Horizontal Standards for Implementation of EU Directives on Sludge, Soil and Treated Biowaste

Project acronym:
HORIZONTAL

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Literature review on levels of pathogens and abatements of them in the field of sludge, soil and treated biowaste

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1 Sludge and Biowaste return to soil *(To be completed)*

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

2 Pathogens

2.1. Description of the 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)

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).

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2.1.1. Bacteria

The spectrum of bacteria depends on the epidemiological conditions in the region where the sewage sludges are collected (Strauch, 1998).

2.1.2. Virus

The type of virus varies as a function of the regions. They 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).

2.1.3. Yeast and fungi

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).

2.1.4. Parasites

Because of the specific characteristics of the life cycle of most of parasites, humans and animals are very important as intermediate and final hosts. So, parasites in sludge essentially come from faecal wastes (Strauch, 1998).

2.1.5. 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 2).

Table 2: 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

2.2. Pathogens in sludge

The agricultural utilisation of sewage sludge is common in many countries. However, these sludges contain pathogens which are excreted by the human population served by the sewers and sewage

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treatment plants. In the sewage purification processes, most of the pathogens are reduced in number but not completely removed. They are enriched by sedimentation processes in the sewage sludge.

In sludge, the most part of pathogens come from human population, companion animals and livestocks (CSHPF, 1998; Strauch, 1991). Depending on the type of wastewater, pathogens will be different (Table 3).

Table 3: Origin of pathogens present in sludge (ADEME, 1994)

Wastewater origin	Pathogens
Urban type wastewater	Pathogens specific of human and animal
Dairy wastewater	Pathogens specific of milk
Slaughter house wastewater	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 4 and Table 5 respectively.

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Table 4 : 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		

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Table 5: 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	$10^2 - 10^4$	3×10^2
	Bacteriophages	10^5	-
Bacteria	Total coliforms	$10^8 - 10^9$	7×10^8
	Faecal coliforms	$10^7 – 10^8$	8×10^6
	Enterococci	$10^6 – 10^7$	2×10^2
	Salmonella spp	$10^2 –10^3$	9×10^2
	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	$10 - 10^2$	3×10^2

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. As the description of sewage sludge pathogens has already been tackled in the previous paragraph, only the pathogens from vegetable wastes are exposed in Table 6.

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Table 6: Description of 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

2.4. Pathogens in soils

ADD SOME DATA ON THE COMPOSITION OF THE MICROBIOLOGICAL POPULATION FOUND IN SOILS

3 Monitoring

As mentioned in paragraph 1, 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.

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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).

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).

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 and EN 12579:1999) 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.

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.

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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. 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.

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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 5.

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 ?

The Table 7 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 7: 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

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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).

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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). Some micro-organisms have been retained for their specific properties (Table 8).

Table 8: 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).

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 (Table 9). However, no correlation was found between *Clostridium* and *Salmonella* or *Clostridium* and *L. monocytogenes*.

Table 9: 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

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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 10 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 10: 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

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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 11.

Table 11: 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*	*Ditylenchus dipsaci* *Longidorus spp* *Xiphiema spp*

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Crytosporidium parvum	

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 even compared to *Ascaris* ova when treatment is performed in liquid conditions. 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. (ADEME, 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 (Chabrier, 19?). Pasteurisation of sludge causes a complete removal of enteric viruses and *Salmonella* (30 minutes at

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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 12.

Table 12: Pathogen reduction obtained after thermal treatment (Mocé- Ilivina *et al*, 2003)

	Pathogen reduction (log unit)	
	Sludge treated at 80°C for 90min	Wastewater treated at 60°C
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 13) Andreadakis (19?).

Table 13: Reduction of microbial load after treatment with quicklime (Andreadakis, 19?)

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

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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 ensured a significant reduction of *E. coli* (99.7%), Enterococci (99.4%) and *Clostridium* spores (99.5%). However, this process did 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 14).

Table 14: 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

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

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Table 15: 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).

The impact of different kinds of digestion processes on pathogen is given in the Table 16. 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 16: 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

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Schwartzbrod (1997) has also studied the effect of digestion on helminth (Table 17) 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 17: 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 18 gives quantitative data about the influence of aerobic and anaerobic digestion on pathogen reduction has been studied by Straub *et al* (1993).

Table 18: Pathogen and indicator concentrations in digested sludge (Straub *et al*, 1993)

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

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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). 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 19). 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 19: 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 20 exposes the pathogen reduction (expressed in log unit) obtained after different biological treatments of biosolids.

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Table 20: Reduction of pathogens in biosolids (UKWIR (2002))

Organism	Mesophilic anaerobic digestion (MAD)	Process			
		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.

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4.3. Strategies of different countries

In Europe, only some countries have set up strategies for the evaluation of sanitisation 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 French, German and Nordic examples will be discussed in details. However, the strategies adopted by other countries will be also tackled.

♦ **<u>Direct process evaluation</u>**

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-bag 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.

♦ **<u>Spot test analysis</u>**

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

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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. French strategy

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4.3.2. Nordic strategy

The Nordic strategy consists of a full-scale investigation on the fate of specific organisms in composting systems by means of direct process evaluation or spot test analysis. The pathogens studied in each case are described in the Table 21.

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 21: 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

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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 Table 22 exposed 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 22: 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. Coliphages have been used as indicator of inactivation of enteric viruses because of their resistance to temperature. *Rhizoctonia solani* has been

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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).

4.3.3. 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 the indicator micro-organisms chosen for the process evaluation.

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).

4.3.4. 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 23).

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Table 23: 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	Salmonella senftenberg W775

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 in some papers (Table 24).

Table 24: Survival of pathogens in soils (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
*Streptococcu*s	38 days at the soil surface and 35 to 63 days in soils.

Several factors can influence the survival of pathogens in wastes spread on land:
- Microbial structure: spore-forming bacteria, non- enveloped viruses, ova and cyst for helminth;
- Environmental factors: sunlight, temperature, moisture, pH;

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- Quality of waste: pathogen level, organic content, competing organism, antimicrobial and toxic substances;
- Sludge spreading: application rate (Carrington, 2001).

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).
- There is some problem for the pathogen level evaluation because of the background induced by animal faeces (ADEME, 1994).

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

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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. 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. This leads to artificially add micro-organisms to a treatment plant and to monitor naturally occurring micro-organisms.

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

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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

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* 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.

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.

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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.

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