are brought back to the soil.

1.3.1.2. Vegetalisation

In order to improve the present rate of recycling nutriments and organic matter contained in biowastes, or to reduce the sludge land spreading pressure, it will be necessary to broaden the scope of the existing regulations and include the management of sludge in outlets such as silviculture, green areas and reclaim land hazards (EU, 2000).

Some limits have been set on sludge that should not be used in natural forests. However, European Union members may allow the use of sludge in plantations (short-rotation plantations, plantations for growing energy crops, Christmas tree plantations and similar) and for re-afforestation purposes where there is a need for an extra input of nutriments and as long as the provisions of Article 4 of Directive 75/442/EEC are complied with.

1.3.2. Alternatives

Mineralisation through thermal oxidation such as incineration and landfilling are also recycling paths that are not taken into account in the Horizontal project as they do not imply a return of organic matter to soil.

1.4. European Regulation

1.4.1. Present regulation for Sludges and Biowaste

Sanitary control of the end-product is not mandatory (Directive 86/278/EEC) but is more and more considered through different approaches. It consists in the control of the residual level of micro-organisms in wastes after treatment. This is performed by the mean of microbiological analyses but as the residual level of micro-organisms is dependent on the kind of treatment process, some regulation has to be established for assessment of microbiological quality. However, according to the country, several approaches can be defined for the control of microbiological quality of treated wastes in terms of regulation.

In the first approach, described in European Union directive, used in France and Germany, there is no real specifications on treatment processes but strict rules have been defined concerning the use of treated sludges.

In the second one, some treatment processes have been validated for their efficient reduction on pathogen level. Once they have been well defined, it is considered that they ensures a good microbiological quality of the end-products. This approach is used in Denmark for example.

The third approach is used in Switzerland and Italy. In this case, specific standards are defined for the microbiological quality of the end-product of sludge treatment. In Switzerland, treated sludge must display less than 100 *Enterobacteria* /gDM and no helminth ova. In Italy, less than 100 *Salmonella* spp MPN/gDM has to be found in the sludge before landing.

The fourth one corresponds to the one used in US and defined by EPA. It is a combination of the previous approaches i.e. micro-organism concentration in sludge and treatment processes used are defined in a regulation. According to their residual level in micro-organisms after treatment, two classes of sludge can be distinguished:

- class A for which sludge must exhibit less than 1000 faecal coliforms MPN/g DS or 4 *Salmonella* spp MPN/4g DS
- class B for which sludge must display less than $2x10^6$ faecal coliforms MPN/g DS.

Due to a great difference between the quality of class A and class B sludges, they are not used for the same agricultural purpose. In addition, in the US regulation, specific treatment processes has been validated to ensure a good sanitation of sludge.

The biological treatment of biodegradable waste and the use of compost and digestion products is currently not subject to EU rules. Some member states as Germany, Switzerland, Denmark, Luxembourg, Netherlands, France and Flemish Belgium, have developed their own regulation system and certification of residues from biologic treatment of biowaste.

1.4.2. Trends of projects of up-dated regulations

The proposal for a directive on sewage sludge will be delayed and issued at the same time as proposals on soil monitoring and biodegradable waste and a communication on soil erosion, decline in organic matter, and contamination. The timetable is likely to be second half of 2004.

1.4.2.1. Sludge

A proposal for a directive on sewage sludge (EU, 2000) would amend Directive 86/278/EEC on sewage sludge use in agriculture. This would aim to promote the use of sludge in areas such as silviculture and land reclamation. This would extend the definition of sewage sludge to cover sludge from urban waste water treatment plant septic tanks, domestic waste waters from dwellings and certain industrial sectors. It would require prior biological, chemical or heat treatment of sludge, according to the specific use to be made of land. A sanitation process (biological contaminants elimination) should be initially validated through a 6 Log reduction of a test organism. The Directive project on sludge, proposes the use of *Salmonella Senftenberg* W 775 as test organism. Furthermore, this Directive project proposes that a sanitised sludge should not contain *Salmonella* spp in 50 g (wet weight) and the treatment should achieve at least a 6 Log reduction in *Escherichia coli* to less than 5 10^2 CFU/g. This working document on sludge does not indicate limit values for pathogenic organisms in non-sanitised sludge, however it defines restrictions use for this class of sludge.

1.4.2.2. Biowaste

In the case of the biodegradable wastes regulation, the final objective is the harmonisation of measures concerning its management. A global recycling politic for this waste class should prevent or reduce impacts on the environment, and thus provide a high level of environmental protection. Also, the creation of limits values for chemical and biological contaminants in composts or stabilised biological wastes, should avoid the soil contamination and permit the enhancement of soil quality (EU, 2001).

1.5. Selection of Sludge/Biowaste conditioning prior to land spreading

Much has been done to minimise the potential transmission of pathogens by waste through effective treatment processes and then matching efficiency of pathogen removal to operational restriction on application practices and land use.

1.5.1. Sludge

A lot of treatments can be applied to sludge. For conditioning, some treatments aim especially to improve physical characteristics of sludge such as dry solids content (dewatering, thickening and

drying). A complementary conditioning can then be an effect on pathogens with a purpose either of stabilisation or sanitation (Hamel, 1997). These aspects are not yet regulated but are taken into account in the draft directive on sludge (EU, 2000). The stabilisation implies the limitation over a certain period of time of any microbiological activity able to generate fermentation odours. The aim of stabilisation is not the degradation of the organic matter but to place the micro-organisms in specific conditions in which their metabolisms will be inhibited (for example: low moisture content, variation of pH). Sanitation of sludge consists in the removal of pathogens up to defined thresholds.

Both stabilisation and sanitation can be achieved by biological (anaerobic disgestion, …), chemical (lime addition) or thermal processes. (Hamel, 1997).
Analyses are used as a tool to verify that the sludge is stabilised or hygienised.

Specific treatment processes are defined to achieve sanitation (Table 1).

Table 1: Sludge treatment processes (Hamel, 1997; Dumontet *et al*, 1999)

Stabilisation processes	Sanitation processes
Thermal drying Lime conditioning Aerobic stabilisation Anaerobic stabilisation Composting	Lime conditioning Thermal drying Gamma irradiation Sludge lagooning Anaerobic digestion Mesophilic aerobic digestion Thermophilic aerobic digestion Composting

Thermal drying removes moisture from sludge by means of evaporation (Strauch, 1998). It is carried out at a temperature higher than 80°C and with a reduction of water content to less than 10% (EU, 2000). Drying is a good disinfection method because of the high temperatures used. However, the investment costs are rather high compared with other processes. The effect of long term storage of dried sewage sludge on the pathogen survival is bad known (Dumontet *et al*, 1999).
In the lime conditioning procedure, stabilisation is ensured by an increase of pH and/ or an increase of temperature, depending on the kind of lime used: lime conditioning with quicklime (CaO) or lime conditioning with slaked lime ($Ca(OH)_2$). The difference between lime conditioning with calcium oxide or calcium hydroxide is the absence of exothermic reaction. The heat generated in lime-sludge mixture contributes to inactivate and destroy pathogens (Dumontet *et al*, 1999; EU, 2000).

The composting process is an aerobic fermentation. The efficiency of composting in transforming the putrescible organic matter of sewage sludge to an odourless and stabilised product is correlated with the biological activities taking place in the solid rather in the liquid phase (Dumontet *et al*, 1999). The stabilisation phase in composting process corresponds to the period where considerable amounts of cellulose and eventually lignin are decomposed leading to a lowering of the biological oxygen demand of the composting material. Temperatures are normally within the range of 35-55°C during this phase. For most composting systems, the stabilisation phase occurs after the sanitation phase (Christensen *et al*, 2001). The two main kinds of composting described in literature are composting of sludge in windrows or piles and composting in reactors (in-vessels composting).

According to the European countries, the operating parameters for composting process are different (Table 2).

Table 2: Composting operating parameters in Europe (UKWIR, 2002)

Countries	Temperature (°C)	Exposure time (days)
Belgium	60	4
Denmark	55	14
France	60	4
Italy	55	3
Netherlands	55	2
United Kingdom	40°C for at least 5 days then at least 4 hours at 55°C	

Pasteurisation process consists of a heating of sewage sludge to temperatures below 100°C but at least 65°C, for at least 30 minutes. The main combinations temperature/time used in sludge treatment are 70°C for 25 minutes, 75°C for 20 minutes and 80°C for 10 minutes (Strauch, 1998). In some cases, for sanitation, pasteurisation can be followed by a mesophilic anaerobic digestion at a temperature at 35°C with a mean retention period of 12 days (EU, 2000).

1.5.3. Biowaste

Conditioning of biowaste is primarily used to achieve a transformation of the organic matter. The main processes available are composting and the anaerobic methanisation.

As described above, the composting process consists of an aerobic fermentation of a mixture of biowastes (manure, sludge, slurry, biodegradable fraction of household wastes). It aims to transform biodegradable waste into good quality compost suitable for the improvement of the soil. For a good composting, different elements are necessary: a nitrogen and carbon sources, some moisture for the micro-organisms development and oxygen (Bigot *et al*, 1997).

Anaerobic digestion consists of the anaerobic decomposition and biodegradation of biodegradable waste under controlled conditions by the action of micro-organisms (methanogenic bacteria) in order

to produce methane in the form of biogas, digestate (fibre fraction) and a liquid fraction (EU, 2001). The principle of anaerobic digestion is to reduce the fermentability of the bio-waste, to increase the biogas production and to ensure that the digestate can be used for the production of compost. It consists of a fermentation of organic matter without oxygen in order to produce biogas and particularly methane and carbon dioxide (ADEME, 2001). The anaerobic digestion can be carried out at mesophilic (30-35°C with a mean retention period of 15 days) or thermophilic (50-55°C) temperatures for 20 hours. It is considered as an effective process for reduction or removal of pathogens (Dumontet *et al*, 1999). During the anaerobic digestion, other factors such as ammoniac production, microbial exoenzymes can influence the pathogen reduction (Couturier, 2002). In order to achieve an hygienisation effect comparable to this one obtained with thermophilic treatment, the mesophilic treatment requires a longer residence time and it is not still sufficient to ensure an acceptable removal of vegetative forms of pathogens (Dumontet *et al*, 1999).

As far as biowaste are concerned, the notion of stabilisation refers to a compost maturity and is not related to any pathogen content. However, new discussions are aiming at including different thresholds in order to reach a level of sanitation. Once more analyses are used to control the process performances.

2 Pathogens

2.1. Description of the main classes of pathogens

2.1.1. Main classes of pathogens

A large number of micro-organisms can be found in sewage sludge and other biowastes. However, for an agricultural application, only pathogen micro-organisms i.e. causing-disease micro-organisms present an interest. The causative agents of many infectious diseases are excreted by the faecal route and also with other excretions or secretions of the body. Some pathogens are also excreted from clinically healthy animals (Strauch, 1991). Pathogens studied are those ones which present a risk for human, animal or plant health. Indeed, this definition of five main classes of pathogens can be described: bacteria, viruses, yeast, fungi and parasites (in this class, two groups can be distinguished: protozoa and helminths). The spectrum of bacteria depends on the epidemiological conditions in the region where the samples are collected (Strauch, 1998). For virus also, the type varies as a function of the regions. Some viruses survive in the digestive tract and they are excreted by infected persons in large amounts. The sources of human viruses are faecal material, urine and sewer-disposed contaminated blood. Their clinical consequences vary from minor to fatal. They can also be excreted

with animal faeces. In this case, they usually come from birds, cats and dogs (Strauch, 1998). The fungi may have different toxicological health impacts (Fischer *et al.*, 2000). Pathogenic yeast and fungi have a low impact on the epidemiological aspect of sludge use. They can infect humans and animals, cause allergic diseases and/or produce mycotoxins (Strauch, 1998).

Other possible risks to human, animal and plant health exist, e.g. bacterial and fungal toxin, antimicrobial resistant bacteria and resistant genes. The increased use of antimicrobials in farming has resulted in a significant numbers of antibiotic resistant bacteria (Young 1993).

Pathogens may be found in sludge/biowaste directly under infectious form such as bacteria or virus but also under a resistance form (spores for bacteria or cysts and eggs for parasites) which will become pathogenic inside human (Schwartzbrod, 1997). The composition in pathogens is depending on the type of waste studied (ADEME, 1994). Products issued from hospital and slaughter-house bio-wastes, swine avian, bovine and other manure may transfer pathogens from animals such as viruses of Swine fever, New-Castle disease, influenza, bacteria such as *Bacillus anthracis*, BSE prion. Furthermore, the climate may strongly influence microbial flora. Products issued from exotic countries may contain some sorts of pathogens very different from known European flora. Unfortunately, most literature data provide from kwon pathogens of kwon European countries.

2.1.2. Risks associated with pathogenic micro-organisms

All the pathogenic micro-organisms described below may cause disease for humans or animals. However, specific risks can be associated to particular micro-organisms (Table 3).

Table 3: List of pathogens associated with specific risks (Dapilly and Neyrat, 1999)

Risk assessment	**Pathogens**
Digestive risk assessment	*Enterococci*, faecal and total coliforms, *E. coli*, *Salmonella*, *Shigella*, *Ascaris*, Enteric viruses, *Aspergillus flavus*, *Aspergillus parasiticus*, aflatoxin
Respiratory risk assessment	Total fungi, *Aspergillus fumigatus*, *Actinomycetaceae* (thermotolerant), *Faenia rectivirgula*
Cutaneous risk assessment	Pathogen and total Staphylococcus

So it is very important to control pathogens which may be present in sewage, sludge, biowastes, fertilisers, growing media and soil improvers, for protection of human beings, animals and vegetables. In addition animal pathogens can often be transmitted to men (zoonoses).

2.2. Pathogens in sludge

The agricultural utilisation of sewage sludge is common in many countries. In the sewage purification processes, most of the pathogens are reduced in number but not completely removed. They are accumulated by sedimentation processes in the sewage sludge.

In sludge, the most part of pathogens comes from human population, companion animals and livestocks (CSHPF, 1998; Strauch, 1991). Depending on the type of wastewater, pathogens will be different (Table 4).

Table 4: Origin of pathogens present in sludge (ADEME, 1994)

Sewage origin	Pathogens
Urban type sewage	Pathogens specific of human and animal
Dairy sewage	Pathogens specific of milk
Slaughter house sewage	Pathogens present in animal blood, faeces, digestive tract

Moreover, the pathogen level in sludge could be influenced by numerous factors such as type of processes, health of the population, presence of hospitals, meat-processing factories, weather (Dumontet *et a*l, 1999). A brief review of the pathogens found in sewage sludge and the density of some pathogens in sludges are described in Table 5 and Table 6 respectively.

Table 5 : Pathogens in sewage sludge (CSHPF, 1997 ; Carrington, 2001; Déportes *et al*, 1998; Strauch,1998)

Virus	**Bacteria**	**Fungi**
Enteric virus	*Arizona hinshawii*	*Aspergillus fumigatus*
- Poliovirus	*Aeromonas spp*	*Candida albicans*
- Coxsachivirus	*Bacillus cereus*	*Candida guillermondii*
- Echovirus	*Bacillus anthracis*	*Candida krusei*
Respiratory virus	*Brucella* spp	*Candida tropicalis*
- influenza	*Campylobacter jejuni*	*Cryptococcus neoformans*
Adenovirus	*Citrobacter* spp	*Epidermophyton* spp
Astrovirus	*Clostridium botulinum*	*Geotrichum candidum*
Calicivirus	*Clostridium perfringens*	*Microsporum* spp
Coronavirus	*Enterobacteriaceae*	*Phiolophora richardsii*
Enterovirus	*Escherichia coli*	*Trichosporon cutaneum*
Parovirus	*Klebsiella* spp	*Trichophyton* spp
Reovirus	*Leptospira*	
Rotavirus	*icterohaemorrhagiae*	
Norwalk virus	*Listeria monocytogenes*	**Helminths**
Hepatitis A virus	*Mycobacterium tuberculosis*	*Ankylostoma duodenale*
Hepatitis E virus	*Pasteurella*	*Ascaris lumbricoides*
	pseudotuberculosis	*Echinococcus granulosus*
	Proteus spp	*Echinococcus*
Protozoa	*Providencia* spp	*multilocularis*
Acanthomoeba	*Pseudomonas aeruginosa*	*Enterobium vermicularis*
Dientamoeba	*Salmonella* spp	*Hymenolepsis nana*
fragilis	*Serratia* spp	*Necator americanus*
Entamoeba	*Shigella* spp	*Strongyloides stercoralis*
hystolitica	*Staphylococcus aureus*	*Taenia saginata*
Giardia lamblia	*Enterococcus* spp	*Taenia solium*
Giardia intestinalis	*Vibrio parahaemoliticus*	*Toxocara cati*
Isospora belli	*Vibrio cholerae*	*Toxocara canis*
Naeglaria fomleri	*Yersinia enterocolitica*	*Trichuris trichura*
Palantidium coli		
Sarcocystis spp		
Toxoplasma gondii		

Table 6: Densities of pathogen and indicators in sludges (Straub *et al*, 1993)

Type	Organism	Density in primary sludges (/g of dry wt)	Density in secondary sludges (/g of dry wt)
Virus	Various enteric viruses	10^2 - 10^4	$3x10^2$
	Bacteriophages	10^5	-
Bacteria	Total coliforms	10^8 - 10^9	$7x10^8$
	Faecal coliforms	10^7 – 10^8	$8x10^6$
	Enterococci	10^6 – 10^7	$2x10^2$
	Salmonella spp	10^2 –10^3	$9x10^2$
	Clostridium spp	10^6	-
	Mycobacterium Tuberculosis	10^6	-
Protozoa	*Giardia* spp	10^2 - 10^3	10^2 – 10^3
Helminths	*Ascaris* spp	10^2 – 10^3	10^3
	Trichuris vulpis	10^2	< 10^2
	Toxocara spp	10 - 10^2	$3x10^2$

2.3. Pathogens in biowastes

As reported above, there are several types of biowastes (yard wastes, household wastes, vegetable wastes, sewage sludge, manure, etc…). The pathogen composition of these different biowastes in term of amount or species is variable. The concentration in mesophilic bacteria and thermophilic bacteria is important at the beginning of the composting process and decreases during composting (Riachi, 1998; Wrong, 2000). Species retrieved in compost are the same as in sludge (*Bacillus*, *Pseudomonas, Serratia*, *Xanthomonas* and *Klebsiella).* Pathogens from vegetable wastes and from household wastes are exposed in Table 7 and 8.

Table 7: Description of plant pathogens found in vegetable wastes (ADEME, 2001)

Pathogens	Host	Pathogens	Host
Bacteria:		*Marssonia panattoniana*	Lettuce
Xanthomonas campestris	Cabbage	*Sclerotina minor*	Lettuce
Pseudomonas marginalis	Lettuce	*Botrytis cinerea*	Lettuce
Pseudomonas phaseolicola	Bean	*Bremia lactucae*	Lettuce
Pseudomonas lacrimans	Cucumber	*Cerspora beticola*	Turnip
Corynebacterium michiganense	Tomato	*Aphanomyces raphani*	Radish
Corynebacterium sepedonicum	Potato	*Alternaria porri*	Carrot
Erwinia phytophora	Potato	*Septoria apii*	Celery
Agrobacterium tumefaciens	Variable	*Turbucinia cepolae*	Onion
		Sclerotium cepivorum	Onion
		Botrytis allii	Onion
Viruses:		*Uromyces appendiculatus*	Bean
Potato virus X	Potato	*Mycosphaerella piodes*	Bean
Potato virus Y	Potato	*Ascochyta pinodella*	Bean
Tobacco mosaic virus	Tobacco	*Eryiphe polygoni*	Bean
Horse bean mosaic virus	Bean	*Cladosporium cucumernum*	Cucumber
		Sclerotinia slerotiorum	Cucumber
		Septoria lycospersici	Tomato
Fungi:		*Alternaria solani*	Tomato
Plasmodiophora brassicae	Cabbage	*Didymella lycopersici*	Tomato
Phoma apiicola	Cabbage	*Rhizoctonia solani*	Potato
Peronspora brassicae	Celery	*Phyophtora infestans*	Potato
Peronspora spinaciae	Spinach	*Synchytrium endobioticum*	Potato
Peronspora destructor	Onion	*Verticillium albo-altrum*	Potato

Table 8: Densities of pathogens in household wastes (Deloraine *et al.*, 2002)

Type	Organism	Density in household wastes(/g of dry wt)
Virus		-
Bacteria	Total coliforms	10^7
	Faecal coliforms	4.10^7
	Enterococci	$5.10^6 – 10^7$
	Actinomycetaceae	8.10^9
Protozoa	*Giardia* spp	-
	Cryptospridium	-
Fungi	*Aspergillus*	10^7
Toxin	Micotoxin	-

Fungi are present in large amount in compost because the organic matter is transformed by moulds (i.e. *Aspergillus fumigatus*, (Millner *et al.*, 1980)). Therefore their is a threat that pathogenic species can develop.

Their is a concern that plant diseases can originate from primary infection by plant pathogens present in waste material recycled to field crops, garden or greenhouse crops. Vegetal production, if introduced to fields with waste material can have significant economic loss such as potato (*Ralstonia solanacearum*, *Clavibacter michiganensis* pv. *sepedonicus*, *Globodera rostochiensis*), sugar beet (rhizomania), oil seed rape (sclerotinia stem rot), carrots (cavity spot) and tomato (fusarium wilt). An other concern is the introduction of exotic plants pathogens with waste material from outside EU or the spread of a pathogen into areas in EU. *Phytophthora ramorum* causing Sudden Oak Death is an example of such a pathogen causing serious problems on oak in California and which recently have been found on *Rhododendron* spp. in the UK (Brasier, 2003).

Most important pathogens for human and animal retrieved in sludge and biowastes are *Enterococci*, *Salmonella*, *Clostridium perfringens*, *Ascaris* and *Aspergillus*.

2.4. Pathogens in soils

Large amounts of animal faecal wastes can be applied to agricultural land because of its fertiliser value. Approximately three-quarters of these wastes are produced by cattle and the remainder by sheep, pigs and poultry. Some 41% of faecal wastes will be in the form of either slurry (including dirty water) and 59% as farm yard manure (Nicholson *et al*. 2000). Livestock faecal wastes may contain pathogenic microorganisms such as *Listeria*, *Campylobacter*, *Salmonella*, *Escherichia coli* 0157, *Cryptosporidium* and *Giardia* (Hinton and Bale 1991; Mawdsley et al. 1995; Pell 1997; Nicholson et al. 2000). Nicholson et al. (2000) in their review of risks of pathogen transfer into the food chain from

manure applications to land state that there is a lack of data on 'typical' levels of pathogens in animal manures.

Prevalence of infection is only one of the factors influencing likelihood of pathogens being available at the soil surface for transport by overland flow. The actual numbers of pathogens shed is important and this has been found to be affected by a number of factors such as animal age, diet, stress and season (Nicholson et al. 2000). The probability of pathogens being available for transport at the soil surface is also likely to be influenced significantly by the duration and conditions of storage prior to land spreading.

In the case of soilborne pathogens (pathogens that cause plant diseases via inoculum that comes to the plant by way of the soil), the most familiar diseases are probably rots that affect belowground tissues and vascular wilts initiated through root infections. Soilborne pathogens can be divided into soil inhabitants which are able to survive in soil for a relatively long time and soil transients which are only able to survive in soil for a relatively short time. Fungi are the most important soilborne pathogens group: Plasmodiophoromycetes, Zygomycetes, Oomycetes, Ascomycetes and Basidiomycetes. Fewer plant disease are caused by soilborne bacterial pathogens (i.e. *Erwinia*, *Rhizomonas*, and *Streptomyces*. *Pseudomonas* and *Xanthomonas* usu sally persist in the soil for only a short time. Few soilborne viruses and parasites (Nematodes) affect vegetable crops (Koike *et al.*, 2003).

3 Monitoring

As already mentioned, a monitoring can take place either to check the quality of the final product (treated Sludge or Biowaste) before land spreading, or to demonstrate the pathogen removal efficiency of a specific treatment process.

3.1. Checking the quality of the final product before land spreading

The analytical monitoring must take into account: sampling on site (especially on heterogeneous matrix), sample conservation, sampling at the lab, extraction/purification steps and analytical method.

The most frequent encountered problems with evaluation of pathogen reduction are (i) to ensure a representative sampling and conservation of samples, (ii) the difference existing in the culture media (*in vivo, in vitro*) and (iii) the existence of a background in the samples studied (ADEME, 1997). A European standard project describes a sampling method for growing media in the CEN/TC 223.

3.1.1. Limits of sampling and sample treatment

The precision of the enumeration result strongly depends on the precision of the sampling and the sample treatment. In some cases, the error in sampling and sample handling may be greater than the difference between analytical results. For example, differences from 50 to 90% in results are not always significant. Sometimes, analytical techniques display some limits and only a small fraction of the present micro-organisms can be quantified. For all these reasons, it has been decided to use the notion of order range and to express results in log unit (ADEME, 1994) but this risks to complicate data comparisons between results of laboratories.

The only international standards for sampling sludges, treated biowastes or soils in the landscape are guidance documents. The guidance on the sampling of sludge and treated biowastes (ISO EN 5667-13:1998) do not contain information about the precision that can be expected. The facilities produce a range of type of material (liquid, paste, semi-dry) and there such wide range of potential process variants that it may not be possible to define standardised methods of sampling that will be appropriate for every type of facility. Moreover, biowastes are extremely heterogeneous. Similarly, the guidance on the sampling of soils (ISO-DIS 10381-1 and ISO-DIS 10381-4) is not specific. The properties of some soils show very little variation over considerable distance whereas some other vary substantially within single field. The properties of soils also vary with the depth. Furthermore, the stability of the material has to be consider. In addition, the conservation of the sample after sampling depends strongly of the conditions in which it is transported and conserved at the laboratory until the analysis are performed. One should fight against the re-growing phenomenon by keeping samples at 4°C during transport.

Homogenisation techniques have included the use of mortar and pestle, sonication, vortex agitation or Waring blender. The ideal dispersion technique is one which can remove pathogens from complex wastes without disintegrating the matrix which could lead to difficulties in recovery of the pathogen (UKWIR, 1998).

3.1.2. Limits of microbiological analytical methods

In the determination of the microbiological quality of treated wastes, several limits correlated to the microbiological analytical techniques can be noted. In routine analysis, only few micro-organisms can be easily monitored. For other micro-organisms, easy techniques are available but assume only qualitative results i.e. presence or absence.

Moreover, the analytical techniques used for assessment of waste sanitation vary with the kind of micro-organism studied. Moreover, depending on the analytical techniques, the results will not be expressed in the same unit. Then, bacteria and virus quantification are based on their ability to grow and to proliferate on specific medium. For example, for bacteria the results are expressed in UFC (Unit Forming Colony), in MPN (Most Probable Number) or in log unit. For parasites, the quantification is

based on a microscopic observation and does not take into account the notion of viability. In this case, the results are expressed in number of eggs or oocysts. However, some viability tests can be performed *in vitro*.

The last limit for microbiological analytical techniques, especially for bacteria and viruses, corresponds to the fact that their quantification are performed at lab-scale, *in vitro* and on rich culture media. Then it is often difficult to assess the behaviour of the same micro-organisms in *in vivo* conditions (ADEME, 1994).

Development of robust standard methods and rapid alternative methods for the determination of micro-organisms in biosolids is an area of active research. Research activities focuses on developing detection methods which are specific and sensitive to insure the good evaluation of pathogen reduction (UKWIR, 1998). Indeed, Skanavis and Yanko (1994) have studied the potential risk for Salmonellosis associated with the use of amendments products derived from sludge. It appeared in their study that whereas no *Salmonella* was detected in the end-product of composting, either in the raw amendment materials (rice hulls, saw dust, etc…), *Salmonella* was retrieved in the soil amendments which consist of a mixture of compost product and raw amendment materials. This result could be explained by the fact that the routine laboratory tests were not enough sensitive to permit the detection of a low number of *Salmonella* in compost. Then, *Salmonella* found in the soil amendments come from the compost products. Moreover some problems can occurs with not enough specific methods for the identification of pathogens. As a matter of fact, in several cases, *Klebsiella pneumoniae* has been identified as *E. coli* in waste samples. As *E. coli* is commonly used as indicator micro-organism, this could induce some errors in result interpretation about sanitary effect of treatments (Brassard *et al*., 1999).

3.1.3. Conclusion

At present, the difficulty is STANDARDISATION of the entire monitoring procedure from one lab to the other and from one country to the other. Lots of data are available but comparisons are difficult. The statue of progress of analytical development is also different from one matrix to the other.

3.2. Demonstrating the pathogen removal efficiency of a process

In addition to the monitoring procedure, standardised protocols are missing to demonstrate the pathogens removal efficiency of a process.

The draft sludge and biowaste Directives require that the treatment plant fulfil validation criteria in terms of log abatements of specific micro-organisms to obtain a sanitised sludge/biowaste. In the case of sludge, the process shall be initially validated through a $6Log_{10}$ reduction of a test organism such as *Salmonella Senftenberg W775.* Treated sludge shall also not contain *Salmonella ssp* in 50 g (wet weight) and the treatment shall achieve at least a $6Log_{10}$ reduction in *Escherichia coli* to less than 5.10^2 CFU/g. This lead to the necessity to artificially add micro-organisms to a treatment plant and to

monitor naturally occurring micro-organisms. Therefore it is necessary to evaluate both the initial contamination level and the level in treated sludge. For *E. coli*, a $6Log_{10}$ reduction to less than 5.10^2 CFU/g means that the initial contamination level is about 5.10^8 CFU/g. This value seems to be overestimated as shown in the Table 9.

Moreover, the questions to be addressed are: is it recommended to spike the sludge and the biowaste prior the treatment? What procedure to apply beyond those existing such as the EPA procedure, the German or Other? However, it is possible to inoculate test organisms in ways that will not contaminate the entire product, e.g. in semi-permeable test chambers.

The Table 9 gives an idea of scale for the evaluation of pathogen reduction in sludge. The pathogen reduction is more often expressed in log unit but it may also be expressed in percentage.

Table 9: Scale for evaluation of pathogen reduction level (ADEME, 1994)

Reduction level	Reduction rate (log unit)	Reduction rate (%)
Low	< 2 log	< 99
Intermediate	2 to 4 log	99 to 99.99
Good (sanitation)	4 to 6 log	99.99 to 99.9999
Very good (sanitation)	> 6 log	> 99.9999

4. Selection of microbiological quality of Sludge/Biowaste

4.1. Criteria to select indicators

4.1.1. Indicators

As described in previous paragraphs, there is a various and important number of pathogens in sludge and biowastes. If only sanitation efficiency has to be considered, evaluation of the efficiency of treatments should be based on the monitoring of pathogenic micro-organisms. However, it is not possible to monitor all these micro-organisms because of their high number but also because there are not always specific techniques for their quantification or identification. There is a few number of micro-organisms for which quantification of the population is possible: *Salmonella* spp, some enteric viruses, cysts of protozoa and helminth eggs. For the other micro-organisms, qualitative criteria such as presence or absence are used (ADEME, 1994). It becomes therefore important to define specific micro-organisms for the evaluation of the efficiency of the different treatments in terms of microbiological quality. For these reasons, two notions have been developed: the concept of indicator micro-organisms, and the one of test micro-organisms.

Indicator micro-organisms are defined as endogenous micro-organisms of the samples studied (sludge or biowastes) and they usually come from the digestive tract. For this latter reason, the presence of these micro-organisms is associated with faecal contamination. Their growth characteristics (i.e. temperature, pH, spore-forming micro-organisms) are similar to those of numerous pathogens for which detection and quantification are difficult or sometimes impossible. They are used to evaluate the sanitation efficiency of some processes (ADEME, 2001).

To be considered as indicator, micro-organisms have to satisfy to other criteria such as:

- to be quantified or identified by means of simple, reliable, precise and inexpensive analytical techniques,
- presenting high resistance to treatments,
- the concentration and evolution of indicator micro-organism has to be correlated to those of pathogen population,
- to be present in sufficiently high number to ensure a precise quantitative analysis,
- to be able to withstand the disinfectant and environmental stresses at the same level as the potentially present pathogens (ADEME, 1994; Dumontet *et al*, 1999).

As a sole, indicator micro-organism does not allow to predict the presence of all pathogens, several indicator micro-organisms are useful (Straub *et al*, 1993). Pathogens such as helminth and protozoa are not always found in wastes. Moreover, as virus cultivation is not quite easy, the best kind of indicator micro-organism appears to be bacteria (Carrington, 2001). Several studies have shown that bacteria are poor indicators of virus. Some test, performed on *Bifidobacterium* were not very conclusive and *Clostridium* spores persist to longer in the environment to be representative of the viral risk (Gantzer *et al.,* 2002; Schwartzbrod *et al.,* 1991). Phages (somatic coliphages, F-specific RNA phages and phage infecting *Bacteroides fragilis*) have been proposed as potential surrogate indicators. Furthermore, they are easy to enumerate and non pathogenic for human (Grabow *et al.,* 1986). Also with recent development in PCR, identification and quantification of virus are now becoming a possibility but is seems necessary to perform epidemiological studies to estimate the impact of virus detected by PCR on the human health (Abbaszadegan *et al.,* 2003). Furthermore, the matrices concerned are likely to be inhibiting and to involve wrongfully negative results and false impression of safety. Some micro-organisms have been retained for their specific properties (Table 10).

Table 10: Indicator micro-organisms (ADEME, 1994)

Possible indicator micro-organisms	Growth characteristics
Enterococci	Good survival and has no trend to regrowth
Sulfito-reducing clostridia	Spore-forming bacteria and present a good survival after treatment
Coliphage (particularly f2)	Good resistance to heat
Ascaris ova	Great resistance to treatment
Enteric virus	Very easy quantification

In most of the European countries, the selected micro-organisms are the following: Coliforms (total, faecal), *E. coli*, *Salmonella*, *Enterococcus*, *Enterobacteria* and *Clostridium*. *E. coli* is similar to vegetative bacteria (*Salmonella*, *Shigella*, *Vibrio* or *Listeria*). *Clostridium* spp is common in raw sludge and resists to heat. So, their removal can be related to removal of spore-forming bacteria such as *Bacillus*. Faecal coliforms and *Enterococci* appear to be good indicators for assessing municipal waste solids compost sanitation (Déportes *et al*, 1998; De Bertoldi *et al*, 1991). AFNOR has worked to establish a list of simplified criteria for Organic soil improvers – Composts containing substances essential to agriculture, stanning from water treatment in order to insure safety of composts for human, rather than animals and vegetables. This list contains treatment indicators : *E. coli*, *clostridium perfringens*, *Enterococcus* and pathogens to research : Viable helminth eggs, *Listeria monocytogenes* and *Salmonella* spp. (NFU-095:2002).

ADEME (2002) have studied correlation between pathogens and indicator micro-organisms. Correlation between some pathogens such as *L. monocytogenes*, Helminth ova and *Aspergillus* were found with indicator micro-organisms in treated wastes (composts for example) (Table 11). However, no correlation was found between *Clostridium* and *Salmonella* or *Clostridium* and *L. monocytogenes*.

Table 11: Relation between pathogens and indicator micro-organisms (ADEME, 2002)

Pathogens	Relation with indicator micro-organisms
Listeria monocytogenes	Absence for *E. coli* < 2log Absence for *Enterococcus* < 2log
Helminth ova	48% of absence when *E. coli* < 2log Absence for *Enterococcus* < 2log
Aspergillus	Absence for *Enterococcus* < 2log Absence for *C. perfringens* < 2log

In conclusion, for the comparison resistance of indicators and pathogens during treatment, is at the present time no good assessment of the levels on indicators and pathogens in different European countries. It appears that, even if they can be pathogenic under certain conditions, *E. coli*, *Enterococci* and *Clostridium* are interesting to be used as indicators. They indicate the presence of faecal material and the efficiency of the treatment process. Considering the Table 12 which gather the abatement of several indicators in the case of different type of treatment, it appears that for each type of treatment the better indicator to choose varies. This highlight the fact that it is impossible to choose one "universal" indicator.

Table 12: Compilation of the Log drops obtained for different indicators during different types of treatment (De Bertoldi *et al*, 1991; UKWIR (2002); Mocé- Ilivina *et al*, 2003; Schwartzbrod, 1997)

	Thermal treatment	**Lime conditioning**		**Mesophilic anaerobic digestion**	**Thermophilic anaerobic digestion**	**Composting**
		Sludge	**Biowastes**			
Pathogens				2.00	4.00	
Faecal coliforms		1.30 - > 6.70				0.68 - > 6.00
Enterococci		2.22				0.71 – 4.00
Sulfitoreducing Clostridia	0.30	2.30				
C. perfringens		0.10 - > 4.00				
S. senftenberg			4.71 – 7.95	4.18		2.09 – 2.39
E.coli	> 3.60	2.57	4.35 - 4.76	3.37		6.18
Enterococci	> 2.70					
Cryptosporidium			0.00 – 1.98	2.67		
L.monocytogenes			6.75	2.23		2.44 – 3.10
C. jejuni			7.23 – 7.49	0.34		5.70
Virus				0.50 – 2.00	6.00	
Somatic coliphage	0.6					
Poliovirus			6.50 – 6.82	4.46		7.85

4.1.2. Test micro-organisms

A test micro-organism corresponds to non endogenous micro-organisms introduced by spiking in the wastes studied. They are used to validate the sanitation phase in treatment processes. They also have to display some specific characteristics such as: resistance to physical and chemical conditions of

treatment, easy conditions of isolation and culture compared to another micro-organisms, low transmission potential and sanitary risk, lower cost for viability analyses (ADEME, 2001).

The principle of the utilisation of these micro-organisms is the following one: a defined amount of test micro-organisms is dosed in the samples before treatment. After treatment, the concentration of this micro-organism is measured. The difference between the initial and the final concentration of test micro-organisms allows calculating the pathogen reduction. As for indicator micro-organisms, the test micro-organisms are chosen for specific growth characteristics (resistance to high temperature for example).

A list of micro-organisms which could be considered as test micro-organisms for biowastes sanitation is shown in Table 13.

Table 13: Potential test micro-organisms (ADEME, 2001)

Human and animal pathogens	**Plant pathogens**
Bacteria	Fungi
Bacillus spp *Campylobacter spp* *Clostridium perfringens* ***Escherichia coli*** ***Enterococci*** *Listeria spp* *Mycobacterium tuberculosis* *Mycobacterium partuberculosis* ***Salmonella spp*** ***Salmonella senftenberg* W775** *Yersinia enterocolitica*	*Chalara elegans* *Cylindrocarpon destructans* ***Fusarium oxysporum*** *Phythophtora cryptogea* ***Plasmodiophora brassicae*** *Pythium ultimum* ***Rhizoctonia solani*** *Sclerotinia sclerotiorum*
Viruses	Viruses
Coliphages Coxcakievirus B Parvovirus	Beet necrotic yellow vein virus **Tobacco mosaic virus** Tobacco necrosis virus
Parasites (viable ova)	Nematodes (oocysts, larva, ova)
Ascaris spp *Taenia spp* *Giardia lamblia* *Crytosporidium parvum*	*Ditylenchus dipsaci* *Longidorus spp* *Xiphiema spp*

Organisms shown on bold are preferred test micro-organisms

Among all the bacteria listed, *C. perfringens* is certainly the most resistant. Therefore, its inactivation rate is not sufficiently important and for these reasons, it has not been retained as test organism by

Germany and Nordic countries. *S. senftenberg* is a more thermoresistant strain than other strains of *Salmonella* spp. In addition, as the inactivation kinetic of *E. coli* is close to the one of *Salmonella*, *S. senftenberg* appears to be a good test micro-organism. *Enterococcus faecalis* displays the most important resistance to temperature. Enteric virus is the virus displaying the better resistant to treatment but Nordic countries recommend the use of coliphage. For parasites, *Ascaris* ova are the most resistant, *Ascaris* ova show higher heat resistance that bacteria (ADEME, 2001).

A Nordic project has investigated the inactivation of plants pathogens during composting as well as their possible use as indicator organisms for the sanitary quality composting process: *Rhizoctonia solani, Fusarium oxyporum, Plasmodiophora brassicae* and Tobacco mosaic virus. *R. solani* survived under relatively low temperatures in combination with a short sanitation phase. *P. brassicae* was inactivated at rather low temperature during composting and *F. oxysporum* survived at the in-vessel plant treating household waste. Tobacco mosaic virus was inactivated during prolonged composting phase (e. g. more than 3 months) at rather low temperatures or when exposed to temperatures above 70°C (Christensen *et al*., 2000; Christensen *et al*., 2001).

4.2. Influences of different treatment processes on micro-organisms

For a good sanitary treatment, a valuable pathogen reduction and the absence of bacterial regrowth after sanitation are required (ADEME, 1994).

Several factors such as heat, moisture, pH can influence pathogen reduction (Carrington, 2001). For virus, the most important factors achieving inactivation seem to be thermal exposure, evaporative drying, microbial antagonism, exposure to high pH and irradiation. This can be achieved by treatments such as thermophilic digestion, pasteurisation, liming, lagooning, thermo-irradiation (Strauch, 1998).

It is important to note that whether all the stabilisation processes have a good impact on the removal of olfactory disorders, their influence on pathogen reduction is quite variable (Hamel, 1997).

4.2.1. Heat treatments

In addition to the fact that thermal treatments ensures a good reduction of sludge volume, it also has a significant effect on the microbiological quality of the sludge because pathogens are inactivated during exposure to heat. The period of exposure is dependent on the temperature and on the species of the organism.

As a matter of fact, after drying, no presence of viable Helminth eggs is established whereas these organisms are described to have a good resistance to most of the treatment processes. Pasteurisation of sludge causes a complete removal of enteric viruses and *Salmonella* (1 to 3 hours at 70°C) (Schwartzbrod, 1997) and inactivation of *Cryptosporidium* oocysts (2h at 55°C) (Whitmore and Robertson, 1995). A Spanish study has been realised on pathogen reduction in sludge and wastewater after thermal treatment. The results obtained are exposed in the Table 14.

Table 14: Pathogen reduction obtained after thermal treatment (Mocé- Ilivina *et al*, 2003)

	Pathogen reduction (log unit)	
	Sludge treated at 80°C for 90min	Sewage treated at 60°C for 30 min
E coli	> 3.6	6
Enterococcus	> 2.7	3.4
Sulfito-reducing *Clostridium*	0.3	0.1
Somatic coliphage	0.6	0.8

4.2.2. pH treatments

The raising of the pH to at least pH 12 by the use of lime has the effect of suspending microbiological activity. Lime conditioning in specific conditions (pH of 12.5 for 2-4 months) can cause a helminth reduction of 98.5% and a virus inactivation of 90% (Schwartzbrod, 1997). Addition of quicklime to dewatered sludge and storage under a pH of over 12 for at least three months ensures a high degree of sanitation depending on the calcium oxide dose, the temperature and the treatment duration (Table 15).

Table 15: Reduction of microbial load after treatment with quicklime

CaO dose (%)	**°C after 1st day**	**Coliforms**			***Clostridium perfringens***				
					Vegetative			**Spores**	
		4 hours	**1 day**	**14 day**	**4 hours**	**1 day**	**14 days**	**1 day**	**14 day**
0	20	-	-	1.3	-	-	0.1	-	0.4
2	20	2.8	3.5	5.3	1.2	1.5	2.0	2.0	> 4.0
4	20	3.2	3.2	6.3	1.3	1.8	2.2	> 4.0	> 4.0
6	20	3.8	5.2	6.3	1.4	1.9	3.0	> 4.0	> 4.0
6	26	-	5.2	6.3		2.2	4.0	> 4.0	> 4.0
8	20	5.2	3.2	> 6.7	1.5	1.7	3.0	> 4.0	> 4.0
8	28	-	5.6	> 6.7		1.9	> 4.0	> 4.0	> 4.0
10	20	3.2	6	6.2	1.2	1.7	> 4.0	> 4.0	> 4.0
10	33	-	5.5	6.3	-	2.1	> 4.0	> 4.0	> 4.0
15	20	-	5.5	> 6.7	-	2.9	> 4.0	> 4.0	> 4.0
15	39	-	6	> 6.7	-	> 4	> 4.0	> 4.0	> 4.0

The impact of lime conditioning on sludge can lead to a total micro-organism a reduction of 2.37 log (ADEME, 1997) and, according to Strauch (1983), it ensures a significant reduction of *E. coli* (99.7%), *Enterococci* (99.4%) and *Clostridium* spores (99.5%). However, this process does not display

good efficiency for *Mycobacterium* and *Ascaris* eggs reduction. For pH comprised between 11 and 12.4, a complete disappearance of *Salmonella* is observed. Viruses are inactivated with a pH of 11.5 (Furet, 1997).

UKWIR (2002) have studied the impact of lime conditioning on pathogen reduction during biosolids treatment (Table 16).

Table 16: Effect of lime conditioning on pathogen reduction (UKWIR (2002))

Organism	Reduction (log unit)
E. coli	4.35 – 4.76
Listeria monocytogenes	6.75
Campylobacter jejuni	7.23 – 7.49
Salmonella senftenberg	4.71 – 7.95
Salmonella typhimurium	8.75 – 9.67
Salmonella enteriditis	-
Salmonella dublin	6.84 – 7.58
Poliovirus	6.50 – 6.82
Cryptosporidium	0 – 1.98

4.2.3. Storage and drying

Other processes have a great impact on virus inactivation. For example, in a dried sludge with 90% of dryness, 99.9% of virus are inactivated. The storage of digested sludge for several months at temperatures between +4°C and –7°C induces a *Salmonella* reduction from 68 to 80% (Schwartzbrod, 1997; Ducray and Huyard, 2000).

In the Table 17, a review of the efficacy of the different kinds of physical treatments on reduction of helminth number is exposed.

Table 17: Efficiency of the different processes on helminth reduction (Schwartzbrod, 1997)

Efficient treatments		**Non efficient treatments**	
Treatment type	Operating conditions	Treatment type	Operating conditions
Irradiation	1Mrad	Drying	Sludge with a dryness of 80%
Storage	16 months at 25°C (*Ascaris*) 10 months at 25°C (*Toxocara*)	Storage	Storage at 4°C (*Ascaris, Toxocara*)

4.2.4. Biological treatments affecting pathogen reduction

4.2.4.1. Digestion

The thermophilic anaerobic digestion at a temperature of at least 55°C during a continue period of 24h and a sludge retention time of at least 20 days has a good impact on pathogen reduction. Therefore, many pathogens (bacteria, viruses, parasites, yeasts and fungi) survive during mesophilic anaerobic digestion. According to Couturier (2002), mesophilic anaerobic digestion induces a pathogen reduction of 99% whereas thermophilic anaerobic digestion ensures a reduction of pathogen of 99.99%. Thermophilic anaerobic digestion appears to be efficient for sanitation of wastes with high content in pathogens. Mesophilic anaerobic digestion will be more interesting for sanitation of waste weakly contaminated. Sludge treated by mesophilic anaerobic digestion have to be submitted to other disinfection treatment before its use in agriculture (Strauch, 1998). Some authors reported that mesophilic treatment was ineffective on *Salmonella* spp and spores of *Clostridium perfringens* (Berg and Bergman, 1980; Olsen and Larsen, 1987).

The impact of different kinds of digestion processes on pathogen is given in the Table 18. During digestion, several factors may influence virus inactivation:

- temperature: thermophilic digestion is more efficient than mesophilic digestion;
- micro-organisms or microbial enzymes,
- ammoniac production (Schwartzbrod, 1997).

Table 18: Effect of digestion on viruses (Schwartzbrod, 1997)

Treatment processes	Virus inactivation (%)
Mesophilic anaerobic digestion (30 – 35°C)	50 to 99
Thermophilic anaerobic digestion (50°C)	99.9999
Thermophilic aerobic digestion (45°C)	98

Schwartzbrod (1997) has also studied the effect of digestion on helminth (Table 19) and *Salmonella.* It appears that mesophilic anaerobic digestion induces a *Salmonella* reduction in a range of 16 to 98% whereas thermophilic anaerobic digestion achieves a *Salmonella* reduction of 99.8%. A high reduction is obtained for faecal *Enterococci* with thermophilic digestion (3 to 4 log). With mesophilic digestion, their reductions vary from 0.05 log to 1 log (ADEME, 1999). Thermophilic aerobic digestion (24h at 55°C) is described as an effective treatment for inactivation of *Cryptosporidium* cysts. Approximately 10% of cyst population were viable after 18 days exposure to mesophilic anaerobically digesting sludge (Whitmore and Robertson, 1995).

Table 19: Impact of digestion on helminth (Schwartzbrod (1997))

Efficient treatments		**Non efficient treatments**	
Thermophilic aerobic digestion	45°C for 20 days 55°C for 2h	Mesophilic aerobic digestion	Ambient temperature (90-95% of viable eggs)
Thermophilic anaerobic digestion	38°C for 30days 49°C for 10 to 20 days	Mesophilic anaerobic digestion	35°C for 10 to 20 days (38-90% of viable eggs)

The Table 20 gives quantitative data about the influence of aerobic and anaerobic digestion on pathogen reduction has been studied by Straub *et al* (1993).

Table 20: Pathogen and indicator concentrations in digested sludge (Straub *et al*, 1993)

	Number of organisms/g dry wt		
Organism	Primary sludge	Anaerobic digested sludge	Aerobic digested sludge
Enteric viruses	10^2 - 10^4	0.2 – 210	0 – 260
Rotaviruses		14 – 485	ND
Salmonella	10^2 –10^3	3 – 10^3	3
Total coliforms	10^8 - 10^9	10^2 –10^6	10^5 –10^6
Faecal coliforms	10^6 – 10^7	10^2 –10^6	10^5 –10^6
Shigella spp		20	ND
Yersinia enterocolitica		10^5	ND
Giardia spp	10^2 - 10^4	10^2 –10^3	ND
Ascaris	10^2 – 10^3	-	-
Trichuris	10^2	-	-
Toxocara	10 - 10^2	-	-

4.2.4.2. Composting

The efficiency of the composting process is based on the heat generated during the process and on the fact that during the mesophilic then thermophilic steps, the composting mass turns into substrate unsuitable for the growth and the survival of most of pathogens (Dumontet *et al*, 1999). The pathogen removal is also linked to factors such as moisture, aeration, pH, nutriment supply, antagonism with indigenous micro-organisms and production of microbial antibiotics (Bigot *et al*, 1997).

It has been demonstrated by Bigot *et al* (1997) that the pathogen reduction observed during composting depends on the quality of the initial wastes (green wastes, sludge) and the efficiency of

treatment (temperature, residence time and aeration). The pathogen reduction is more important in a closed system with aeration (Boutin *et al.,* 1997). For Coliforms and *E. coli*, residence time has an effect, probably related to the re-colonisation phenomenon. For *Salmonella*, the reduction is important where the temperature is more than 65°C (Pereira-Neta, 1986).

According to Strauch (1998), safety in sludge is assumed by composting for a period of two weeks at a temperature of at least 55°C or for one week at 65°C.

Quantitative data have been obtained about reduction of pathogen after composting of wastes consisting of 40% of raw sludge and 60% of solid municipal wastes. Depending on the operating parameters (number of turning of compost material, residence time, use of withdrawal, aeration, composting in windrows or in vessels) the pathogen reduction varies. Composting has a better impact on reduction of *E. coli* and *Salmonella* than *Enterococci* (Table 21). Composting is effective on helminth reduction if conditions applied are 4 hours at 60–76°C or 8 days at 60–70°C (Schwartzbrod, 1997).

Table 21: Pathogen reduction in biowastes treated by composting (De Bertoldi *et al*, 1991)

	Salmonella	**Faecal coliforms**	***Enterococci***
Minimal reduction	0.5 log	0.68 log	0.71 log
Maximal reduction	> 6 log	> 6 log	4.72 log

The Table 22 exposes the pathogen reduction (expressed in log unit) obtained after different biological treatments of biosolids.

Table 22: Reduction of pathogens in biosolids (UKWIR (2002))

Organism	Process				
	Mesophilic anaerobic digestion (MAD)	Pasteurisation + MAD		Composting	
		70°C – 30 min	55°C – 240 min	55°C – 4h	40°C – 5 days
E. coli	3.37	7.90	7.90	6.18	6.18
Listeria monocytogenes	2.23	8.12	6.06	3.10	2.44
Campylobacter jejuni	0.34	6.76	4.56	5.70	5.70
Salmonella senftenberg	4.18	7.89	6.95	2.39	2.09
Salmonella typhimurium	-	7.39	6.42	-	-
Salmonella enteriditis	-	8.24	8.24	5.68	5.68
Salmonella dublin	-	-	-	5.58	5.58
Poliovirus	4.46	8.30	8.42	7.85	7.85
Cryptosporidium	2.67	1.4	1.4	-	-

Deportes *et al* (1998) have monitored a municipal solid waste composting from raw material to mature compost and long term storage (1 year). The following pathogenic (*Ascaris* eggs, *Salmonella*, *Shigella*) and indicator micro-organisms (*Enterococci*, total coliforms, faecal coliforms and *E. coli*) were studied. Based on the results observed at the end of the composting, the following conclusions can be obtained:

- no seasonal variations of the micro-organisms were observed,
- the concentration of faecal coliforms and *Enterococci* is highly decreased during composting,
- no *Shigella* was observed,
- a disappearance of *Salmonella* and Ascaris eggs was noted during composting before 27 days,
- during storage, indicators and pathogens micro-organisms remained either undetectable or at low level.

In conclusions, treatment appearing efficient on pathogen reduction are thermophilic digestion, thermophilic stabilisation, composting and pasteurisation (ADEME, 1994). However, it was very difficult to compare the results obtained from different studies because they are obtained with different methods and expressed in different units.

4.3. Strategies of different countries

In Europe, only some countries have set up strategies for the evaluation of sanitation of biowastes (particularly for compost). Two main kinds of strategies can be distinguished: the direct process evaluation and the spot test analysis (ADEME, 2001).
In this report, only German and Nordic examples will be discussed in details. However, the strategies adopted by other countries will be also tackled.

- **Direct process evaluation**

The Direct process evaluation has been first defined for composting process. In the direct process evaluation, defined organisms (micro-organisms and/or seeds) are inoculated in the raw material by the mean of inoculation-bags at the beginning of the process. At a later stage, the recovery of micro-organism is investigated in order to determine the efficiency of treatment for pathogen reduction (Christensen *et al*, 2001).

This method offers the possibility to evaluate the inactivation of defined micro-organisms. It is a good tool for identifying and monitoring parameters for process optimisation. This method presents also some disadvantages: production of inocula, use of inoculation-bags and the sampling procedure are very laborious and expansive. Another problem with direct process evaluation is that the environment in inoculation-bags can vary from the one of the surrounding material. So, it is not obvious to obtain very representative samples. This is usually due to the fact that mixing or turning of the material is impossible in the inoculation-bags. These phenomena may induce significant errors in the interpretation of the results.

In conclusion, direct process evaluation is a valuable tool for identifying parameters for process optimisation in different decomposition zones and for detecting pathogens not normally present in the raw material. However, it is an unreliable method for evaluating the overall sanitary process, since it is very difficult to adequately represent a heterogeneous environment when inoculating a limited number of decomposition zones.

- **Spot test analysis**

The spot test analysis consists of microbiological analyses of the raw material, the hygienisated compost, and/or the finished compost for the same parameters with the purpose of achieving a qualified estimate of the changes during the process. The composition of the raw material must have

been kept steady (Christensen *et al*, 2001). Opposed to direct process evaluation, in the spot test analysis micro- organisms studied are naturally present in the samples.

The best advantage of this evaluation method is that information obtained concerns the actual content of organisms present in the biowaste. Moreover, the stabilisation phase can be analysed by comparison of the results of the finished compost and the sanitised compost.

In conclusion, if the samples are collected just after the sanitary phase, the spot test analysis is an accurate method for the analysis of the sanitary process. In addition, it is simpler and cheaper to perform than direct process evaluation (Christensen *et al*, 2001).

4.3.1. Nordic strategy

Nordic projects have tested both approaches direct process evaluation and spot test analysis in full-scale investigations on the fate of specific organisms in composting systems. The pathogens studied in each case are described in the Table 23.

The plants selected for the full-scale investigation were located in Norway, Finland, Sweden and Denmark. It should be noted that Iceland has participated to the set up of this investigation. Plants presenting different operating parameters were selected (composting in windrow, static tunnel, semi-permeable cover, household wastes, yard wastes, sewage sludge). The most common composting processes used in Nordic countries were represented among the plants investigated.

Table 23: Pathogens monitored in Nordic strategy (Christensen *et al*, 2001; Paulsrud *et al.*, 2001)

	Direct process evaluation	**Spot test analysis**
Plant pathogens	*Rhizoctonia solani* *Fusarium oxysporum* *Plasmodiophora brassicae* Tobacco mosaic virus	*Rhizoctonia solani*
Human and animal indicator pathogens	*Escherichia coli* *Enterococcus faecalis*	*Escherichia coli* *Enterococcus* Thermotolerant coliform bacteria Coliphages *Salmonella* spp Infective parasite eggs
Seeds	Tomato White clover	Weeds

Direct process evaluation was performed by means of inoculation-bags containing raw material. Smaller fibre bags, full with raw material infested with the indicator organisms or seeds, were placed

inside these inoculation-bags. The raw material used was a composite sample i.e. a sample constituted of several sub-samples. The inoculation-bags were incubated into the waste at different localisation. The duration of the sanitary phase was defined for each participating plants depending on the composting system. However, it does not exceed 4 weeks (Christensen *et al*, 2001). For some plants, wastes had to be turned or moved during the composting process. In such cases, the inoculation-bags were removed and replaced in their initial positions after these operations. At the end of the sanitary phase, the inoculation-bags were collected and the compost surrounding the fibre bag was used for chemical, physical and microbiological analysis.

For **spot test analysis**, compost samples were taken at 3 different stages of the composting process:

- raw material was collected at the start of the direct process evaluation,
- sanitised compost was collected simultaneously with inoculation-bags at the end of the sanitation phase,
- finished compost was collected after the expiration of both sanitation and stabilisation phases.

All these samples were analysed for the organisms described in table 10 but analyses for parasite eggs and seeds were carried out only on finished compost.

The Spot test analysis was chosen as being the most critical. It provides a better description of the actually achieved sanitation and is more cost effective. Therefore, this method was tested at 16- full scale composting facilities of different times throughout the Nordic countries (Christensen *et al*., 2002).

The Table 24 shows the results obtained in terms of pathogen reduction for *E. coli* and *Enterococcus* in direct process evaluation and spot test analysis on composting systems in Nordic countries.

Table 24: Results for spot test analysis and direct process evaluation on compost (Christensen *et al*, 2002)

	***E. coli* reduction (log unit)**	***Enterococcus* reduction (log unit)**
Spot test analysis	2.6 – 3.3	0.7 – 3.94
Direct process evaluation	4.89 - > 6.59	>2.9 - > 5.71

- **Selection of indicator micro-organisms**

Nordic countries have not chosen *Salmonella senftenberg* for evaluation of pathogen reduction because they took into account the risk of a dissemination of this bacterium in surrounding environment even though this organism is not described as pathogen. *E. coli* was selected for its resistance to temperature close to the one of *Salmonella*. *E. faecalis* was chosen for its high resistance to heat and chemical treatments. *C. perfringens* has not been selected because it displays a too much important resistance to heat due to its ability to form spores, thus not comparable to most pathogens in

question. Coliphages have been used as indicator of inactivation of enteric viruses because of their resistance to temperature. *Rhizoctonia solani* has been selected as plant pathogen because it is a fungi infecting numerous plants and presenting a great survival in soil (from 5 to 6 years*). Fusarium oxysporum* displays a great resistance to temperature and its removal is quite difficult (ADEME, 2001).

4.3.2. German strategy

The German strategy is based on similar principle to those of Nordic countries and has been also defined for biowastes. Nowadays, German is the most advanced country in terms of development of strategy for pathogen reduction evaluation (ADEME, 2001). The major difference between the Nordic and the German strategies is that Germany use Direct process evaluation and the Nordic countries recommend and use the spot test analysis.

As a matter of fact, Germany has selected essentially micro-organisms with specific growth characteristics such as resistance to heat and these micro-organisms has been artificially introduced in the wastes.

As in Nordic strategy, a direct process evaluation and a spot test analysis were performed. The main difference corresponding to the fact that in spot test, the micro-organisms studied were the same than the one for direct process evaluation.

The collected samples were used for analyses of human, animal and plant pathogens. The following micro-organisms were monitored for determination of sanitation efficiency:

- *Salmonella senftenberg* W775,
- *Plasmodiophora brassicae*,
- Tobacco mosaic virus,
- Tomato seeds virus.

Tobacco mosaic virus have been chosen as indicator because of their resistance to heat. *P. brassicae* is interesting for its resistance to temperature and its low sensitivity to microbial antagonism (ADEME, 2001).

4.3.3. Strategies in the other European countries

Strategies for evaluation of sanitation efficiency also exist in countries such as Switzerland, Austria, Spain or United Kingdom. The main difference among their strategies is the micro-organisms selected as indicator (Table 25).

Table 25: Micro-organisms selected for evaluation of sanitation efficiency (ADEME, 2001; Carrington, 2001; Mocé- Ilivina *et al*, 2003)

Countries	Micro-organisms
Switzerland	Enterobacteria, helminth eggs
Austria	*E. coli*, *Salmonella* spp, *Campylobacter*, *Listeria* spp
Spain	Bacteriophage, spores of sulfito-reducing bacteria, *Salmonella choleraesuis*
United Kingdom	*E. coli* and *Salmonella* spp
France	*E. coli*, Enterovirus, viable helminth eggs

5. Fate of pathogens on soils and plants after landspreading

5.1. Pathogens in soils

There is no quantitative data about the amount of pathogens in soils but some data about the survival of pathogens in soils are described (Table 26).

Table 26: Survival of pathogens in soils in France (Dapilly and Neyrat, 1999).

Pathogens	Survival conditions
Coliforms	38 days at the soil surface and 14 days on grass
Salmonella	70 days in deep areas of soils and 40 days at the soil surface
Shigella	42 days on grass
Streptococcus	38 days at the soil surface and 35 to 63 days in soils.

One of the major factors influencing the availability of waste-derived pathogens for overland flow transport is their survival in the soil environment. The survival in soil of pathogens originating from animal manures and sewage sludge has been reviewed by Van Donsel et al. (1967), Sorber and Moore (1987), Smith (1996), Nicholson et al. (2000) Carrington (2001) and Tyrrel *et al.*, (2003). Although a number of factors have been shown to influence the survival of microorganisms in soil. Those are

environmental factors (e.g. temperature, moisture content, sunlight, pH), quality of waste factors (e.g. pathogen level, organic content, competing organism, antimicrobial and toxic substances and the availability of organic matter). Microbial structure factors have been shown to influence the survival of microorganisms in soil (e.g. spore-forming bacteria, non- enveloped viruses, ova and cyst for helminth). Temperature is the most significant factor. In general, survival time increases with decreasing temperature. Nicholson et al. (2000) conclude that the majority of pathogens in manures applied to land will decline below detectable limits after 3 months. Therefore more pathogens are likely to be transported to a watercourse if an overland flow event occurs soon after faecal waste is applied to land.

The way that faecal wastes are applied to the land may also have a bearing on the survival and hence the chances of pathogen transport to watercourses. A modelling work assumed that only 50% of microorganisms are available if manures are incorporated to a depth of 10 cm (Walker *et al.,* 1990). On the other hand, the environment at the soil surface may be more hostile because of the effects of insolation (e.g. u.v. irradiation, heating and desiccation) than the environment at depth. There is relatively little evidence in the literature of studies that have addressed the question of whether or not incorporation or injection will reduce pathogen losses. In a comparative study of runoff of faecal microorganisms and nutrients from grass plots that had received either injected or surface-applied slurry Heinonen-Tanski and Uusi-Kämppä (2001) concluded that there was no clear difference between application methods. Daniel et al. (1995) found no significant difference in runoff quality (no microbial indicators measured) between surface-applied and incorporated manure. Small scale rainfall simulation experiments conducted by the authors however suggest that there is approximately a 10-fold increase in faecal coliform transport if waste is surface-applied rather than incorporated (unpublished data).

5.2. Land disposal of sewage sludge

Amendment of sewage sludge to non-food agricultural production land is perhaps the most economical means of sewage disposal. There are three methods in which liquid sludge is applied to land:

- surface spreading by tankers,
- surface spreading by rain gun,
- sludge injection (Straub *et al*, 1993).

It is very difficult to assess an acceptable risk level from sludge–borne micro-organisms when they have been released in the environment for many reasons:

- death of micro-organisms in soils is influenced by several factors such as temperature, pH, moisture, nutrient supply, sun ultra-violet exposure, climate, antagonisms with competing microflora and method of sludge application (Dumontet *et al*, 1999).
- However, the washing action of the rain can cause contamination of the land below.
- There is some problem for the pathogen level evaluation because of the background induced by animal faeces (ADEME, 1994).

Furthermore, this method can bring olfactive nuisance if the product to be spread out is not sufficiently sanitised.

Usually, after landing, pathogens are retrieved on the soil surface, on plants or at a small depth in soils. 90 to 95% of micro-organisms are accumulated up to 5cm of depth. The pathogen population decrease faster when sludge is spread on soils and plants than when they are buried (ADEME, 1998).

The evolution of indicator micro-organisms during the storage of stabilised sewage sludge has been monitored for one year (Gibbs *et al*, 1997). They concluded that a too much long period of storage was not an effective method for biosolids disinfection and this could favour bacterial regrowth.

The parasitic eggs in sludge cause problems in areas with pasture farming because they survive on soils and plants for many months. For example, *Ascaris* eggs can survive up to 14 years in soils. They are resistant to chemicals but they are quickly killed by temperatures above 55°C (Strauch, 1998).

There is very poor data on the survival of viruses on soils but one can consider that they survive at least as in sewage i.e. about a hundred days (Strauch, 1998). In general, virus survival decreases as temperature increases. However each kind of virus has quite different survival characteristics. For bacteria, temperature, pH, moisture and nutrient supply are the factors which have the greatest impact on their survival. Other important factor is microbial antagonism (Straub *et al*, 1993). The viability of *Cryptosporidium* cysts decreased within the range 20-40% in sludge treated soils over 30 days (Whitmore and Robertson, 1995). Gale (2003), concludes that root crops were exposed to 0.07log of *Salmonella* per kg and 0.033 log of *Cryptosporidium* per Kg.

Sometimes a significant number of coliform bacteria can be detected on soils were amendment products have been used. In their study, Skanavis and Yanko (1994) show that this is the result of a coliform regrowth after composting favoured by the nutrient content in the amendment materials.

6 Conclusion

The regulation on the level of micro-organisms in biowastes implicates two approaches for the control of the microbiological quality. The first consists in the control of the hygienised final product in order to minimise the dissemination of potential pathogenic germs. In this field, the severity and the nature of the microbiological criteria to respect could vary according to the type of culture for which the

product to scatter are intended (e.g.: vegetables, strawberries or pasture require higher precaution). The second approach consists in the validation of an sanitation process by the enumeration of the reduction of specific pathogens. The draft sludge and biowaste Directives requires that treatment process fulfil validation criteria in terms of log abatements..

It is important to take into account that the evaluation of the microbiological quality or of the pathogen reduction appears to be limited by different factors such as composition and conservation properties. First, an error in sampling and sample treatment is induced by the range of type of material (liquid, paste, semi-paste) for sludge and very heterogeneous material for domestic and green wastes and also the heterogeneity of the microbial composition. Moreover, there is such wide range of potential process variants that it may not be possible to define standardised methods of sampling appropriate for every type of facility. The determination of the microbiological quality of treated wastes is also limited to the analytical techniques used in routine analysis. Development of robust standard methods and rapid alternative methods for the determination of micro-organisms in biosolids is an area of active search. It is essential to have standardised methods at the European scale to be able to evaluate the microbiological quality of the different matrices.

A wide range of pathogens are likely to be present in sludge/biowaste, particularly those that contain large amounts of faecal material. In most of the data about the effect of the treatments on the reduction of pathogens, micro-organisms studied are *E. coli*, *Enterococci*, *Clostridium*, *Salmonella*, helminths and *Cryptosporidium.* It appeared to be very difficult to compare the results obtained from different studies because those are obtained with different methods and expressed in different units.

Several factors such as heat, moisture, pH can influence pathogen reduction. Heat treatment inactivates pathogens such as enteric viruses, *Salmonella*, *Cryptosporidium* oocysts and viable helminth eggs when heat is coupled with drying. The pH treatment consists to raise the pH to pH 12 by the use of lime. It allows to suspend the microbiological activity of viruses, Helminth, *E. coli*, *Enterococci* and *Clostridium* spores from 90 to 99.7%. *Salmonella* completely disappears. However, this process does not display good efficiency for *Mycobacterium* and Ascaris eggs reduction.

Thermophilic anaerobic digestion of at least 55°C has a good impact on pathogens while mesophilic anaerobic digestion does not inactivate all pathogens. On the other hand, aerobic digestion seems to be quite more efficient on total and faecal coliforms than anaerobic digestion.

The pathogens reduction observed during composting depends on the quality of the initial wastes (green waste, sludge) and the efficiency of the treatment (temperature, residence time and aeration). Composting has a better impact on reduction of *E. coli* and *Salmonella* than faecal *Enterococci*, but can also have a very efficient impact on reduction of *Enterococci.* Reactor composting has the best impact while windrow composting needs a number of turnings and a long stabilisation/maturation phase to achieve a sufficient reduction in *Enterococci* (Christensen *et al.,* 2002)

Because it is not possible to monitor all micro-organisms and because there are not always specific techniques for their quantification and identification, the concept of indicator has been developed. Indicators are endogenous micro-organisms of the sample studied which are easy to analyse and represent high resistance to treatment. Their concentration and evolution must be correlated to those of pathogens. For the moment, this last point miss some data. It appears that even if they can be pathogenic under certain conditions, *E. coli*, *Enterococci* and *Clostridium perfringens* are the most interesting to be used as indicators. On the other hand, it seems that each treatment requires specific indicator and that it would not be impossible to have an universal indicator. It seems obvious that several indicators should be chosen altogether. It would give a good opportunity to have one or more indicator actually present in the product to be sanitised and analysed. The use of indicators with different resistance levels can give a good idea for the efficiency of the sanitising treatment. The most relevant microbiological analysis would be the following: *E. coli, Enterococcus, Clostridium perfringens, salmonella* spp, *Aspergillus*, eggs of viable Heminths, *Cryptosporidium* and *Listeria monocytogenes. E. coli*, is well suited as an indicator organism for a finished product regarding presence of fecal bacteria and the stability of the product (i.e. less risk of pathogen regrowth) and also for the sanitary treatment when sampled just after expiration of the sanitary phase. *Enterococcus* is well suited as an indicator organism for the sanitary treatment when sampled just after expiration of the sanitation phase but less suited for the testing of final products due to some regrowth during the composting stabilisation phase. *Salmonella* is suited as an indicator organisms for a finished product regarding revealing possible recontamination.

For the moment, in Europe, only few countries have set up a strategy for the evaluation of stabilisation/sanitation of sludge/biowastes. Those are based on the direct process evaluation which consists to inoculate defined organisms at the beginning of the process and on the spot test analysis which consists to analyse micro-organisms naturally present in the samples. The first approach is a good tool for process optimisation but is laborious and expansive. Furthermore, it is not obvious to obtain representative samples. The second approach allows to obtain information concerning the actual content of micro-organisms present in the biowaste and is simpler and cheaper to perform than direct process evaluation.

The development of horizontal and harmonised standards will facilitate regulation. The objective of the project is to develop methods available to determine specific micro-organisms and also to reach a details protocol for validating plant performance and end product in term of hygienic microbiological parameters. Research will focus on *Enterococci*, *Clostridium perfringens*, helminth ova, *E. coli* and Salmonella, micro-organisms which have been the most studied. It seems also relevant to consider the use of bacteriophage as indicators of viruses.

However, it is important to consider that microbiological diversity is linked to climates, regions, types of biowastes, type and origin of soils, fertilisers and growing media. The product must be safe for

human but also for animals and vegetables. Horizontal project will consider the aspect of phytopathogens.

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