DISINFECTION OF HEALTHCARE WASTE (HCW) BY ALKALINE HYDROLYSIS, ITS EFFICIENCY AND EMISSIONS

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DISINFECTION OF HEALTHCARE WASTE (HCW) BY ALKALINE HYDROLYSIS, ITS EFFICIENCY AND EMISSIONS

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To my family,

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ABSTRACT

The present work aimed at providing additional knowledge about the application of wet treatments to healthcare waste (HCW), namely alkaline hydrolysis, through the study of transformations of some components usually present in HCW and characterization of effluents produced.

Samples of components usually present in HCW were subjected to autoclaving or alkaline hydrolysis under the same conditions of temperature and time. Alkaline hydrolysis caused appreciable degradation of most of the components, particularly in adhesives and diapers. The autoclaving treatment degraded the components in a much lesser extent than alkaline hydrolysis. The effluents obtained showed an appreciable organic load. Nevertheless, the effluents produced by autoclaving showed a lower organic load and were less biodegradable than the ones resulting from the alkaline hydrolysis treatment. The minimum conditions of temperature, time and concentration of NaOH to achieve the total destruction of animal tissues (pork and beef) were studied. The bone and meat containing samples were completely destroyed when subjected to temperatures higher than 95 °C with 1 M NaOH solution in less than 60 minutes. The effluents generated, although with very high pH and organic load, were biodegradable after neutralization.

Geobacillus stearothermophilus spores were used to assess the disinfection efficiency of alkaline hydrolysis. The survival curves and the D–values (decimal reduction time) were determined. The complete inactivation of spores (6 log10 reduction) was achieved at a temperature of 110 °C with 1 M NaOH in less than 5 minutes.

Based on the conditions obtained for the total destruction of animal tissues as well as for the inactivation of *Geobacillus stearothermophilus*, i.e. temperature of 110 °C, time 35 minutes and 1 M NaOH, animal tissues and discarded medical components usually present in healthcare waste were hydrolyzed and the effluents were characterized according to Portuguese legislation laying down emission limit values for discharges of waste water, and their aerobic and anaerobic biodegradability was evaluated. The effluents showed values lower than the discharge limit values for almost all the parameters, except pH, total nitrogen, TOC, COD and BOD_5. The effluents showed a coefficient of total aerobic biological degradation of 50.5 % and 52.9 % and an anaerobic biodegradability of 22.3 % and 42.2 %, for discarded medical components and animal tissues, respectively.

A real sample of infectious HCW was subjected to alkaline hydrolysis at a pilot scale, using the conditions of the treatment defined in the laboratorial study. The characteristics of the resultant effluent corroborate with studies carried out in the laboratory.

KEYWORDS: Healthcare waste; Alkaline hydrolysis; Medical waste; Autoclaving

RESUMO

O presente trabalho pretendeu acrescentar conhecimento adicional sobre a aplicação dos tratamentos de calor húmido, isto é, hidrólise alcalina, aos resíduos hospitalares, através do estudo das transformações de alguns componentes usualmente presentes nos resíduos hospitalares e da caracterização dos efluentes produzidos.

Amostras de componentes normalmente presentes nos resíduos de hospitalares foram submetidas a autoclavagem e a hidrólise alcalina nas mesmas condições de temperatura e tempo usadas no actual processo de autoclavagem. A hidrólise alcalina causou uma degradação apreciável da maior parte dos componentes, sendo esta mais expressiva para o adesivo e a fralda. Embora na autoclavagem a degradação dos componentes tenha sido bastante inferior à hidrólise alcalina, os efluentes obtidos apresentaram uma carga orgânica elevada, mas menor biodegradabilidade do que os efluentes resultantes do tratamento por hidrólise alcalina.

Nos ensaios de hidrólise alcalina realizados com tecidos de origem animal (carne de porco e carne de boi) foram avaliadas as condições mínimas de temperatura, tempo e concentração de NaOH necessárias para a destruição total dos tecidos animais. Estes foram completamente destruídos, quando sujeitos a temperaturas superiores a 95 °C e NaOH 1 M em menos de 60 minutos. Os efluentes gerados embora com elevados valores de pH e carga orgânica são biodegradáveis após neutralização.

Nos ensaios efectuados para avaliar a eficiência de desinfecção da hidrólise alcalina foi usada a estirpe do microrganismo *Geobacillus stearothermophilus*. A eficiência do tratamento alcalino foi determinada pelas curvas de sobrevivência e pelos valores D (tempo de redução decimal). A completa inactivação dos esporos (redução 6 log10) foi conseguida à temperatura de 110 °C e a uma concentração de NaOH 1 M em menos de 5 minutos.

Com base nas condições obtidas para a destruição total dos tecidos de origem animal bem como para inactivação do *Geobacillus stearothermophilus,* isto é temperatura de 110 °C, tempo de 35 minutos e concentração de NaOH 1 M, as amostras foram hidrolisadas e os efluentes produzidos foram caracterizados de acordo com a legislação portuguesa que estabelece os valores limites de emissão de descarga de águas residuais. A biodegradabilidade aeróbia e anaeróbia dos efluentes também foi avaliada. Os efluentes, na maioria dos parâmetros excepção para o pH, azoto total, COT, CQO e CBO_5, apresentaram valores inferiores aos valores limites de emissão. Os efluentes apresentaram um coeficiente de degradação biológica aeróbia total entre 50,5 % e 52,9 %, e uma biodegradabilidade anaeróbica entre 22,3 % e 42,2 %

Uma amostra de resíduos hospitalares do grupo III foi submetida ao tratamento por hidrólise alcalina, à escala piloto, nas condições de tratamento definidas nos ensaios laboratoriais. As características do efluente resultante do tratamento corroboraram os resultados obtidos nos ensaios laboratoriais.

PALAVRAS-CHAVE: Resíduos Hospitalares; Hidrólise alcalina; Autoclavagem

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LIST OF ACRONYMS AND SYMBOLS

BAT – Best Available Techniques

BEP – Best Environmental Practices

BOD – Biochemical oxygen demand

CDJ – Creutzfeldt–Jajob disease

CL – Carbon loss

COD – Chemical oxygen demand

DGS – Direção Geral Saúde

DO – Dissolved oxygen

DPA – Dipicolinic acid

DSC – Differential Scanning Calorimetric

EPA – Environmental Protection Agency

EU – European Union

EWL – European Waste List

GEF – Global Environment Facility

HBV – Hepatitis viruses B

HCV –Hepatitis viruses C

HCW – Healthcare waste

HHW – Household hazardous waste

HIV – Immunodeficiency virus

KOH – Potassium hydroxide

NaOH – Sodium hydroxide

PAHs – Polycyclic aromatic hydrocarbons

PCA – Principal Components Analysis

PCDDs – Polychlorinated dibenzodioxins

PCDFs – Polychlorinated dibenzofurans

PET – Polyethylene terephthalate

POPs – Persistent organic pollutants

PVC – Polyvinyl chloride

SDS – Dodecyl sulphate

STAATT – State and Territorial Association on Alternate Treatment Technologies

TC – Total carbon

TG – Thermogravimetric

TOC – Total organic carbon

TSE – Transmissible Spongiform Encephalopathy

UK – United Kingdom

USA – United States of America

USEPA – United States Environmental Protection Agency

VOCs – Volatile organic compounds

WHO – World Health Organization

WL – Weight loss

CHAPTER 1 – INTRODUCTION

1.1 FRAMEWORK

1.1.1 RELEVANCE OF THE STUDY

Waste management is a matter of concern in developed countries due to environmental consequences, public health and valuable resources waste, many of them strategic. In the European Union, 2.5 billion tonnes of waste are produced annually out of which 100 million tonnes are hazardous waste (Eurostat, 2010).

Healthcare waste (HCW) is a small fraction of solid waste produced, since it represents only 0.3 % of total generated in Europe (Eurostat, 2010), but it requires special attention due to the danger it represents to public health and environment. Nevertheless, the amount of HCW is increasing compared to other types of waste, mainly due to the use of medical disposable products, higher number of healthcare facilities and medical services.

HCW includes all the waste generated by healthcare establishments, research facilities and laboratories (Prüss et al., 1999). It is a heterogeneous mixture of waste with different components produced in variable amounts, differing among countries according to their particular medical management practices (Prüss et al., 1999).

Characterisation, composition and quantities of HCW generated per year and per country or region can be found in the literature (Lee and Huffman, 1996; Diaz et al., 2008). The majority of HCW, of about 75 – 90 % is not infectious, thus can be treated as municipal waste posing no additional risk to health or the environment. The remaining 10 – 25 % is classified as hazardous waste, and it represents the fraction that requires special attention and specific treatments (WHO, 2005). In Portugal, in recent years, the quantity of hazardous HCW generated was about 30 000 ton (Figure 1.1).

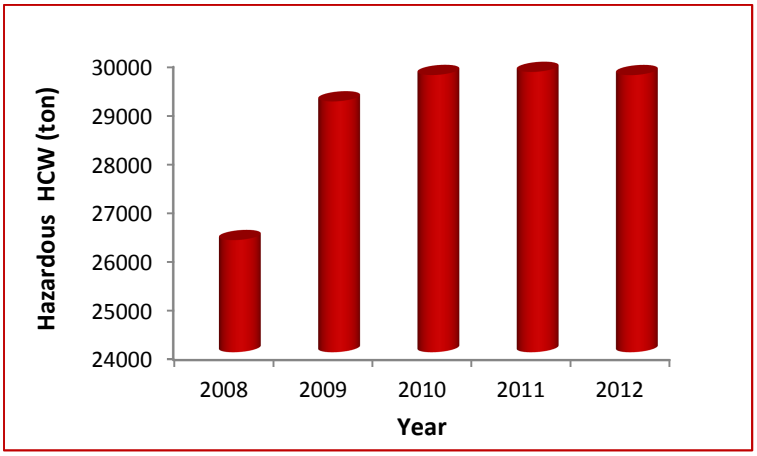

Figure 1.1 – Hazardous healthcare waste generated in Portugal (source: INE, 2014).

The implementation of efficient and environmentally friendly technologies for HCW waste treatment requires considerable technical and funding resources and a legal framework, all which are mostly lacking in developing countries. For these reasons, in these countries, is common to watch an inappropriate treatment and uncontrolled deposition of HCW that, due

to the infectious nature of the waste, may contaminate surface and groundwater, exposing the population to diseases and parasites and increasing the epidemics risk (Hossain et al., 2011).

There are several HCW treatment processes, including incineration, pyrolysis, autoclaving, chemical disinfection, encapsulation, microwave disinfection, irradiation and gasification. Autoclaving and incineration are the main processes used for treating HCW (Sukandar et al., 2006), the last being the oldest and, until now, the most used (Lee and Huffman, 1996). However, the incineration process presents a drawback, and may emit a wide range of hazardous pollutants including dioxins, furans, heavy metals, fine dust particles and other pollutants resultant of incomplete combustion (Alvim-Ferraz and Afonso, 2005; Sukandar et al., 2006; Singh and Prakash, 2007).

In 2004, the Stockholm Convention on Persistent Organic Pollutants put a focus on eliminating or reducing releases of 12 persistent organic pollutants (POPs) including dioxins and furans. Accordingly, stricter emissions limits for incinerators were introduced in the European Union (EU). Additionally, World Health Organization (WHO) has been promoting the use of non-incineration technologies to treat medical waste, eliminating the risk of dioxins and furans emissions that may be generated by uncontrolled medical incinerators.

Also, the small producers of HCW, due to issues of current technique as the storage areas needed for the HCW and the high transportation costs of untreated waste to the treatment unit, are seeking for viable alternatives technologies.

Some of the treatments applied to HCW, as autoclaving and microwave, require a mechanical process to reduce the waste volume, which increases the operating costs. Additionally, due to the low mass reduction achieved in these treatments, there is a considerable mass of waste that is disposed of in landfills; and, in some countries as Korea, there was reluctance to accept the disposal of the sterilized products in their landfills (Jang et al., 2006).

Under such guidelines, alkaline hydrolysis may be an alternative treatment process for HCW in some countries (Health Care Without Harm, 2004). This treatment has been shown to have significant advantages compared to other HCW treatments, because it sterilizes and destroys at once, and it also reduces the total waste volume. Also, alkaline hydrolysis may have a range of application larger than autoclaving, since it can also accept organic tissues.

However, the effects of various materials present in HCW during the autoclaving process as well as the final effluent composition and its treatability are not well known. This lack of knowledge makes authorities to have some reluctance in licensing autoclaving plants and in permitting their effluents to be discharged into domestic sewage treatment systems.

Thus, once the consequences of application of alkaline hydrolysis are well known, it may advantageously substitute autoclaving and incineration to treat some types of HCW and offer an interesting basis of decentralised treatment for reducing the risks of infection from handling and transporting HCW.

1.1.2 OBJECTIVES OF THE WORK

The present work aimed at providing some additional light on the application of wet treatments to HCW, namely autoclaving and alkaline hydrolysis. It intends to help waste treatment operators, regulatory authorities, general public concerned with wastes and as well as researchers to clarify some particularities of those two processes through carefully obtained experimental data. Some of these aspects include answers to questions as the following: how common materials in HCW behave under these treatments, and, in particular, which are the minimum conditions of alkaline hydrolysis to destroy animal tissues? Which conditions guarantee disinfection when HCW contain very resilient infectious microorganisms? What kind of effluents is produced and are they compatible with discharge in public sewage network? Thus, this work established some objectives and tasks as follows:

1 – Study the transformations of some components generally present in HCW and characterize the effluents produced, at laboratory scale using similar conditions to autoclaving.

2 – Study the transformations of some components generally present in HCW and characterize the effluents produced by alkaline hydrolysis, i.e. using similar conditions of temperature and time to those used in autoclaving but with different NaOH solution concentrations, at laboratory scale.

3 – Make the alkaline hydrolysis process an alternative treatment for animal tissues and discarded medical components present on HCW by determining in laboratory:

i. milder conditions than those described in the literature for destroying animal tissues with bones and make them unrecognizable.
ii. effective minimum sterilization conditions using a very resilient biological indicator.
iii. the characteristics of the effluents from the treatment.
iv. the aerobic and anaerobic biodegradability of the effluents obtained.

4 – Test the alkaline hydrolysis process with a real HCW sample under pilot scale conditions:

i. design and build a reactor to scale up the laboratorial process to the range of tenths of liters not far from 100 L.
ii. make at least one test with a real sample of hazardous HCW using the conditions of treatment defined in the laboratorial study and determine the characteristics of the effluent and waste obtained.

1.1.3 OUTLINE

The outline of the work is shown in Table 1.1, including the synthetic description of the content approached in each chapter.

Table 1.1 – Outline of the work

CHAPTERS	CONTENT
CHAPTER 1 INTRODUCTION	Introduces the subject relevance, objectives and outline of the work. Presents an overview of HCW as: definition; classification; generation; composition and management.
CHAPTER 2 HEALTHCARE WASTE TREATMENT TECHNOLOGIES	Presents and describes the technologies used on the treatment of HCW.
CHAPTER 3 ALKALINE HYDROLYSIS	Describes the alkaline hydrolysis process.
CHAPTER 4 EFFECTS OF ALKALINE HYDROLYSIS AND AUTOCLAVING ON DISCARDED MEDICAL COMPONENTS PRESENT IN HEALTHCARE WASTES	Studies the behaviour of some discarded medical components usually present in healthcare waste when subjected to treatments of alkaline hydrolysis or autoclaving.
CHAPTER 5 APPLICABILITY OF ALKALINE HYDROLYSIS TO DESTROY ANIMAL TISSUES PRESENT IN HEALTHCARE WASTES	Studies the behaviour of animal tissues usually present in HCW when subjected to alkaline hydrolysis treatment.
CHAPTER 6 INACTIVATION OF *Geobacillus stearothermophilus* SPORES BY ALKALINE TREATMENT	Studies the effects of NaOH concentration and temperature on the inactivation of *Geobacillus stearothermophilus* spores**.**
CHAPTER 7 CHARACTERIZATION OF EFFLUENTS RESULTANT FROM ALKALINE HYDROLYSIS TREATMENT	Characterize the effluents produced when alkaline hydrolysis is applied to animal tissues and discarded medical components common in HCW and assess their aerobic and anaerobic biodegradability.
CHAPTER 8 SCALE-UP EXPERIMENTS	Alkaline hydrolysis tests with a real infectious HCW sample using a designed reactor with 70 L of capacity.
CHAPTER 9 CONCLUSIONS AND FUTURE WORK	Presents the main conclusion and suggests future work.

1.2 HEALTHCARE WASTE CHARACTERISTICS

1.2.1 DEFINITION AND CLASSIFICATION

The **definition** of healthcare waste is not universal and is relatively variable worldwide. Many terms as “medical waste”, “clinical waste”, “hospital waste”, “biomedical waste” and “infectious waste” have often been used in the literature. These terms can have different meanings according to its designation; the severe differences between them often depend on which legal or regulatory authority is defining the term. Indeed, in some countries the definition of medical waste is often different due to the existing regional legislation. In Spain, most of regional laws use the common term “medical waste” and only one region, of Cantabria, uses the term “hospital waste”.

Medical waste is defined, according to the WHO, as “all the waste generated within healthcare facilities, research centers and laboratories related to medical procedures. In addition, it includes the same types of waste originating from minor and scattered sources, including waste produced in the course of healthcare undertaken in the home (e.g. home dialysis, self-administration of insulin, recuperative care)”. In the United States of America (USA), the Medical Waste Tracking Act of 1988 (MWTA, 1988) defines medical waste as "any solid waste that is generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals”. This definition is commonly used in some countries, such as Greece, (Hellenic legislation, Ministerial Decision 37591/2031) or India, although in the later the term used is “biomedical waste” (Biomedical Waste Rules, 1998). According to the Chinese legislation the medical waste is defined “as waste characterized by infectious, toxic and other hazardous properties deriving directly or indirectly from medical treatment, prevention, health protection, and other related activities in healthcare institutions” (PR China State Council, 2003). The current legal definition of clinical

waste in the United Kingdom (UK) is taken from The Controlled Waste Regulations 1992, issued under the Environmental Protection Act 1990. It has remained unchanged since it was first issued under the Collection and Disposal of Waste Regulations 1988, issued pursuant to the Control of Pollution Act 1974. Clinical waste is defined as: (a)"...any waste which consists wholly or partly of human or animal tissue, blood or other bodily fluids, excretions, drugs or other pharmaceutical products, swabs or dressings, syringes, needles or other sharp instruments, being waste which unless rendered safe may prove hazardous to any person coming into contact with it; and (b)"…any other waste arising from medical, nursing, dental, veterinary, pharmaceutical or similar practice, investigation, treatment, care, teaching or research, or the collection of blood for transfusion, being waste which may cause infection to any person coming into contact with it."

The Portuguese legislation defines hospital waste as "waste resultant from healthcare activities to humans or animals, in the areas of prevention, diagnosis, treatment, rehabilitation or research and education, as well as other activities involving invasive procedures such as acupuncture, piercings and tattoos" (Decree Law No 73/2011).

Medical waste is often considered as a subcategory of healthcare waste or hospital waste which contains the waste with infectious character. WHO defined infectious waste as "the waste type suspected to contain pathogens (viruses, bacteria, parasites or fungi) in enough concentration or quantities to cause disease in susceptible hosts" (Prüss et al., 1999).

The **classification** of healthcare waste is not uniform worldwide and brings some confusion and disagreement between countries, regions and institutions. Several classifications have been used in legislation and in the literature. Healthcare waste may be classified into different types according to the source of production, chemical and physical composition, type and risk factors associated with their handling, storage and ultimate disposal. In the early 1980s, WHO

suggested a simple classification of HCW into eight categories; currently there are seven categories as shown in Table 1.2 (Chartier et al., 2014).

Table 1.2 – Healthcare waste classification according to WHO.

Waste type	Description
General Waste	Wastes that have similar properties to the household solid waste.
Infectious to potentially infectious waste	Waste contaminated with blood and its by-products, cultures and stocks of infectious agents, waste from patients in isolation wards, discarded diagnostic samples containing blood and body fluids, infected animals from laboratories, and contaminated materials (swabs, bandages) and equipment (such as disposable medical devices).
Pathological waste	Human tissues or fluids, e.g. body parts, blood and other body fluids, fetuses.
Sharps waste	Syringes, needles, disposable scalpels and blades, etc.
Chemicals waste	Waste containing chemical substances, e.g. laboratory reagents, film developer, disinfectants that are expired or no longer needed, solvents.
Pharmaceuticals waste	Expired, unused and contaminated drugs; vaccines and sera.
Radioactive waste	Such as glassware contaminated with radioactive diagnostic material or radiotherapeutic materials.

According to United States Environmental Protection Agency (EPA 40 CFR Part 60.51c), the wastes are divided into six categories (Table 1.3). In Greece, according to the Hellenic legislation (HMWC, 2003), the wastes are classified into three categories; in Turkey, the Medical Waste Control Regulation (MWCR, 2005) categorized wastes in four categories; Chinese national legislation (PR China MOH, SEPA, 2003) and the federal resolutions of Brazil (ANVISA, 2004 and CONAMA, 2005) classify wastes in five categories; in Korea, under Waste Management Act in 1999, exist six categories, and India define ten categories in accordance with Biomedical Waste Rules, 1998 (Table 1.4 to Table 1.9). In the case of Spain, wastes classification varies from region to region in the range of three to seven categories, according to the regional laws of the Autonomous Communities (Table 1.10).

Table 1.3 – Healthcare waste classification according U. S. Environmental Protection Agency.

Waste type	Description
Cultures and stocks	Cultures and stocks of infectious agents and associated biologicals, including cultures from medical and pathological laboratories; cultures and stocks of infectious agents from research and industrial laboratories; wastes from the production of biologicals; discarded live and attenuated vaccines; and culture dishes and devices used to transfer, inoculate, and mix cultures.
Pathological and chemo wastes	Human pathological wastes, including tissues, organs, and body parts and body fluids that are removed during surgery or autopsy or other medical procedures and specimens of body fluids and their containers.
Human blood and blood product	Liquid waste human blood; products of blood; items saturated and/or ripping with human blood; or items that were saturated and/or dripping with human blood including serum, plasma, and other blood components.
Sharps	Sharps that have been used in animal or human patient care or treatment or in medical, research, or industrial laboratories, including hypodermic needles, syringes (with or without the attached needle), Pasteur pipettes, scalpel blades, blood vials, needles with attached tubing, and culture dishes (regardless of presence of infectious agents).
Animal wastes	Contaminated animal carcasses, body parts, and bedding of animals that were known to have been exposed to infectious agents during research (including research in veterinary hospitals), production of biologicals, or testing of pharmaceuticals.
Isolation wastes	Biological waste and discarded materials contaminated with blood, excretion, exudates, or secretions from humans who are isolated to protect others from certain highly communicable diseases or from isolated animals known to be infected with highly communicable diseases.

Table 1.4 – Healthcare waste classification in Greece according to the Hellenic legislation (HMWC, 2003).

Waste type	Description
Household type medical wastes	Wastes that have similar properties to the household solid waste.
Hazardous medical wastes	Waste with solely infectious properties, such as body tissues, blood, fecal and urine samples from patients with an infectious disease, needles etc. Waste with both infectious and toxic properties, such as chemotherapy related waste, waste from bio-pathology and histology laboratories, etc. Waste with solely toxic properties, such as waste that contains mercury, hazardous organic waste, expired drugs, filters, etc.
Other types of medical waste	Radioactive waste, batteries, pressurized cans, etc.

Table 1.5 – Healthcare waste classification in Turkey according to the Medical Waste Control Regulation (MWCR, 2005).

Waste type	Subgroup	Description
Municipal Wastes	General wastes	Wastes generated from offices, warehouse, kitchen, etc.
	Packaging wastes	Recyclable materials such as paper, cardboard, plastics, glass, metals, etc.
Medical wastes	Infectious wastes	Microbiological laboratory wastes, blood, objects and products contaminated with blood, used surgical gloves, dialysis wastes, quarantine wastes, air filters that contain bacterium and viruses, infectious organ pieces.
	Pathological wastes	Tissues, organs, placenta, blood, wastes produced from surgical operations.
	Sharps objects	Needles, syringes, broken glass, blades and other objects that could cause a cut or puncture.
Hazardous wastes		Hazardous chemicals, cytotoxic, amalgam wastes, gynotoxic and cytotoxic wastes, pharmaceutics wastes, heavy metal containing wastes, pressurized vessels.
Radioactive wastes		Collected and removed according to Turkey atomic energy council act.

Table 1.6 – Healthcare waste classification according to the China Ministry of healthcare, 2003.

Waste type	Description
Infectious waste	Hazardous medical wastes carrying pathogenic microorganisms which can cause the infectious disease transmission.
Pathological waste	Medical waste generated in the human body and medical laboratory animal carcasses during the diagnosis and treatment, including tissues, organs, blood and body parts and fluids.
Sharp objects	Needles, syringes, broken glass, blades and other items that could cause a cut or puncture.
Pharmaceutical waste	Overdue, eliminated or contaminated waste drugs.
Chemical waste	Hazardous chemicals, heavy metal containing wastes, toxic, corrosive, flammable and explosive waste drugs.

Table 1.7 – Healthcare waste classification in Brazil according to federal resolutions (ANVISA, 2004 and CONAMA, 2005).

Waste type	Description
A	Waste representing risk to public health and the environment due to presence of biological agents such as: blood, bodily fluids, drainage fluids or excreta (ex. surgical gloves, gauze, cotton, bandages; laboratory plates and blades; microorganism cultures; vaccines discarded and devices used for transference, inoculation or stir cultures).
B	Substances representing risk to public health and the environment, depending on their characteristics of inflammability, corrosiveness, reactivity and toxicity (ex. some chemical products and pharmacological substances).
C	Radioactive waste.
D	General waste represented by recyclable materials (e.g. paper, cardboard, plastic, metals, and glass) and non-recyclable (e.g. organic substances, food leftover, and toilet paper).
E	Sharp devices (ex. needles, syringes, lancets and similar tools).

Table 1.8 – Healthcare waste classification in Korea according to Waste Management Act in 1999.

Waste type	Description
Tissue	Human or animal pathological wastes, including tissues, organs, blood, pus, and body parts and fluids that are removed during autopsy or surgery.
Absorbent cotton	Items (e.g. cotton pads, bandages, disposable diapers, or bedding) saturated or stained with human or animal blood, pus, discharge, or secretion.
Discarded medical plastics	Disposable syringe, blood bag or waste from blood dialysis.
Pathological waste	Culture and stocks of infectious agents from test or examination, culture dishes, discarded blood fluids and containers; items that were in contact with infectious agents, such as used slides and cover glass.
Waste sharps	Discarded sharps, hypodermic needles, syringes, surgical blades and blood lancets.
Waste mixed with infectious waste	Wastes that are not classified into the above categories but mixed or in contact with waste tissue and waste sharps.

Table 1.9 – Healthcare waste classification in India according to the Biomedical Waste Rules, 1998.

Waste type	Description
Human anatomical waste	Human tissues, organs and body parts.
Animal waste	Animal tissues, organs, body parts, carcasses, bleeding parts, blood and experimental animals used in research.
Microbiology and biotechnology waste	Waste from lab culture, specimens from microorganisms, vaccines, cell cultures, toxins, dishes, devices used to transfer cultures.
Waste sharps	Syringes, needles, disposable scalpels and blades, glass.
Discarded medicines and cytotoxic drugs	Contaminated, expired, discarded drugs.
Soiled waste	Waste contaminated with blood and body fluids including cotton, dressings, and soiled plasters.
Solid waste	Tubes and catheters.
Liquid waste	Waste generated from laboratory and washing, cleaning, disinfection.
Incineration waste	Ash from incineration of any medical wastes
Chemical waste	Chemicals used in production of biologicals, disinfection

Table 1.10 – Healthcare waste classification in Spanish Autonomous Community of Madrid.

Groups	Waste type	Description
I	Municipal waste	Waste comes from no polluted and infectious risk areas of the hospital (ex. papers, glass, plastic, metals, wood or food remains).
II	Non-specific waste	Includes waste from medical activities without infectious or toxic risk, such as dialysis filters, gloves, probes, and bandages, which have not been in contact with patients.
III	Anatomical substances	These substances belong to category 1 of the hazardous waste listed by Directive 91/689/EEC and Royal Decree 833/1988.
IV	Large pieces of human remains	
V	Chemical products	Includes chemical substances, pharmaceuticals, medicines and veterinary compounds, biocides and phyto-pharmaceutical substances.
VI	Cytostatic waste	Waste is only generated by medical activities related to cancer treatment as carcinogenic, mutagenic and teratogenic drugs.
VII	Radioactive waste	This waste refers to radioactive chemical elements that do not have a practical purpose, as well as those products that have come in contact with them, including solid and liquid substances.

Portuguese legislation classifies HCW in four categories (Table 1.11), the two first are considered non-hazardous waste and the third and fourth ones are hazardous waste (Order No 242/96).

Table 1.11 – Healthcare waste classification according Portuguese legislation (Order No 242/96).

Groups	Waste type	Description
I	Wastes similar to municipal ones	Wastes that do not present special requirements in their treatment such as: wastes from general services, support services, kitchen and packaging (paper, cardboard, etc).
II	Non-hazardous hospital wastes	Wastes that do not require specific treatment and can be considered similar to municipal wastes. Orthopedic supplies, diapers and disposable guards, personal protective equipment from general and support services without contamination and no traces of blood. Serum bottles without contamination except those from group IV. Empty containers of drugs except those included in group III and group IV.
III	Hospital wastes with biological risk	Waste contaminated or suspected of contamination susceptible of incineration or other effective pre-treatment before elimination as municipal wastes, such as waste from rooms and wards for infectious patients or suspected of infectious, materials contaminated with blood or traces of blood, anatomical not identified pieces, materials used in dialysis, etc.
IV	Specific hospital wastes	Wastes with compulsory incineration, such as animal tissues, organs, body parts, carcasses of animals used in research, sharps materials, chemical substances, pharmaceuticals and cytostatic waste.

In some countries of European Union, as United Kingdom, the healthcare classification is based in the European Waste List (EWL). In order to standardize the waste classification, in December 1993, the European Union publishes the EWL adopted by Commission Decision 2000/532/EC, as amended by Commission Decisions 2001/118/EC, 2001/119/EC and 2001/573/EC. In the EWL, wastes are referred to using 6 digit numerical codes, the first two digits of the code relate

to the EWL chapter, the second two digits relate to sub-grouping within the chapter, and the final two digits are specific to the waste (Table 1.12). HCW are identified with code 18 in the EWL, which includes various categories. Wherein, the first sub-grouping corresponds to the waste generated in diagnosis, treatment or prevention of disease in humans and the second sub-grouping corresponds to the waste generated from research, diagnosis, treatment or prevention of disease involving animals. In general, the healthcare wastes are categorized in two types: general waste and special waste or non-infectious and infectious waste, thus defined as non-hazardous and hazardous wastes. Therefore, the EWL considers both types of waste and defines a separate category for cytotoxic agents and chemicals. Indeed, the HCW can be further characterized to a more universal form as shown in Figure 1.2 (Chartier et al., 2014).

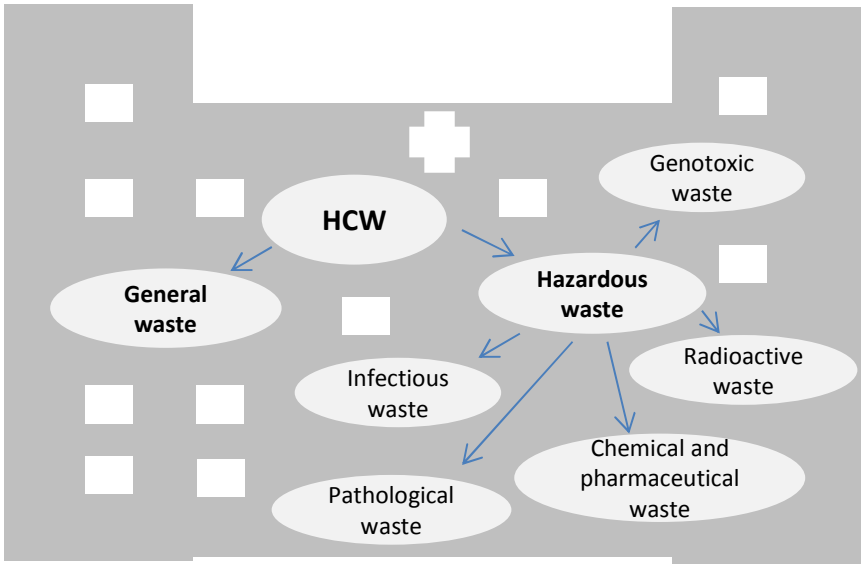

Figure 1.2 – Healthcare waste classification.

Table 1.12 – Healthcare waste classification according to the European Waste List.

18 01	**Wastes from natal care, diagnosis, treatment or prevention of disease in humans**
18 01 01	Sharps (except 18 01 03)
18 01 02	Body parts and organs including blood bags and blood preserves (except 18 01 03)
18 01 03*	Wastes whose collection and disposal is subject to special requirements in order to prevent infection
18 01 04	Wastes whose collection and disposal is not subject to special requirements in order to prevent infection (e.g. dressings, plaster casts, linen, disposable clothing, diapers, 'Controlled Waste' as defined in NZS 4304:2002 Management of Healthcare Waste)
18 01 06*	Chemicals consisting of or containing hazardous substances
18 01 07	Chemicals other than those mentioned in 18 01 06
18 01 08*	Cytotoxic and cytostatic medicines
18 01 09	Medicines other than those mentioned in 18 01 08
18 01 10*	Amalgam waste from dental care
18 02	**Wastes from research, diagnosis, treatment or prevention of disease involving animals**
18 02 01	Sharps (except 18 02 02)
18 02 02*	Wastes whose collection and disposal is subject to special requirements in order to prevent infection
18 02 03	Wastes whose collection and disposal is not subject to special requirements in order to prevent infection
18 02 05*	Chemicals consisting of or containing hazardous substances
18 02 06	Chemicals other than those mentioned in 18 02 05
18 02 07*	Cytotoxic and cytostatic medicines
18 02 08	Medicines other than those mentioned in 18 02 07

1.2.2 GENERATION

There has been an increase in the quantities of HCW compared to municipal waste mainly due to changes in healthcare policies, higher number of healthcare facilities, medical services and to the use of medical disposable products. In USA more than 3.5 million tons of HCW are produced each year (Lee et al., 2004); India generates about 3 million ton/year (Mohankumar and Kottaiveeran, 2011), while China produces 650 000 ton/year (Yang et al., 2009). In

Portugal, near 90 000 ton are generated every year (DGS, 2006). Thus, the quantities of HCW generated do not depend only on the population but also on several factors such as the medical situation of each country and its waste management systems.

The major sources of HCW are hospitals, but other sources as clinics, healthcare establishments, laboratories and research facilities, mortuary and autopsy centers, animal research, blood banks, veterinary surgeons, dentists, nursing homes are also generating extensive quantities of HCW (Prüss et al., 1999). In Portugal, about 94 % of the total HCW production has been generated by hospitals (DGS, 2006).

In recent years, there have been an increasing number of patients receiving a home healthcare and medical services. The home-based healthcare services also produce substantial quantities of HCW with a variety of waste materials. Some researchers (Ojeda-Benítez et al., 2013; Yasuda and Tanaka, 2006) have studied the characteristics, generation and composition of household hazardous waste (HHW). Gu et al. (2014) found that approximately 17.7 % of the HHW stream was medicines in Suzhou, China. These wastes are still included in general household waste even though they are infectious (Blenkharn, 2008). Nevertheless, there is no management guideline and legislation for this type of waste even in developed countries as Japan (Miyazaki et al., 2007).

The quantities of HCW generation differ among countries by their waste medical management practices allied on the level of economic development. The developing countries generate lower quantities of HCW than the developed countries (Prüss et al., 1999). The quantities of HCW generated are usually depicted as a function of the bed capacity of healthcare services (kg/bed/day); however, in healthcare facilities where the beds are not available the production is expressed as kg/patient/day. For the low-income countries, WHO estimated a HCW generation range of 0.5 – 3 kg/bed/day and for the high income countries a HCW generation range of 1.1 – 12 kg/bed/day (Prüss et al., 1999). The high-income countries generate on

average up to 0.5 kg/bed/day of hazardous waste, while low-income countries generate on average 0.2 kg/bed/day of hazardous waste. However, the real quantity of hazardous waste can be much higher in low-income countries because the HCW are often not separated into hazardous or non-hazardous wastes (WHO, 2014). According to WHO, the North America generates 7 – 10 kg/bed/day of HCW, while South America produces just 3 kg/bed/day. In Asia, richer countries generate 2.5 – 4.0 kg/bed/day and poorer countries produce 1.8 – 2.0 kg/bed/day of HCW. The same happens in Europe, on Western the production rate is about 3 – 6 kg/bed/day while on Eastern the production rate is 1.4 – 2.0 kg/bed/day of HCW (Prüss et al., 1999). Diaz et al. (2008) reported that the quantities of HCW in various types of facilities located in developing countries are lower than in some industrialized countries. They described that the range of HCW and infectious wastes in developing countries varies from 0.016 to 3.23 kg/bed/day and 0.01 to 0.65 kg/bed/day, respectively. This wide variation is due to the fact that some facilities, used in their study, provide very basic services and thus quantities of the waste generated are relatively small. In fact, the quantities of HCW generated depend on the level of economic development, and the developed countries generate more quantities of HCW mainly due to the the use of medical disposable products, higher number of healthcare facilities and medical services and greater consumption of disposable instruments and packing materials. Moreover, in developing countries the HCW are still handled, stored and disposed together with municipal wastes for which reason the amount of HCW is smaller than really produced (Rudraswamy et al., 2013; Hossain et al., 2011; Bendjoudi et al., 2009; Sawalem et al., 2009).

The quantities of HCW generated per country in kg/bed/day can be found in the literature and Table 1.13 shows the values found in some references.

The quantity of HCW generated varies not only from country to country, but also within the same country mainly due to the type of healthcare establishment, the collection and disposal

methods adopted by healthcare services managers, hospitals departments or services, and the use of medical disposable products as a result of developing healthcare technology. Prüss et al. (1999) estimated HCW generation according to source size of healthcare establishment, namely university hospital, general hospital, district hospital and primary healthcare center.

Table 1.13 – Healthcare waste generation in different countries.

Country/city	Waste generation (kg/bed/day)	Reference
Algeria	0.7 – 1.22	Bendjoudi et al. (2009)
Bangladesh/Dhaka and Khulna	0.55 – 1.10	Akter and Tankler (2003)
China	0.5 – 3.0	Changping et al. (2012)
Croatia	1.2	Marinkovic et al. (2008)
El Salvador/San Salvador	0.37	Johnson et al. (2013)
Greece/Attica	0.24 – 0.27	Komilis et al. (2011)
Iran/Tabriz	3.48	Taghipour and Mosaferi (2009)
Italy	1.3 – 2.87	Giacchetta and Marchetti (2013)
Jordan (northern)	0.83	Abdulla et al. (2008)
Jordan (southern)	0.73	Fraiwan et al. (2013)
Korea	0.48	Jang et al. (2006)
Kuwait	3.87 – 7.44	Alhumoud and Alhumoud (2007)
Mongolia/Ulaanbaatar	0.78	Shinee et al. (2008)
Nigeria/Lagos	0.43 – 0.67	Longe and Williams (2006)
Portugal	3.3	Botelho and Pinto (2010)
Sudan/Khartoun	0.80 – 1.71	Saad (2013)
Taiwan	2.41 – 3.26	Cheng et al. (2009)
Tanzania	0.75	Manyele and Anicetus (2006)
Turkey/Istambul	2.11	Eker and Bilgili (2011)
USA	5 – 7	Medical Waste Committee (1994)*

*Taken from Lee et al. (2004).

The highest generation rate was in university hospital with 4.1 – 8.7 kg/bed/day followed by general hospital, 2.1 – 4.2 kg/bed/day, district hospital and primary healthcare center with a generation rate about 0.5 – 1.8 kg/bed/day and 0.05 – 0.2 kg/bed/day, respectively. Shinee et

al. (2008) reported medical waste generation rate (kg/patient/day) in the inpatient services of public healthcare facilities was 1.4 to 3.0 times higher than in the outpatient services a sample of 56 healthcare facilities at Ulaanbaatar, capital of Mongolia. Sawalem et al. (2009) studied the amounts of HCW generated in fourteen different healthcare facilities, in three cities with different size and population, in Libya. These cities were: Tripoli a large city, Misurata a medium-sized city and Sirt a small city. They have showed that the highest generation rate was found in Tripoli and the lowest rate was found in the clinics and rural health centers. This fact was due to the medical centers in Tripoli were more developed general public facilities and serve a higher number of patients. Abdulla et al. (2008) and Fraiwan et al. (2013) studied the medical waste management practices in northern and southern Jordan. The average amount of medical waste generated in both regions is not the same, the northern produced 0.83 kg/bed/day and southern produced 0.73 kg/bed/day.

In the Portuguese hospitals, according to DGS (2006), the region of Lisbon and Tejo Valley produced the highest generation rate of 3.8 kg/bed/day followed by the northern region with 2.6 kg/bed/day. Those two regions are the ones with higher population density. Coker et al. (2009) characterized and quantified the medical wastes generated in Ibadan, Nigeria. They selected 52 healthcare facilities based in size and function. Their results indicated that large hospitals and clinics generated the greatest amounts of medical wastes followed by the small healthcare units, which have facilities to treat only outpatients, and diagnostic service laboratories. Different results were obtained by Cheng et al. (2010) when investigated the quantities of medical waste generated in small clinical facilities (private clinics, medical laboratories, blood centers and public clinics) in Taiwan. They reported that the production rate of medical wastes was higher at the small clinics (3.97 kg/bed/day) than at the large hospitals (2.41 – 3.26 kg/bed/day). The highest quantities of infectious wastes generated were from blood centers (3.14 kg/bed/day) and the lowest were from public clinics

(0.053 kg/bed/day). Graikos et al. (2010) evaluated the production rate of HCW generated in two laboratories and in two departments of the healthcare facility of social insurance institute of Xanthi, Greece. The injection therapy showed the highest HCW production rate of 0.145 kg/patient/day while the surgery department had a production rate of 0.041 kg/patient/day. The clinical pathology laboratory showed higher HCW production rate (0.071 kg/patient/day) than X-ray laboratory (0.023 kg/patient/day). Taghipour and Mosaferi (2009) determined the quantity and generation rate of medical waste generated in 10 hospitals at the city of Tabriz, Iran. The maximum quantities produced of medical waste and hazardous–infectious waste were associated with non-governmental organization and private hospitals. A similar conclusion was reported by Eker and Bilgili (2011) when studied the amounts of HCW generated in 375 healthcare services in Istanbul, Turkey. Their study indicates that the major producers of medical waste were the private hospitals, while the amount of hazardous waste generation is much higher at state hospitals. Komilis et al. (2011), in the study conducted in 95 public and private medical facilities in the Attica region, Greece, concluded that no correlation among the number of beds and the unit medical waste generation rate could be established. Each hospital should be studied separately due to the differences in the type of hospitals, the number of occupied beds, number of external patients and the types of departments or laboratories.

The implementation of standardized and optimized management practices leads to the reduction of HCW quantities, mainly a decrease of generation rate of hazardous waste. According to Chartier et al. (2014), 75 – 90 % of HCW is classified as general waste and 10 – 25 % is classified as hazardous waste, of which 1 % is made of cutting and perforating materials, 3 % are discarded chemicals and pharmaceuticals and less than 1 % is radioactive and genotoxic matter and heavy metal content. However, some authors reported different

fractions of hazardous waste on the total HCW generated in several countries Table 1.14 summarize their results.

Table 1.14 – Hazardous and non-hazardous wastes in different countries.

Country/city	Hazardous Waste (%)	Non-Hazardous Waste (%)	Reference
USA	15	85	Lee and Hufman (1996)
Bangladesh/Dhaka and Khulna	20	80	Akter and Tankler (2003)
Croatia	14	86	Marinkovic et al. (2008)
Mauritius	10	90	Mohee (2005)
Brazil, São Paulo	25	75	Moreira and Gunther (2013)
Iran/Tabriz	29.9	70.1	Taghipour and Mosaferi (2009)
Libya	28	72	Sawalem et al. (2009)
Kuwait	10 – 15	85 – 90	Alhumoud and Alhumoud (2007)
Nigeria/Lagos	26 – 37	63 – 74	Longe and Williams (2006)
Turkey/Istambul	28.8	71.2	Eker and Bilgili (2011)

1.2.3 COMPOSITION

HCW is a heterogeneous mixture of wastes, with different components in different countries, depending on their medical situation (Zhao et al., 2009). A sample of HCW can contain plastics, food waste, paper, pathological waste, animal carcasses, blood soaked-bandages and many other types of materials (Lee and Huffman, 1996).

In order to develop appropriate waste management policies, it is essential to characterize the composition of the waste stream. With this purpose, several authors have studied the composition of HCW generated in several types of healthcare establishments or hospitals worldwide (Prüss et al., 1999; Lee et al., 2004; Shinee et al., 2008; Diaz et al., 2008; Graikos et al., 2010). Some studies, where HCW were not differentiated into hazardous and non-

hazardous waste, showed that plastics have the highest contribution in total HCW with 35 % to 20.1 %, (Fraiwan et al., 2013; Coker et al., 2009) followed by paper with about 12.6 – 38 % (Taghipour and Mosaferi, 2008; Abdulla et al., 2008;). Others indicated that the major component present in HCW was organic waste/food waste, with about 31 % (Dehghani et al., 2008; Taghipour and Mosaferi, 2008. Metals constitute the minor component in HCW with a percentage of 1.1 – 6.7 % (Figure 1.3). Coker et al. (2009) reported the composition of medical waste into eleven fractions, specifying the various components in each fraction, including animal infected anatomical fraction (Figure 1.3).

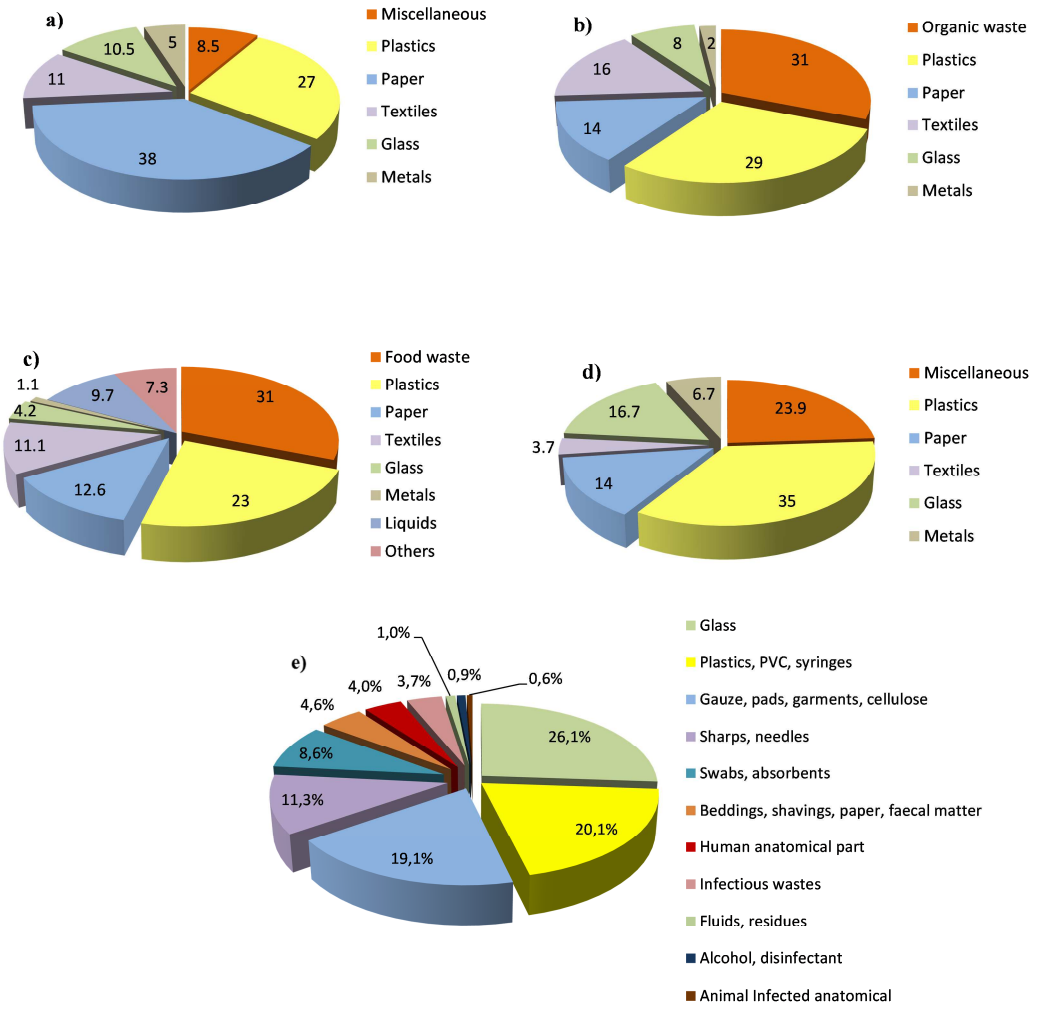

Figure 1.3 – The HCW composition reported by four studies: a) Abdulla et al. (2008); b) Dehghani et al. (2008); c) Taghipour and Mosaferi (2008); d) Fraiwan et al. (2013); e) Coker et al. (2009).

The knowledge of the waste stream contents, essentially the composition of non-hazardous fraction of the waste, is advantageous in the development of reduction and recycling programs (Diaz et al., 2008). Therefore, the composition of non-hazardous waste in different countries have been examined in various studies (Mohee, 2005; Alhumoud and Alhumoud, 2007; Taghipour and Mosaferi, 2008; Sawalem et al., 2009). Again, these studies indicated that organic waste or food waste, plastics and paper have the highest contribution for non-hazardous HCW (Figure 1.4). The high plastic content found is due to the widespread use of disposables rather than reusable for various purposes such as bottles, packing materials and bags.

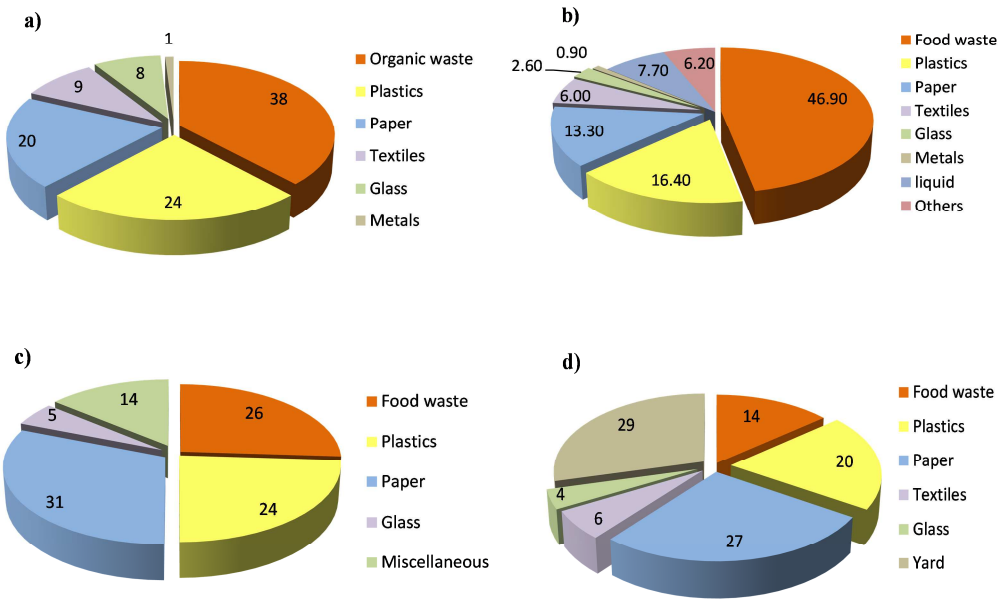

Figure 1.4 – The non-hazardous waste composition reported by: a) Sawalem et al. (2009); b) Taghipour and Mosaferi (2008); c) Alhumoud and Alhumoud (2007); d) Mohee (2005).

Taghipour and Mosaferi (2008), in their study of the composition of hazardous waste (Figure 1.5), reported plastics as having the highest contributions in hazardous waste with 35.7 % followed by the textiles with 20.8 %. Sharps represent about 1 % within hazardous waste. Other study (Marinkovic et al., 2008) reported that sharps represented 8 % of the hazardous

waste generated in Croatia. The sharps fraction is inserted into the metals component in the HCW composition.

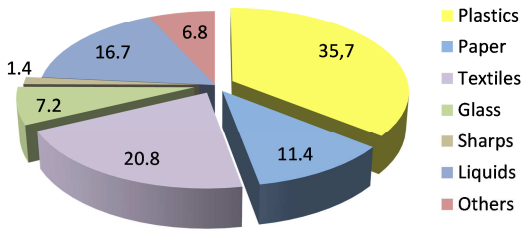

Figure 1.5 – The hazardous waste composition reported by Taghipour and Mosaferi (2008).

These studies permit to conclude that waste composition varies among different countries. This variability can be explained by different HCW management systems mainly by preventing or minimizing production, appropriate segregation and recycling programs.

1.2.4 OTHER CHARACTERISTICS

Waste specific weight is defined as the weight of uncompacted material relative to its volume. Waste specific weight is expressed as mass per unit of volume, e.g., kg/m^3. The knowledge of this parameter is fundamental in waste management, such as for determination of storing space; definition of size for the collection vehicle and estimation of requirements for compaction, size reduction, disinfection, and other equipment to treat this type of waste. Table 1.15 shows the waste specific weight of HCW reported in some studies. The data show that the average specific weight for total waste varies from 99.6 to 218 kg/m^3 while for general waste ranges from 101.3 to 211 kg/m^3 and for hazardous–infectious waste the values fluctuated from 96.2 to 262 kg/m^3. The reason for such variation is mainly due to different amounts of plastics and paper/cardboard in the composition of generated HCW.

Table 1.15 – Specific weight of healthcare waste in different studies.

Country /Reference	Average specific weight (kg/m^3)		
	Total waste	General waste	Hazardous–infectious waste
Iran/Taghipour and Mosaferi (2008)	99.6	101.3	96.2
Peru/Diaz et al. (2008)	218	211	226
Philippines/Diaz et al. (2008)	-	151	262

The specific weight of various components present into HCW was also reported in literature (Lee and Huffman, 1996; Diaz et al., 2008).

Table 1.16 shows the specific weight for several components of HCW generated in Guayaquil, Ecuador and in USA. The specific weight vary from 56 to 960 kg/m^3 in the first study (Diaz et., 2008) and in the second study (Lee and Huffman, 1996) the data show the wide range of values for some of materials present into HCW, the sharps and needles having the highest value.

Other two important variables, in the case of incineration of the HCW, are the **moisture content,** i.e., the quantity of water contained in a material, and the **heating value**, the amount of heat produced by combustion of a unit quantity of a fuel. Table 1.16 lists typical moisture contents and heating value of several components of HCW. The reported values show a wide variety allied to the high heterogeneity of healthcare waste.

Table 1.16 – Specific weight, moisture content and heating value of various components of healthcare waste in Ecuador, India and USA.

Component	Specific weight (kg/m^3)	Moisture content (%)	Heating value (kcal/kg)	Country/Reference
				Ecuador/Diaz et al.
General waste	56.22	–	–	**(2008)**
Kitchen waste	322.19	47.07	2087	
Yard waste	126.25	–	–	
Paper/cardboard	65.14	16.20	2899	
Plastic/rubber	85.35	14.87	7076	
Textiles	120.27	30.41	1985	
Sharps	429.11	–	–	
Food waste	580.19	44.95	3269	
Medicines	959.71	64.18	3340	
Garden waste	–	40.24	1863	
				India/Gupta and Boojh
Human anatomical	–	70–90	50–800	**(2006)**
Kitchen	–	70	1400	
Clinical	–	0–30	3600–4500	
Paper	–	0–10	4700	
Cotton	–	0–10	4700	
Plastics	–	0–1	9000–11100	
				United States/Lee and
Human anatomical	810–1215	70–90	444–2000	**Huffman (1996)**
Plastics	80–2330	0–1	7700–11100	
Swabs, absorbents	80–1000	0–30	3100–6700	
Alcohol, disinfectants	780–1000	0–0.2	6100–7800	
Infected animals	490–1300	60–90	500–3600	
Glass	2840–3650	0	0	
Bedding, shavings, paper, fecal matter	320–750	10–50	2200–4500	
Gauze, pads, garments, paper, cellulose	80–1000	0–30	3100–6700	
Sharps, needles	7300–8100	0–1	0–33	
Fluids, residuals	1000–1020	80–100	0–1100	

1.3 HEALTHCARE WASTE MANAGEMENT

The management of HCW is a challenge, principally in most healthcare facilities of the developing world. Poor practices, inappropriate handling and HCW disposal methods as well as inappropriate treatments can expose the population to parasites and diseases, like typhoid fever, tuberculosis, diarrhoea and skin infections, thus increasing the epidemics risk (Diaz et al., 2005; Coker et al., 2009). The development of upgraded waste management practices in middle- or low-income countries are hampered, essentially, by insufficient financing and management commitment to develop improved treatment and disposal facilities (Rushbrook, 1999; Ananth et al., 2010). In countries as Nigeria, Ethiopia, Indonesia and Sudan, where the HCW has not received sufficient attention, there is no HCW waste legislation at the national level, existing only some federal general environmental regulations. The HCW is still handled and disposed together with domestic waste causing high health risks to all health professionals, patients, community and the environment (Coker et al., 2009; Anagaw et al., 2012; Kühling and Pieper, 2012; Saad, 2013). An inadequate healthcare handling operation is a potential contamination source principally for health professionals because a poor handling can originate infections inside hospitals (Park et al., 2009). This type of infection originated in hospitals is called a nosocomial infection. Studies conducted in some developing countries have reported hospital-wide nosocomial infection rates mostly higher than 15 % with a range from 6 % to 27 % (Mayon-White et al., 1988; Pittet et al., 2008). The hands are the main route of transmission, and approximately 21 % of nosocomial infections are caused by microorganisms present in the medical personnel hands. These infections explain around 25 % of the extra hospitalization days (Silva, 2001).

In 2002, the results of a study made by the WHO in 22 developing countries showed that the possibility of contracting an infection, promoted by improper handling and disposal HCW

methods, varies from 18 % to 64 %, in these countries the risk is two to twenty times higher than in developed countries (WHO, 2004; Pittet et al., 2008).

Several authors have studied the risk of exposure to serious infection, such as *Mycobacterium tuberculosis* (Johnson et al., 2000), pathogenic viruses and bacteria, including *Pseudomonas* spp., *Lactobacillus* spp., *Staphylococcus* spp. (Park et al., 2009), hepatitis B and hepatitis C (Franka et al., 2009; Anagaw et al., 2012) in medical waste. Health professionals, as nurses and waste workers, are especially at risk of acquiring infections caused by injury with syringe needles contaminated with human blood, or laboratory specimens containing immunodeficiency virus (HIV), hepatitis viruses B (HBV) and hepatitis viruses C (HCV) (Anagaw et al., 2012). Epidemiological studies indicate that the percentage of an individual contracting an infection when in contact with an infected syringe being with HBV, HCV and HIV is 30 %, 1.8 % and 0.3 %, respectively (WHO, 2014). In 2000, the WHO estimated the population that was infected with contaminated syringes: 21 million infected with the hepatitis B virus (HBV), 2 million with the hepatitis C virus (HCV) and 260 thousand with HIV (Chartier et al., 2014).There is strong evidence that these infections can occur when sharps waste is poorly managed (Chartier et al., 2014). In Africa, this fact is more patent due to high cost of safety boxes for proper disposal of sharps that limits their use (Rudraswamy et al., 2013). For healthcare workers, the fractions of infections that are due to percutaneous occupational exposure to HBV, HCV and HIV are 37 %, 39 % and 4 %, respectively. It is estimated that more than two million healthcare workers are exposed to percutaneous injuries with infected sharps every year (Prüss-Üstün et al., 2005). The annual number of HBV infections in the USA resulting from exposure to healthcare waste was between 162 and 321 (Chartier et al., 2014).

The production of disposable syringes was assumed as a solution to minimize the risk of infections. However, in developing countries, there has been an opposite effect with the re-use and recycling of disposable syringes, thereby increasing healthcare risks. Tamplin et al.

(2005) studied the issues and options for the safe destruction and disposal of syringes reused in developing countries.

Developed countries, and organizations like WHO and USEPA, due to the high risk of contracting diseases by contaminated needles and syringes, promote a public awareness and solutions for safe disposal of self-injections, such as home needle destruction device, household hazardous waste collection and drop-off collection sites.

Many studies have focused on HCW management practices in developing countries. The studies were carried out in countries such as Bangladesh (Akter and Tränkler, 2003), Mauritius (Mohee, 2005), Nigeria (Longe and Williams, 2006 and Coker et al., 2009), Tanzania (Manyele and Anicetus, 2006), Mongolia (Shinee et al., 2008), Algeria (Bendjoudi et al., 2009), Iran (Taghipour and Mosaferi, 2009), China (Yong et al., 2009), Libya (Sawalem et al., 2009), India (Mohankumar and Kottaiveeran, 2011), Ethiopia (Debere et al., 2013) and Sudan (Saad, 2013).

Several developing countries are faced with a serious problem that is poor scavengers; women and children collect some of the HCW for reselling, despite the health risks to which they are subjected (Diaz et al., 2005; Blenkharn, 2006; Coker et al., 2009; Patwary et al., 2011). The scavengers, involved with waste collecting, scavenging, recycling and resale to the community, have access to potentially hazardous medical waste due to poor waste segregation and inadequate disposal of HCW (Blenkharn, 2006; Coker et al., 2009). Patwary et al. (2011) studied the illicit economy associated with scavenging and recycling of HCW in Dhaka, Bangladesh. They reported that in some cases the scavengers' motivation for being involved in the collection of HCW is often related to drug use, but exist other motivations, including service to the community. Outside the scavengers and cling operators, other groups are involved in scavenging and recycling HCW as healthcare facilities employees that sell hazardous items directly to scavengers. According to Patwary et al. (2011), many people benefit from the Bangladesh unregulated and informally managed HCW system. More recently, Patwary et al. (2012) reported that HCW scavengers in Bangladesh who were

involved in unauthorised scavenging and reselling of HCW are essentially young people with problems as homelessness sexual abuse and drug use.

The need to develop a healthcare waste management in order to reduce the environmental risks caused by an inadequate treatment or disposal of wastes brings together various organizations such as WHO, UNICEF, USEPA and national governments. National governments have an important role in this intervention through implementation of HCW management guideline and a strict legislation, as well as defining responsibilities and training requirements for agreement with the resources of each country.

The WHO defines some guidelines to be adopted through legislation to this waste, namely: a) a clear definition of hospital waste and their categories; b) a precise indication of the legal obligations regarding the production, handling and safe disposal; c) specifications regarding the maintenance of records and reports; d) specification for implementing a control system in order to ensure law enforcement and appropriate penalties to be imposed for violation of these; e) name of the judge responsible for the handling of disputes arising from the application or non-compliance with the law.

WHO also suggests the establishment of a document to state the reasons for enforcement and to mention the national targets and key steps to achieve these targets, based on the following items: a) description of the health and safety risks arising of poor management of HCW; b) list of treatment and disposal methods adopted for each waste category, c) warning against incorrect practices for disposal of hazardous waste such as disposal of this type of waste in landfills for municipal waste; d) the producer responsibility within the management and outside the health establishments; e) assessment of costs associated with the management of HCW; f) key steps for waste management, technical specifications and guidelines for implementation of each stage, i.e., minimization, separation, storage, transport, treatment and

final disposal; g) record keeping and documentation; h) training requirements standard safety and security applied to individuals who directly handle the HCW.

Several countries adopt the WHO recommendations and guidelines; however, the directives of USEPA are used, too. In many developed countries, specific guidelines and regulations have been implemented for HCW management systems that are more effective than those in many developing countries (Yong et al., 2009). The major reasons of HCW poor management are the lack of appropriate legislation, lack of specialized healthcare professionals, lack of awareness and effective control (Hossain et al., 2011). Some studies reported that healthcare workers do not have education enough and most of them do not have any special training on the management of HCW, often performing most activities without correct guidance and insufficient protection (Shinee et al., 2008; Diaz et al., 2008; Coker et al., 2009; Ananth et al., 2010; Nema et al., 2011; Anagaw et al., 2012).

Although some developed countries already implemented guidelines for HCW management there are still some flaws in hospitals of UK (Blenkharn, 2007), Croatia (Marinkovic et al., 2008) and Italy (Giacchetta and Marchetti, 2013). The Jordan healthcare facilities represents one of the most advanced and comprehensive healthcare systems in the Middle East. Nevertheless, the practices of medical waste management in major hospitals in southern Jordan were suffering from serious defects, namely the segregation that is performed inaccurately due to the lack of both training and proper education (Fraiwan et al., 2013).

Fundamental principles of HCW management include preventing or minimizing production, appropriate segregation of general HCW from hazardous-infectious waste, aggregation of general HCW to the municipal waste stream for final disposal and using effective treatments for hazardous-infectious HCW. Preventing the formation of HCW contributes significantly to the reduction of waste management costs in healthcare. The cost to handle and dispose of hazardous HCW is indeed substantially higher than municipal waste handling (Tudor et al.,

2005). When domestic waste is mixed with HCW it must be treated as part of the HCW stream which handling costs about 20 times more than municipal waste (Giacchetta and Marchetti, 2013). The implementation of sustainable decision making regarding resource use, including methods of waste minimisation at the source and recycling, lead to the reduction of waste. Healthcare professionals training and awareness underpin several of the short and medium/long term solutions suggested to reduce the waste at the source and recover value from that produced, could potentially reduce disposal quantities by as much as 20 – 30 % (wt.) and costs by around 25 – 35 % (Tudor et al., 2005).

As HCW contains high amounts of reusable and recyclable materials (Marinkovic et al., 2008), developing of reusing and recycling programs for non–hazardous wastes can serve as a means of reducing rising quantities of waste generation and treatment costs (Lee et al., 2004; Blenkharn, 2005). Presently, many healthcare facilities in developed countries as USA and UK have recycling programs for non–hazardous wastes materials as paper, cardboard, metal and glass (Hossain et al., 2011).

1.3.1 SEGREGATION, COLLECTION AND STORAGE

The most important step in waste management of medical waste is the appropriate **segregation** of hazardous-infectious waste from the general waste. Segregation and sending of general HCW to municipal waste disposal can reduce at least 70 % of the total quantity of generated waste (Taghipour et al., 2014). The hazardous waste portion is usually separated into two portions: used sharps and potentially infectious items. Since sharps may cause injuries, both contaminated and uncontaminated sharps should be collected in a puncture-proof and impermeable container that is difficult to break or open after closure.

One of the practical ways for segregation is colour coded method. Many countries have national legislation that prescribes the waste segregation categories to be used, and where there is no national legislation WHO has available an easy color coding (Table 1.17) for healthcare professionals to put waste items into the correct container**.** The segregation of infectious waste is made in yellow bags, despite some countries, such as Greece, Korea and Turkey, use red bags. In Spain, non-specific wastes are collected in green bags and, in some Autonomous Communities, are used color blue containers for cytostatic waste. In Portugal the segregation is made according the Order No 242/96, the two first groups are collected in black bags, the third group in white bags and fourth in red bags, exception for the sharps which are collected in impermeable container. The colour bags and containers (Figure 1.6) for segregation of HCW should be available to staff in each medical and other waste-producing area in a healthcare facility. This system allows to segregate and dispose waste at the point of generation, as well as reduces the need for healthcare professionals to carry waste through a medical area. In USA, the segregation of HCW is usually made considering three types: red bag; pathological and general waste.

Table 1.17 – WHO – Recommended segregation scheme (Chartier et al., 2014).

Type of waste	**Colour of container and markings**
Highly infectious waste	Yellow, marked “HIGHLY INFECTIOUS”, with biohazard symbol
Other infectious waste, pathological and anatomical waste	Yellow with biohazard symbol
Sharps	Yellow, marked “SHARPS”, with biohazard symbol
Chemical and pharmaceutical waste	Brown, labelled with appropriate hazard symbol
Radioactive waste	Labelled with radiation symbol
General healthcare waste	Black

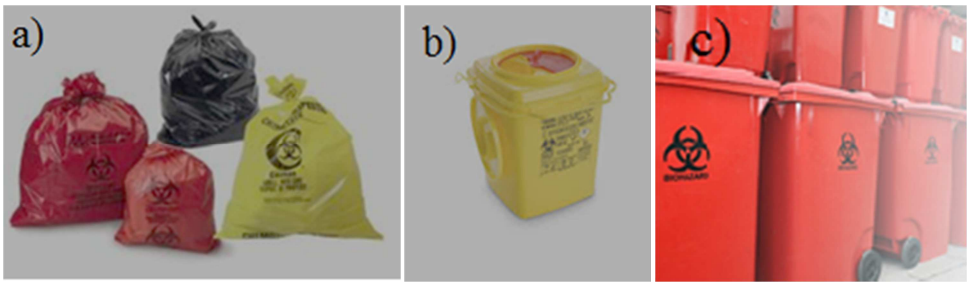

Figure 1.6 – a) Colour plastics bags for waste collection; b) Container for sharps collection; c) Waste containers.

The **collection** stage includes the process of packaging and labelling. The bags are collected only after being filled up to three-fourths of its volume to avoid overloading and prevent possible spills. Once this level is reached, they should be sealed and are ready for collection. Collection times should be fixed and adequate to the quantity of waste produced in each area of the healthcare facility. General waste should not be collected at the same time or in the same trolley as infectious or other hazardous wastes (Chartier et al., 2014).

According to WHO, the collection should be daily for most wastes, with collection timed to match the pattern of waste generation during the day. The hazardous waste generated in medical areas should be stored in utility rooms, to avoid the contact between HCW and patients before removal, then collected and transported to a central storage facility.

After segregation and collection of the HCW, the wastes are temporary **stored** in a large container, properly labelled and packed. These containers can be of varied shapes and sizes and be made from diverse materials. Many modern waste containers are designed for automated systems that empty their contents into the waste disposal system and wash and disinfect them mechanically. Additionally, waste containers may also be made of reused plastic and metal. Moreover, they should be sturdy and leak-proof, and lined with a sturdy plastic bag. The container and the bag should have the same color for the waste they are intended to receive, to avoid potential confusion and poor segregation (Chartier et al., 2014).

1.3.2 TRANSPORT, TREATMENT AND DISPOSAL

Onside **transport** of HCW is carried out through pre-established routes, which include specific corridors and elevators, and deposited in an adequate area designated for that purpose before treatment and final disposal. Despite the fact that most of the hospitals have not any special place for waste storage, the infectious waste should be kept in a refrigerator at a temperature preferably no higher than 3 °C to 8 °C if stored for more than a week to avoid biodegradation, odours, insects and rodents.

Offsite transport of hazardous HCW should comply with national regulation and with international agreements if wastes are shipped across an international frontier for treatment. In Portugal, the transport of waste within the national territory is established by ordinance No. 335/97. If there is no national regulation, responsible authorities may refer to recommendations on the transport of dangerous goods published by the United Nations.

The international agreement which regulates the transboundary movements of hazardous waste produced worldwide is the Basel Convention. Composed by 181 parties, specifically refers to transportation of clinical wastes from medical care in hospitals, medical centers and clinics and waste pharmaceuticals, drugs and medicines.

Drivers of vehicles carrying hazardous HCW should have appropriate training about risks and handling of hazardous waste. Vehicles or containers used for transporting HCW should be exclusively used on transport of this type of wastes. Vehicles should be kept locked at all times, except when loading and unloading, and kept properly maintained. Vehicles and transporting containers used for the transportation of waste should be cleaned and disinfected daily after use (Chartier et al., 2014).

There are several processes that can be used to treat hazardous HCW, including chemical disinfection, encapsulation, microwave disinfection, irradiation, gas sterilization, pyrolysis and

oxidation. However, autoclaving and incineration are the most used to treat this type of waste (Sukandar S., 2006). According to some studies, about 40 – 60 % of wastes are incinerated, and 14.5 – 37 % are treated by autoclaving (Lee et al., 2004; Moreira and Günther, 2013). Some countries, such as Poland (Gielar and Helios-Rybicka, 2013), Croatia (Marinkovic et al., 2008) and Korea (Jang et al., 2006) used both methods to treat the HCW. Nevertheless, incineration has been the most used process to treat HCW all around the world until now (Lee, 1996; Changping et al., 2012). In developing countries, the major hazardous HCW fraction is incinerated and is disposed on the land (i.e. open dump). The remaining waste, mainly domestic waste, generated by the healthcare facilities is disposed of as municipal waste.

1.4 HEALTHCARE MANAGEMENT IN PORTUGAL

Until mid-eighties of the XX century, the management of HCW was incipient, and generally these wastes were deposited in dumps or landfilled together with the municipal waste, despite being considered specific waste. In 1985, the Decree Law No 488/85 determined the requirements for institutions that generated HCW to set up an inventory updated with the quantity, nature, origin and destination of wastes; and those institutions were still responsible for the collection, transport and disposal of these wastes. Later, in 1990, the Order No 16/90 classified the HCW in two groups: contaminated waste (group A) and non-contaminated waste (Group B), and also defined the type of treatment to which they must be subjected. The waste of group A should be incinerated, while the wastes of group B were considered general waste and thus did not require any special treatment. In the nineties, all hazardous HCW were incinerated in more than 40 on-site incinerators without any environmental monitoring, causing serious environmental problems. In the last two decades, due to the increase of HCW production and the impossibility of compliance with the air emissions limits it was considered essential to implement new rules for HCW management.

In 1996, through Order No. 242/96, the HCW was classified in four groups (see Table 1.11), with the main objective of reducing the amount of waste to be treated and of introducing alternative processes to incineration. This legislation follows the main guidelines recommended by the WHO with concern to HCW management, including the steps for segregation, collection, storage, transport, treatment and disposal. As a result of a better screening, taking into account the classification of the HCW, the quantities of hazardous waste decreased from 25 000 tons in 1995 to 16 000 tons in 2004 (DGS, 2006).

According to WHO recommendation, in 1999 it was approved, through Joint Order No 761/99, the **Strategic Plan for Healthcare Waste 1999-2005**. It was the first national legislation for HCW, prepared by both Ministries of Health and Environment. This plan defines these important guidelines: accentuate the responsibility, supervision and control of administrators of the HCW units; encourage the development of management plans; improve the safety of segregation and packaging at production; reduce and environmentally adapt the existing treatment units; rationalize the collection circuits and storage units; concentrate the treatment by incineration in a small number of units, with responsiveness to current and future production; reinforce the use of new technologies to treat the waste of Group III; promote training and/or information of stakeholders in the process, professionals, users and the general public; create a permanent Commission to monitor the implementation of the Plan.

In 2005, 75 – 90 % of HCW were treated as municipal waste (groups I and II), out of which 20 % were recycled and the remaining were disposed of in landfills or incinerated by municipal waste incinerators. Concerning waste of Group III, it was incinerated or subjected to an effective treatment that allowed its disposal in a municipal landfill. During 2005, approximately 12 000 tons were produced, 92 % of which were autoclaved, 8 % incinerated and only a small fraction was treated by chemical disinfection (DGS, 2006). Currently, the HCW of group III are treated in five units of autoclaving throughout the country. More recently (April 10th, 2013)

opened a new unit to treat HCW of group III by microwave technology, located in Eco Parque do Relvão, Chamusca. All wastes from group IV are incinerated in continental Portuguese territory in an incineration plant, licensed since 2007, located in the park of Health in Lisbon and another incinerator is located in Madeira Island.

In 2010, the Strategic Plan for Healthcare Waste 1999-2005 was revised and in 2011 was approved through Order No. 43/2011 the second **Strategic Plan for Healthcare Waste 2011-2016**. This Plan aims at emphasizing prevention measures, introducing the approach to lifecycle of product and materials, highlighting the reduction of environmental impacts from the production and management of HCW, and strengthening the notion of economic value associated with them. Also, it emphasizes the incentive to waste recovery and the use of the materials resulting from the recovery, considering elimination the last management option.

Thus, the latter plan included the following categories of waste for recovery: paper and cardboard; organic matter; packaging and packaging waste; waste electrical and electronic equipment; batteries and accumulators; used cooking oil; bulky waste; computer consumables, dental amalgam; chemicals rejected and other waste resulting from radiology activities. Thereby increasing the amount of waste collected, in 2006 the selective collection already represented over 20 % of HCW of group I and group II.

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CHAPTER 2 – HEALTHCARE WASTE TREATMENT TECHNOLOGIES

2.1 INTRODUCTION

The essential purpose of HCW treatment is to reduce the potential health and environmental hazard (Chartier et al., 2014). Indeed, the treatment should inactivate pathogens using different disinfection methods for potentially infectious waste. The State and Territorial Association on Alternate Treatment Technologies (STAATT) define the following microbial inactivation levels, shown in Table 2.1 (Health Care Without Harm, 2004).

The recommended minimum criterion for HCW disinfection, based on the STAATT, is the level III although, the microbial inactivation achieved in some processes is the level IV.

A large number of processes are available to treat HCW. Most of them use the same fundamental principle of heat, chemicals, irradiation, biological and mechanical.

The **thermal processes** use heat to destroy pathogens present on HCW and can be subdivided into low-heat and high-heat processes. In these two processes, occur different

thermochemical reactions and physical changes on the waste as well as different atmospheric emissions depending on the diverse types of equipment. Generally, low-heat thermal technologies operate between 95 °C and 250 °C (Salkin et al., 2000), and take place in either moist or dry-heat environments. The low-heat technologies are autoclaving, dry heat and microwave. The high temperatures thermal technologies operate from approximately 500 °C to greater than 6 000 °C (Salkin et al., 2000). Incineration, pyrolysis, gasification and plasma are considered to be high-heat technologies.

Table 2.1 – Levels of microbial inactivation.

Level I	Inactivation of vegetative bacteria, fungi and lipophilic viruses at a 6 $\log_{10}$ reduction or greater.
Level II	Inactivation of vegetative bacteria, fungi, lipophilic/hydrophilic viruses, parasites and mycobacteria at a 6 $\log_{10}$ reduction or greater.
Level III	Inactivation of vegetative bacteria, fungi, lipophilic/hydrophilic viruses, parasites and mycobacteria at a 6 $\log_{10}$ reduction or greater; and inactivation of *G. stearothermophilus* spores and *B. subtilis* spores at a 4 $\log_{10}$ reduction or greater.
Level IV	Inactivation of vegetative bacteria, fungi, lipophilic/hydrophilic viruses, parasites and mycobacteria, and inactivation of *G. stearothermophilus* spores and *B. subtilis* spores at a 6 $\log_{10}$ reduction or greater.

Chemical processes use disinfectants such as dissolved chlorine dioxide, sodium hypochlorite, or other chemicals to eliminate pathogens.

Irradiation processes employs electron beams irradiation, cobalt-60 or ultraviolet sources to treat HCW.

The **biological processes** consist on the degradation of organic matter by natural living organisms when applied to HCW treatment. Enzymes are sometimes used to accelerate the destruction of organic waste containing pathogens.

Mechanical processes, although they cannot destroy pathogens, these methos reduce substantially waste volume using technologies as shredding, grinding, mixing and compaction. Normally these processes are supplement of other treatment methods as microwave or steam sterilization**.**

Incineration is the oldest method, and until now, the most used in the world (Lee and Huffman, 1996). According to Lee et al. (2004), about 59 – 60 % of hazardous waste are incinerated, 20 – 37% are steam sterilized, and 4 – 5 % are treated by other methods. In developed countries the most used processes are incineration and autoclaving, these two technologies are considered mature technologies (Salkin et al., 2000; Sukandar et al., 2006). In developing countries, due to low costly compared to other methods, the most common methods for treating or disposing of HCW are open dumping and landfilling (Soares et al., 2013; Bendjoudi et al., 2009; Coker et al., 2009; Sawalem et al., 2009; Shinee et al., 2008; Rogers and Brent, 2006). The production of degraded waste, leachate and gas generated by landfilling are, also, the highest source of public health infection and environmental pollution.
In these countries, incineration is also used, however the full combustion is not always completely achieved creating further negatives environmental impacts (Bendjoudi et al., 2009; Coker et al., 2009). Figure 2.1 shows a simple incinerator used to treat HCW in a developing country.

Figure 2.1 – Incinerator used to treat HCW in a developing country (source: Diaz et al., 2005).

Table 2.2 shows the most usual processes to treat and dispose of HCW in different countries.

Table 2.2 – Treatment and disposal processes of HCW in different countries.

Country	Treatment or disposal processes	Reference
Algeria	Open dumping Incineration	Bendjoudi et al. (2009)
Brazil	Open burning Incineration Autoclaving Microwave	Soares et al. (2013)
Croatia	Incineration Sterilization	Marinkovic et al. (2008)
India	Chemical Disinfection Autoclaving Hydroclaving Microwave Incineration Compacting and shredding	Gupta et al. (2009)
Korea	Autoclaving Incineration	Jang et al. (2006)
Lybia	Dumping Incineration	Sawalem et al. (2009)
Mauritius	Incineration Landfill	Mohee (2005)
Mongolia	Open dumping or open burning Incineration	Shinee et al. (2008)
Nigeria	Dumping Burning Incineration	Coker et al. (2009)
Portugal	Autoclaving Microwave Incineration	
Spain	Steam sterilization Autoclaving Incineration	Insa et al. (2010)
Tanzania	Open burning Incineration Autoclaving	Manyele and Anicetus (2006)

According WHO, the selection of HCW treatment processes must be cost effective, easily implemented and environmental friendly. The selection of treatment involves also consideration of waste characteristics, quantity of waste, types of waste as well as reduction of their volume.

Incineration requires high financial start-up costs and occupational capital to implement incineration facilities (Hossain et al., 2012; Coker et al., 2009; Sawalem et al., 2009; Blenkharn, 2005). The capital costs needed for autoclaving are lower, followed by microwave technologies. Karagiannidis et al. (2010) reported that costs of HCW sterilization in Greek hospitals ranged from 0.52 to 1.76 € kg^{-1}. Soares et al. (2013) based on the life cycle assessment, reported that costs for microwaves treatment of HCW were US$ 0.12 kg^{-1} and costs for the HCW treated by autoclaving were US$ 1.10 kg^{-1}. Tudor et al. (2009) showed that the costs of HCW sterilization varied from US$ 470 to US$ 627 ton^{-1} and for incineration ranged US$ 783–1253 ton^{-1}.

The treatment can be carried out on-site or off-site of healthcare facilities. Normally, the middle-sized and large healthcare facilities treated their HCW on-site while the small-sized healthcare facilities use off-site systems. Still, the selection of each system is a controversial subject. Some studies compared both methods and determined their advantages and disadvantages for hazardous HCW treatment (Taguipour et al., 2014).

The off-site systems have the disadvantages of having to transport infectious waste through public roads and the added cost of transport, such as fuel costs. However, it has the advantage of the economy of scale and it is the preferred approach in many developed countries (Health Care Without Harm, 2004). Currently, the quantities of off-site incineration systems have been increasing due to several regulations concerning on-site incineration (Lee et al., 2004). The on-site systems have the advantage of disinfecting infectious waste close to the source of generation. By avoiding the problem of transporting infectious waste through public roads, the

potential for accidental release of infectious materials is reduced (Health Care Without Harm, 2004). More recently appeared a new system – mobile treatment – that is mounted on trucks and is brought to different health facilities to treat their waste. This treatment unit has the disadvantage of high capital and operating costs.

Another important factor is the public acceptability. Due to risk of pollution, the public acceptance for incineration process is quite low, and for this reason many countries, such as USA, Greece and Canada, decreased the number of units used to treat hazardous HCW (Soares et al., 2013; Karagiannidis et al., 2010). Due to problem of pollution caused by HCW incineration through the production of dioxins and furans, and considerable amounts of heavy metals (Gielar and Rybicka, 2013; Singh and Prakash, 2007; Sukandar et al., 2006), many government and state regulatory agencies, namely European Union, introduced more stringent emission standards for HCW incinerators. To meet these enhanced requirements, many incinerators units should be provided with air pollution control devices that represented an important additional cost, for which reason several units were simply deactivated. In USA, since 1997, over 2 000 incinerators were shut down and in some states, such as California (Walton et al., 2008), the incineration of infected waste was effectively prohibited. In Portugal more than 40 on-site HCW incinerators were closed in the nineties of the last century.

In order to meet the Stockholm Convention on Persistent Organic Pollutants and to protect public health, the WHO promotes the use of non-incineration technologies (also called alternatives technologies to incineration) to treat hazardous HCW. Consequently, the Global Environment Facility (GEF) in partnership with WHO and Health Care Without Harm funded the Global Healthcare Waste Project with the objective to protect public health and the global environment from the impacts of dioxin and mercury releases by incinerators. The project also involves a number of other partners at a global, regional and national level. This project convers seven countries: Argentina, India, Latvia, Lebanon, Philippines, Senegal and Vietnam

and focuses primarily on activities, such as promoting the use of non-burn waste treatment technologies, improved waste segregation practices and the use of appropriate alternatives to mercury-containing devices.

In recent years, new non-incineration technologies being considered for the treatment of HCW include autoclaving, microwaving, irradiation, alkaline hydrolysis and biological treatment. However, no single technology can treat all types of HCW, i.e., more than one treatment technology may be needed to treat all types of the stream waste. Each technology has its advantages and disadvantages, so it is still necessary to determine the most appropriate type of waste technology to be used to minimize the consequences for environment, protect the public health and improve the occupational safety (Health Care Without Harm, 2004).

2.2 THERMAL PROCESSES

2.2.1 AUTOCLAVING

Autoclaving or steam sterilization have been used, since 1876, when Charles Chamberland built the first pressure steam sterilizer for the sterilization of surgical instruments, medical devices, heat stabile liquids, as well as numerous applications in medical laboratories and private industry (Salkin et al., 2000).

In autoclaving the waste is loaded into the unit and saturated steam (steam holding water as a vapor) is introduced, forcing the air out of the chamber. The removal of the air from the chamber is done in three ways: gravity-displacement; pre-vacuum (or high-vacuum) and pulse or multi-vacuum cycle. The high vacuum method is the most effective and fastest. With steam accumulation, the pressure and temperature within vessel increases until the minimum temperature and pressure are sufficient for the wastes treatment and these conditions are maintained during a determined time, referred to as the exposure period. Generally, the

minimum time and temperature for waste treatment are 30 minutes at 121 °C to achieve level III disinfection (Table 2.1). However, these parameters should be established by each facility based on the typical waste composition, type of containers used and method of stacking the waste in the autoclave. At the end, the steam is slowly released through a condenser until the pressure reaches the atmosphere pressure. The sterilized HCW is removed and taken to the disposal landfill, treated mechanically or it can also be incinerated in municipal waste incinerators as happen in some countries like Germany (Karagiannidis et al., 2010). Mechanical treatment generally involves size reduction, compaction or both. Some autoclaves are designed to shred waste during the treatment cycle; other systems rely on the use of a pre-treatment process to macerate the waste before the waste is heated. Wastes that are treated in an autoclave do not change considerably from their original state and the waste mass may even increase, due to the addition of water depending upon the type of unit.

Disinfection in an autoclave is carried out by batches: the unit is loaded, the disinfection is carried out, and then the contents are removed from the unit. The entire process, from loading to unloading, is called a cycle.

The major concern associated with the use of autoclaving is that standard autoclaves cannot be used to treat a wide variety of waste including: wastes from chemotherapy treatment, mercury, volatile and semi-volatile organic compounds, and radioactive wastes (Lee et al., 2004; Diaz et al., 2005). It is also not suitable to treat large body parts, animal carcasses, or other large items, due to their mass and other characteristics, makes it difficult or time consuming for the entire material to reach the prescribed temperatures (Diaz et al., 2005). A typical autoclave would release liquid and gaseous discharges that must be properly managed prior to release into the environment. A poorly segregated hazardous HCW may release toxic contaminants into the air. Since pressurized steam is an excellent method of volatilizing organic compounds and many organic reactions are accelerated at elevated temperatures,

possible volatile organic compounds (VOCs) and others maybe emitted into the air, depending upon the quantity and composition of the waste (Salkin et al., 2000). The odors can also be another problem with autoclaves if these are not equipped with proper ventilation for odors removal, such as enzyme-based deodorants (Health Care Without Harm, 2004). Table 2.3 summarizes the main advantages and disadvantages of autoclaving process.

Table 2.3 – Advantages and disadvantages of autoclaving process (Yang et al., 2009; Salkin et al., 2000).

Factors	Advantages	Disadvantages
o Waste characterization o Temperature and pressure o Steam penetration o Size of waste load o Length and number of treatment cycles o Degree of vacuum in the chamber	👍 Low investment cost 👍 Low operation cost 👍 Ease of biological tests 👍 Low hazard residue 👍 PCDD/PCDFs emission free	👎 Appearance, volume unchanged 👎 Not suitable for all waste types 👎 Possible air emissions 👎 Ergonomic concerns 👎 Possible incomplete disinfection

The inactivation of pathogens is achieved by the combination of three variables: temperature, pressure and time. The degree of bacteria inactivation depends on contact time and temperature, so, for a higher temperature less contact time is required (Hossain et al., 2012).

The steam sterilization can be carried out in a *retort*, this unit is similar to autoclaves (Figure 2.2). The major difference between them is that the retort does not incorporate a steam jacket, resulting in inefficient heat transfer and, consequently, higher temperatures are required for a retort than are required for an autoclave (Diaz et al., 2005).

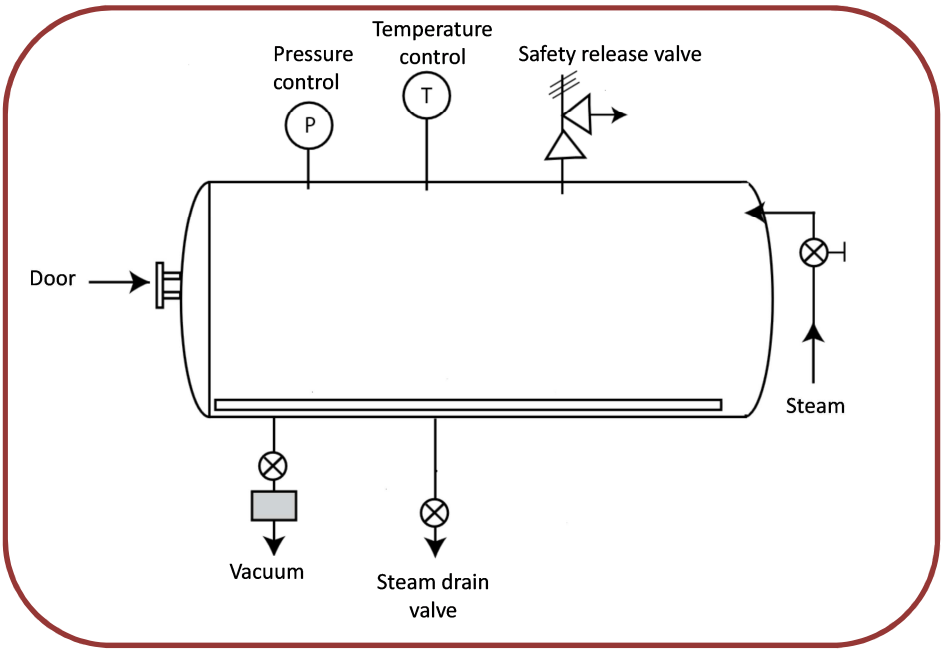

Figure 2.2 – Typical autoclave (adapted from Chartier et al., 2014).

Even with the numerous alternatives available, autoclaves continue to be one of the most popular methods to HCW treatment because of their history of use and track record within healthcare. If followed by shredding it can reduce volume by 60 – 80 % (Gupta et al., 2009; Diaz et al., 2005).

Example – Autoclaving unit in city of Vila Nova de Gaia, Portugal

The unit treats HCW of group III using 2 sterilizers with capacity of 6 200 L.

These sterilizers treat daily 5 796 kg and perform 7 cycles at each 8 hours.

Operation conditions: Temperature – 135 °C

Saturated steam pressure – 3 bar

Cycle time > 60 min.

After sterilization the HCW is shredded and compressed in a crusher with capacity of 1 ton/h and in a compactor with a volume of 20 m^3. The compression cycle is 30 seconds.

A new generation of more efficient autoclaves has been developed to improve the heat transfer, decreasing the processing time, achieving more uniform heating of the waste and eliminating cold spots, rendering the waste unrecognizable, reducing waste volume significantly (up to 85 %) making most of the operation automatic, and/or making the treatment system a continuous process. These technologies now incorporate maceration or shredding during the treatment process to ensure better penetration of steam. Additionally, these systems combine post-treatment drying and compaction, and some also have odors elimination using activated carbon or high efficiency particulate air filters. However, these autoclaves have higher capital costs than standard autoclaves for the same capacity (Health Care Without Harm, 2014; Emmanuel, 2007).

To test the effectiveness of the disinfection process, either biological (for example, *Geobacillus stearothermophilus* or *Bacillus subtilis* spore strips) or chemical indicators are inserted in waste loads, introduced into the autoclave and removed after the process is finished. Although, a few studies have documented that autoclaving inactive pathogenic microorganisms, Hossain et al. (2012) observed in their study a re-growth of bacteria as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa* after some days of sterilization.

2.2.2 DRY HEAT

The dry heat process uses the electrically generated heated air, oil or molten plastic to inactivate potentially pathogenic microorganisms present in the wastes. The waste is heated by conduction, natural or forced convection and thermal radiation. This technology operates at temperatures between 100 °C to 180 °C, though without the intervention of steam. Dry heat technology generally operates at higher temperatures than steam technology and requires

longer exposure times to meet minimum disinfection levels. Above 180 °C, chemical alteration and, ultimately, combustion can occur, creating hazardous by-products (Health Care Without Harm, 2014).

In radiation process, the shredded HCW is introduced in a heart, an air-tight stainless steel chamber, which operates under a negative pressure. After, the waste is exposed to high velocity heated air pumped into the bottom of the chamber through a ring of vanes or slots similar in design to turbine blades. The hot air is directed in a way that causes the waste particles to rotate turbulently around a vertical axis in a toroidal mixing action. Under these conditions, high rates of heat transfer take place. Within four to six minutes, dry unrecognizable waste is ejected into a compactor and put in sealed containers to be disposed at a landfill. With shredding and compaction, the waste volume is reduced by about 80 %.

The types of waste treated are similar to those treated in autoclaving: cultures and stocks, sharps, materials contaminated with blood and body fluids, isolation and surgery wastes, laboratory wastes (excluding chemical waste), liquids such as blood and body fluids. The waste that should not be treated by a dry heat process are: volatile and semi-volatile organic compounds, chemotherapeutic wastes, mercury, other hazardous chemical wastes, and radiological wastes. Table 2.4 summarizes the main advantages and disadvantages of dry heat process.

Table 2.4 – Advantages and disadvantages of dry heat process (Yang et al., 2009; Salkin et al., 2000).

Advantages	Disadvantages
Dry and unrecognizable waste	Not suitable for all waste types
Significant volume reduction	Possible air emissions
Absence of liquid effluents	Any large or hard metal objects may interfere with the shredder
Air emission free	
Automated and easy	
Decrease moisture content of waste	

2.2.3 MICROWAVE AND MACROWAVE

The **microwave** process has been an alternative to incineration for certain categories of hazardous HCW (Hoffman and Hanley, 1994).

Microwaves are defined as those with a frequency between radio and infrared waves in the electromagnetic spectrum. When used in the treatment of medical waste, they stimulate the pre-shredded and moistened waste to generate heat (95 °C or greater) and release steam. The combination of the two – microwaves and moisture – creates the thermal process, which is required to generate the thermal energy to effectively treat the medical waste. The disinfection by microwave units is not a result of exposure of waste to the microwaves, reason why it is important that the waste be wet. Some treatment processes use microwaves to heat water to form steam, which is then applied to the infectious waste stream. “Dry” microwave systems are also available. These use direct microwave energy in a nitrogen atmosphere to treat the waste and produce higher treatment temperatures than those used by “wet” microwave technologies.

In microwave process, the waste is placed in carts and transported to the treatment facility. The carts are lifted by a hydraulic mechanism, the waste is discharged into the hopper and the steam is injected into the hopper and the air is extracted from the unit. All extracted air is passed through a high efficiency particulate air filter. The waste in the hopper is forced into a shredder. The shredded waste is then transported via a rotating conveyor screw, exposed to steam, and then heated to between 95 °C and 100 °C by means of microwaves, during a minimum of 30 min to ensure proper disinfection. Still, microwaving is not sufficient for sterilization temperature above 120 °C (Lee et al., 2004). In some units, the treated waste may be passed through a secondary shredder to achieve a higher degree of size reduction which is mainly important in the event that sharps are part of the waste stream. The treated waste is

conveyed using a second conveyor screw or auger, after it is discharged into a bin or roll-off container and sent to a landfill.

The classic microwave units consist of three types of apparatus: material handling equipment; disinfection equipment itself; and environmental control equipment (Figure 2.3).

This process can operate as a batch process or in semi-continuous mode and can be of various sizes, ranging from a few kg/h to more than 400 kg/h.

The microwave technology is not suitable for large scale treatment and the cost is also expensive. Moreover, some offensive odors around the microwave units may occur. This process is usually not applicable for laboratory and chemotherapy wastes, pathological waste and radioactive wastes (Lee et al., 2004). Table 2.5 shows the advantages and disadvantages of microwave process.

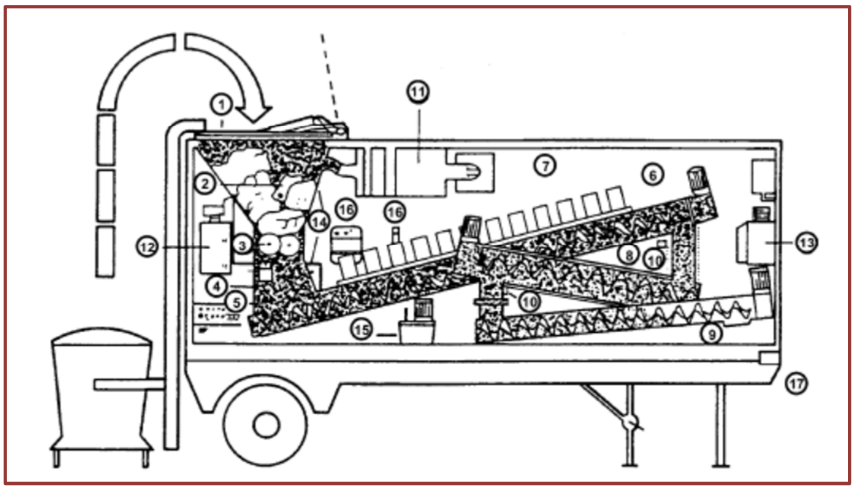

Figure 2.3 – Diagram of a mobile microwave unit: 1. Feeding hopper, 2. Feeding crank, 3.Shredder, 4. Connecting hopper, 5. Level sensors, 7. Microwave generators, 8. Temperature holding section, 9. Discharge conveyor auger, 10. Temperature sensors, 11. Filter system, 12. Water tank with pump and spraying connection, 13. Steam generator, 14. Steam connection, 15. Hydraulic aggregate, 16. Room heater, 17. Container (source: Diaz et al., 2005).

Table 2.5 – Advantages and disadvantages of microwave process (Yang et al., 2009; Salkin et al., 2000).

Factors	Advantages	Disadvantages
o Moisture content of waste o Microwave strength o Duration of exposure o Extent of waste mixture	👍 Unrecognizable waste 👍 Significant volume reduction 👍 Absence of liquid discharge 👍 PCDD/PCDFs emission free	👎 Moderate-High investment 👎 Not suitable for all waste types 👎 Possible air emissions 👎 Possible incomplete disinfection

Some **macrowaves** systems apply low-frequency radio waves to heat shredded, moistened, compacted medical waste to 90 °C for an extended period of time, thereby inactivating microbes contained within the waste. The macrowaves heat the waste from the inside of the materials to their external surfaces.

2.2.4 INCINERATION

Incineration is a high-temperature dry oxidation process that converts organic and combustible waste to inorganic and incombustible matter. The process involves the chemical and physical breakdown of organic material through the processes of combustion. HCW is burned in incineration units under controlled conditions to yield ash and combustion gases. It is carried out at a temperature from 800 °C to more than 1 000 °C, resulting in significant reduction of waste volume , of about 85 – 90 % (Rushbrook, 1999; Alvim-Ferraz and Afonso, 2003; Gielar and Rybicka, 2013) and weight, of about 70 % (Singh and Prakash, 2007). HCW usually requires long incineration times to ensure thorough waste burnout and that the residue quality is good (BREF, 2006). The correct practices of operation, such as controlling the mixing of solids, gas turbulence, the residence time and the incineration temperature as well the air emission limit values are conceded out in accordance with European legislation,

Directive 2010/75/EC and Directive 2000/76/EC. In Portugal, the legislation concerning to incineration of waste are Decree Law No 127/2013 and Decree Law No 85/2005.

The best available techniques (BAT) and best environmental practices (BEP) guideline describes and recommends the proper design and operation parameters for different of incinerators.

Two types of hazardous HCW incinerators are currently used: modular and rotary kilns.

Modular incinerators

The modular incinerators work on **starved air** or **excess air** conditions and usually consist in two furnace chambers. In the **starved air incinerators**, the most used to treat hazardous HCW, the waste is burned in the primary chamber, usually at temperatures between 800 °C and 900 °C with less than the stoichiometric air requirement. Depending on the size of the installation, the residence time can vary from 1 to 4 hours. Airborne contaminants, such as volatile organics, that are released from the primary chamber, are combusted in the secondary chamber at temperatures from 1 100 °C to 1 600 °C, with 100 – 140 % of stoichiometric air needs. This second combustion reduces the smoke, carbon monoxide and odors. Figure 2.4 shows a modular controlled air incinerator.

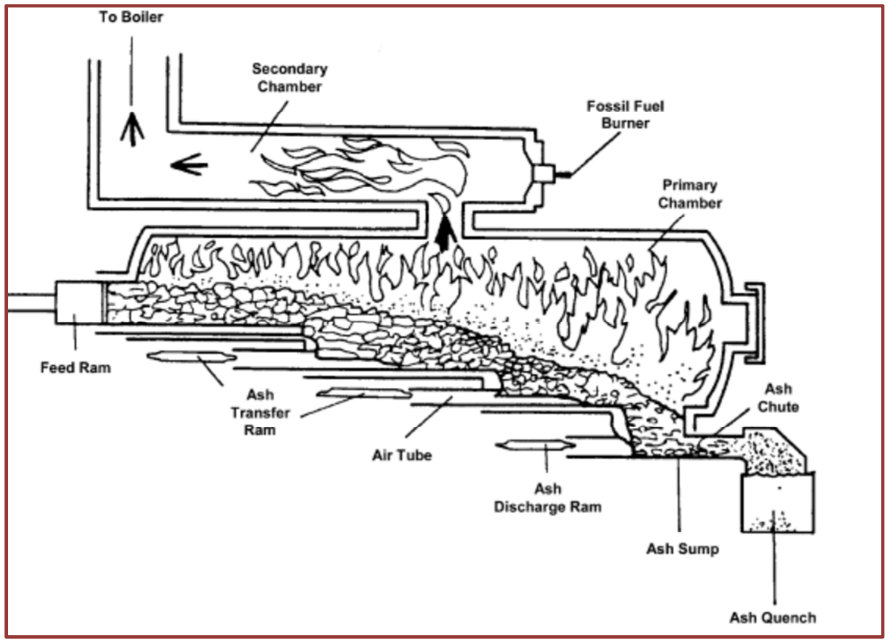

Figure 2.4 – Controlled air incinerator (source: Diaz et al., 2004)

In the **excess air incinerators,** the waste is burned out in the primary chamber and in the secondary chamber is provided the residence time, temperature and supplementary fuel to burn off VOCs in the flue gas. The incinerator contains multiple internal baffles to guide the combustion gases through 90° turns in both lateral and vertical directions. At each turn, ash drops from the gas stream. The air is injected into the primary and secondary combustion chambers through the supplementary fuel burners to reach temperatures of around 800 – 1000 °C.

Rotary kiln

The rotary kiln consists in a rotating oven (in a horizontal refractory lined cylinder that rotates on horizontal axis) that rotates 2 – 5 times per minute and a post-combustion chamber. The waste is charged directly into the kiln with excess of the air to burn. The off-gas from the kiln contains volatiles that have not burnt out on the initial stage and their burning is completed in a secondary chamber that usually has a long residence time of two or more seconds. The incineration temperatures are between 900 °C and 1 200 °C. Figure 2.5 shows a rotary kiln incinerator, which is commonly used for hazardous HCW.

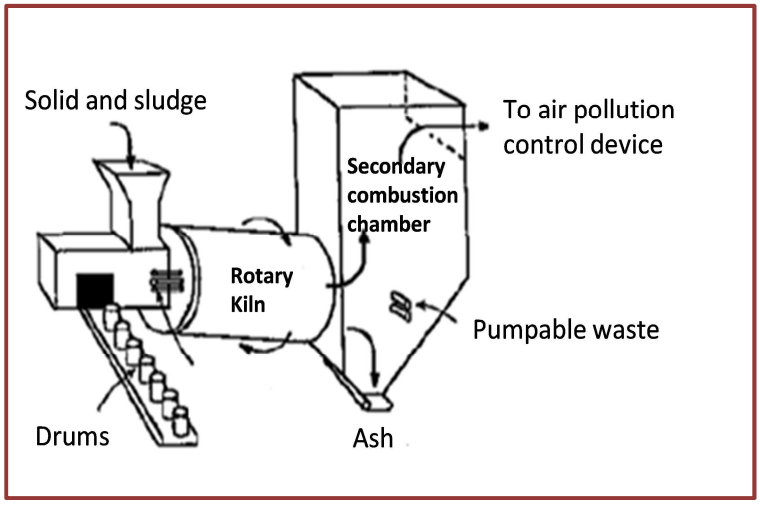

Figure 2.5 – Rotary kiln incinerator (adapted of BREF, 2006).

Several modern large-sized incinerator units can recover the heat energy generated from the combustion of waste, offering an attractive advantage. For an effective incineration is essential

that waste has the following characteristics: heating value above 2 000 kcal/kg; calorific values within the regulatory and design requirements (e.g. the desired residence time, system operating temperature and excess air levels); content of combustible matter above 60 %; content of non-combustible solids below 5 %; content of non-combustible fines below 20 %; moisture content below 30 %. The heating value for HCW containing high levels of plastics can exceed 4 000 kcal/kg, but some of these may contain a high moisture content and consequently, much lower calorific values. A simple schematic of the incineration process is shown in Figure 2.6.

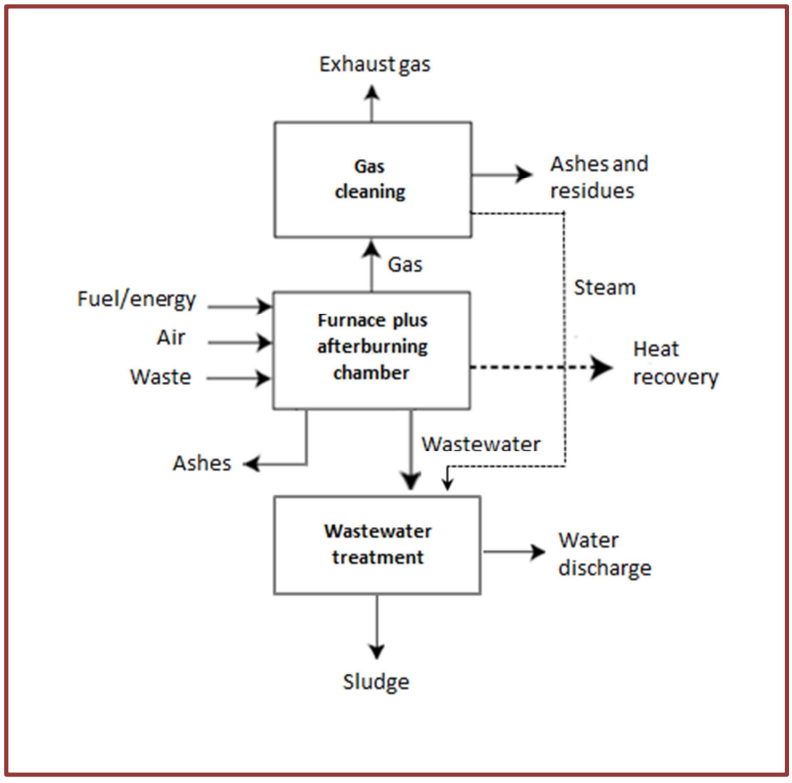

Figure 2.6 – Simplified flow scheme of the incineration process (adapted of Chartier et al., 2014).

The advantages of incineration process shown in Table 2.6 easily explain the success of this technology.

Table 2.6 – Advantages and disadvantages of incineration process (Yang et al., 2009; Salkin et al., 2000).

Factors	Advantages	Disadvantages
o Turbulence and mixing o Moisture content of waste o Filling combustion chamber o Temperature and residence time o Maintenance and repair	👍 Volume and weigh reduction 👍 Heat recovery for large systems 👍 Large scale system waste 👍 Unrecognizable waste 👍 Mature and widely used technology 👍 Complete disinfection 👍 Acceptable for all waste types	👎 High investment and operation costs 👎 High maintenance costs 👎 Bottom and fly ash may be hazardous 👎 Public opposition 👎 Polluting emissions in case of inadequate operation 👎 Skilled operators needed

However, one of the disadvantages of these technologies is the release of combustion by-products into the atmosphere and the generation of residual ash. The HCW combustion produces mainly gaseous emissions containing: fly ash (particulates); inorganic acidic gases such as hydrogen chloride, hydrogen bromide, hydrogen fluoride; nitrogen oxides; sulfur oxides; heavy metals; polycyclic aromatic hydrocarbons (PAHs), dioxins; furans and particulate matter, plus solid residues in the form of ashes, as bottom ash. In addition, the gaseous emissions may contain carbon monoxide as a result of incomplete combustion. Several studies reported some of these pollution emissions from medical waste incinerators (Gielar and Rybicka, 2013; Sukandar et al., 2006; Chen et al., 2003; Lee et al., 2002). Inorganic acidic gases, nitrogen oxides and sulfur oxides are generated due to the presence of elements such as chloride, bromide, fluorine, sulfur and nitrogen in HCW and are emitted during incineration. The direct combustion of nitrogen and oxygen produces also nitrogen oxide, this reaction is accelerated at high temperatures. The presence and forms of heavy metals in fly ash, be contingent operation conditions of the incinerator such as temperature, residence time, gas composition and the presence of reactive compounds. Those conditions influence the final heavy metal speciation and particle size of the residue (Sukandar et al., 2006). Normally the

heavy metals associated with HCW incineration are cadmium, chromium, lead, arsenic and mercury (Singh and Prakash, 2007). The highest amount is usually present on fly ash and bottom ash with the exception of mercury where the greater amount is vented via the flue stack (Singh and Prakash, 2007). Several heavy metals are toxic at low concentrations, persistent and bio-accumulative. PAHs are formed mainly by incomplete combustion and their emissions are directly affected by temperature and excess air during incineration as well as the HCW composition (Singh and Prakash, 2007). Chen et al. (2003) reported in their study that PAHs concentrations of the stack flue gas for two animal carcass incinerators were about 1.5 times higher than for medical waste incinerator. Many of PAHs have been shown to be carcinogenic and mutagenic in experimental animal studies (Chen et al., 2003).

The main sources of dioxin emissions are the incineration of plastics (widely used as disposable materials) and chlorinated materials as paper and inks present in HCW. The dioxins consist of 75 chlorinated dibenzodioxins (PCDDs) and 135 chlorinated dibenzofurans (PCDFs) (Alvim-Ferraz and Afonso, 2003). The dioxins and furans are carcinogenic and toxic at extremely low concentration, producing effects in humans and animals. These chemicals are persistent in the environment due to accumulation in the food chain and to distribution globally (Singh and Prakash, 2007). In modern hospitals, more than 40 % of plastic wastes are chlorinated plastics (Chartier et al., 2014). Because of this and because the plastic content in HCW is significantly higher than in municipal waste, it have been implemented recycling programs. Decreasing of percentage of HCW halogenated plastics reduces the amount of hydrogen chloride and other halogenated pollutants. Thereby, reduces the treatment and disposal costs of HCW that are much more expensive than of municipal waste (Lee et al., 2004).

On the other hand, incinerator emissions should comply with national standards and in accordance with the Stockholm Convention's guidelines for BAT and BEP limit, the levels of dioxins and furans in air emissions should not exceed 0.1 ng I-TEQ/Nm^3 at 11 % O_2. All countries that have signed the convention are required to use the BAT for new incinerators.

Incinerators require emission controls equipment to meet modern emission standards. Alvim-Ferraz and Afonso (2005) determined that without emission controls, dioxin concentrations in combustion gases were 93 to 710 times higher than the European Union legal limit (0.1 ngTEQ/m^3), depending on variations in the waste composition. The atmospheric emissions released by hazardous HCW incineration are strongly influenced by the segregation methods, incineration conditions and waste type classification (Alvim-Ferraz and Afonso, 2005).

Moreover, the hazardous HCW products from incineration – fly ash – is disposed of in hazardous waste landfill, whose costs are several times higher than disposal of decontaminated hazardous HCW at landfills for the municipal waste. The bottom ash is characterized by a leaching test to determine the appropriate ultimate disposal landfill. Some researchers have studied the stabilization/solidification of medical waste incineration ash. Tzanakos et al. (2014) studied the use of both ashes (fly and bottom) as a raw material for the production of geopolymers. They proved that solidified matrices by geopolymerization were able to reduce the leachability of the heavy metals which existed at the medical waste ash. The stabilization of fly ash with colloidal silica solution, suitable for chloride–rich fly ash as it is the fly ash derived from incineration of hospital wastes, could be another treatment method (Karagiannidis et al., 2010).

However, incineration had been the preferred option for the treatment of infectious waste in countries such as Japan, mainly because the area for landfill is limited, only 10 % of the land is suitable for residential purposes this technology is extremely advantageous due to production of minor amount of waste. In Croatia, for ethical reasons, the pathological waste, namely the recognizable body parts is incinerated in crematoria or buried in cemeteries (Marinkovic et al., 2008). Zhao et al. (2009) compared the environmental performances of hazardous HCW incineration and autoclaving with sanitary landfill using life cycle. Their results showed that

from a life cycle perspective the conventional waste hierarchy implying incineration with energy recovery is better than landfilling.

In some cases a distinction is made between the incineration routes for pathological (potentially infectious waste) and non-pathological waste. The incineration of pathological waste is sometimes restricted to dedicated incinerators, while non-pathological waste is, in some cases, treated in other installations, for example with mixed municipal or hazardous wastes (BREF, 2006). In some countries exist national regulations that limit the ratio of HCW that may be incinerated in combined incinerators, as France where ratio is <10 % thermal load (BREF, 2006). Some countries also practice the **co-incineration**, high-temperature incineration of hazardous HCW, namely chemical and pharmaceutical waste, in industrial cement kilns or steel furnaces (Chartier et al., 2014).

Example – Incineration unit in city of Lisbon, Portugal

The unit treats HCW of group IV using a pyrolytic incinerator with capacity of 7 200 kg/day.

HCW is loaded to the incinerator by a hydraulic lifting and tipping of containers.

The incineration is carried out in 2 stages: primary chamber and post-combustion chamber.

The primary chamber is a static horizontal chamber with 3 combustion uneven levels and it works at 850 °C in oxygen absence.

The post-combustion chamber is a horizontal chamber, on the primary chamber, and it operates at 1 100 °C, with an oxygen concentration of 6 % and residence time of 2 seconds.

The post–combustion chamber is equipped with 2 natural gas burners, it is connected to a heat exchanger to cooling the gases and energy recovery.

Gaseous emissions treatment

The gases are cooled to 180 °C and neutralized by injection of powdered sodium bicarbonate. Then the gases pass through a ceramic filter to remove particulate matter.

2.2.5 PYROLYSIS

Pyrolysis process is a novel technology to treat hazardous HCW; it operates at high temperatures, between 550 °C and 1 000 °C, in absence of oxygen (Salkin et al., 2000). It makes the waste into innocuous and converts it into fuel (Na et al., 2008).

At these temperatures the systems treat, destroy and reduce the volume of HCW. Na et al. (2008) studied the thermal decomposition of several usual HCW. Their results showed a weight loss up to 95 % at 800 °C for most of the test samples, thus proving that the pyrolysis can reduce the mass of waste. Table 2.7 shows the main advantages and disadvantages of pyrolysis process.

Table 2.7 – Advantages and disadvantages of pyrolysis process (Yang et al., 2009; Salkin et al., 2000).

Advantages	Disadvantages
👍 Almost no waste remains	👎 Novel technology
👍 Heat recovery for large systems	👎 Air emissions must be treated
👍 Unrecognizable waste	👎 Skilled operator needs

The products generated during the process are directly affected by temperature: gases; ash and coke (in solid phase) pyrolysis oil and water (in liquid phase). The combustible gases are obtained by high temperature pyrolysis (above 1 000 °C); a medium temperature pyrolysis (600 °C – 700 °C) produces mainly oils, and materials such as scrap tires, waste plastics are transformed into a kind of heavy oil materials; and the product obtained by low temperature pyrolysis (under 600 °C) is principally coke (Wei et al., 2012). The gases remaining after pyrolysis can also be used as energy resources. The pyrolysis oil can be further used as a furnace fuel or a fuel for diesel generators.

There are many types of pyrolysis reactors, including muffle furnace, tube furnace, fixed bed, fluidized bed, and entrained flow reactors. Typical pyrolysis reactors employ energy input from conventional sources of heat.

2.2.6 GASIFICATION

Gasification is not a new technology; it was implemented during the nineteenth century in factories to produce town gas (Tchobanoglous et al., 1993). Nowadays, gasification is the main technology for biomass conversion to energy and an attractive alternative for the thermal treatment of solid waste (Fabry et al., 2013).

Gasification differs from incineration because incineration combusts completely the waste is burned with the purpose to produce carbon dioxide and water. In gasification, the objective is to produce carbon monoxide and hydrogen which are intermediate products of combustion; during the process the following exothermic and endothermic reactions occur (Tchobanoglous et al., 1993):

$$C + O_2 \rightarrow CO_2 \quad [2.1]$$

$$C + H_2O \rightarrow CO + H_2 \quad [2.2]$$

$$C + CO_2 \rightarrow 2CO \quad [2.3]$$

$$C + 2H_2 \rightarrow CH_4 \quad [2.4]$$

$$CO + H_2O \rightarrow CO_2 + H_2 \quad [2.5]$$

To achieve that, the operating conditions in gasification must be appropriately maintained in order to avoid complete combustion.

In the gasification process the wastes are thermally decomposed in an oxygen starved (sub stoichiometric) atmosphere. The gasification operates at high temperatures, between 500 °C to 1 600 °C, at a pressure ranging between 1 bar and 45 bar with gasification agent O_2 or H_2O (Chartier et al., 2014). When gasification occurs in the presence of an oxidant gasification agent, that is used to partially oxidize the feedstock and produce heat, it is called direct

gasification. If the process does occur with an oxidizing agent, and needs an external energy source, it is called indirect gasification. Steam is the most commonly used indirect gasification agent, because it is easily produced and increases the hydrogen content of the combustible gas (Belgiorno et al., 2003).

In gasification, the wastes are ignited and reduced in a self-sustaining process. The products generated during gasification of wastes are "syngas", so called "producer gas", and, this depending on the waste content, various vaporised tar oil fractions. The syngas is mainly composed of carbon monoxide (CO) and hydrogen (H_2) with low quantities of carbon dioxide (CO_2), water (H_2O), methane (CH_4), hydrogen sulfide (H_2S), ammonia (NH_3), and under certain conditions, solid carbon (C), nitrogen (N_2), argon (Ar) and some tar traces (Fabry et al., 2013). The syngas is cleaned through its passage by a series of scrubbers/filters and cyclonic separators. Syngas is a desirable product because of its versatility, carbon monoxide and hydrogen can be used in a number of ways to produce heat and electricity and can also be compressed for later use. Additionally, syngas can be used to produce methanol using a Fischer-Tropsch process (Fabry et al., 2013).

There are three fundamentally types of reactors, namely: fixed bed, fluidized bed and indirect gasifier.

Vertical fixed bed reactors are the most competitive fixed bed gasifiers, and can be updraft and downdraft gasifiers. Updraft is a counter-current gasifier, where the feedstock is loaded from the top while air is introduced from the bottom of the reactor. In a downdraft reactor, co-current, the carbonaceous material is fed in from the top, the air is introduced at the sides above the grate while the combustible gas is withdrawn under the grate.

Fluidized bed has a fixed bed of fine solids, typically silica sand, which is transformed into a liquid-like state by contact with a gasification agent. Fluidized bed gasification arises to resolve problems related to feed stocks with a high ash content in the fixed bed and, principally, to

increase the efficiency of the process. The efficiency of a fluidized bed gasifier is about five times higher than a fixed bed (Belgiorno et al., 2003).

Indirect gasifiers are the reactors used for the steam indirect gasification and are grouped as char indirect gasifiers and gas indirect gasifiers, depending on the type of internal energy source. Gas indirect gasifiers use a steam fluidized bed gasifier within bed heat exchange tubes. A fraction of combustible gas is burned with air in a pulse combustor and the hot combustion products provide heat to gasify the feed (Belgiorno et al., 2003).

The main advantage of indirect gasification is the high quality of the combustible gas produced in contrast with greater investment and maintenance cost of the reactor (Belgiorno et al., 2003).

2.2.7 PLASMA

In a plasma system, an electric current is used to ionize an inert gas (e.g., argon) causing the formation of an electric arc to create temperatures as high as 6 000 °C. The waste within the system is brought to temperatures between 1 300 °C to 1 700 °C, destroying potentially pathogenic microbes and converting the waste into a glassy rock or slag, ferrous metal, and inert gases (Gomez et al., 2009; Rezaiyan and Cheremisinoff, 2005).

A plasma-arc employing carbon electrode was first used in 1960s as a source of intense heat (Nema and Ganeshprasad, 2002).

The plasma technology was applied to destroy highly toxic compounds and to modify refractory compounds in an environment-friendly way. The abundant ultraviolet radiation in thermal plasma can dehydrogenate organic chlorine. The reactors can process gaseous, liquid and solid materials (Nema and Ganeshprasad, 2002).

It is an environment friendly technology, which converts organic waste into commercially useful by-products. Plasma-arc technology is a well proven, well-demonstrated, commercially

viable technology, which is utilized in industrial plants to treat different waste materials, worldwide. Medical waste is pyrolyzed into CO, H_2 and hydrocarbons when it comes in contact with the plasma-arc (Gomez et al., 2009). These gases are burned and produce a high temperature (around 1 200 °C).

Plasma pyrolysis integrates the thermo-chemical properties of plasma with the pyrolysis process. This technology makes use of an ionized gas in the plasma state to convert electrical energy to temperatures of several thousand degrees using plasma arc torches or electrodes. The high temperatures are used to pyrolyse waste in an atmosphere with little or no air. Hot plasmas are particularly appropriate for treatment of solid waste and can also be employed for destruction of toxic molecules by thermal decomposition.

This technology provides an efficient treatment of hazardous HCW, and it does not require segregation of chlorinated hydrocarbons. The quantity of dioxins and furans was found to be well below the accepted emissions standards. Another advantage of plasma pyrolysis is the reduction in volume of organic matter, which is more than 99 %.

2.3 CHEMICAL PROCESSES

Chemicals have an extensive and well-documented history in the clinical setting in disinfecting environmental surfaces and medical devices. Table 2.8 summarizes the advantages and disadvantage of chemical processes.

Inherent to the operation of such systems is the fact that the waste must first be shredded prior to exposure to such agents as sodium hypochlorite, chlorine dioxide, peracetic acid, formaldehyde, glutaraldehyde, quaternary ammonium compounds, calcium oxide, ozone, etc., in order to bring all surfaces of the waste into direct contact with the chemicals. The process appears to be able to treat: cultures and stocks, sharps, liquid human and animal wastes

including blood and body fluids (in some technologies this may be limited to a certain percentage of the waste), isolation and surgery wastes, laboratory waste (excluding chemical waste). Chemical sterilization may be considered as an alternative to autoclaving mainly in disinfection of scalpels, syringes with needles and other recyclable sharps (Prüss et al., 1999). However, this process may be problematic due to production of toxic effluents by the used chemical disinfectants. Recently, some controversy arises regarding the use of chlorine-based chemicals, namely hypochlorite and its by-products in wastewater, which may be cause long-term environmental effects (Health Care Without Harm, 2014). There may be some offensive odors around some chemical treatment units.

Table 2.8 – Advantages and disadvantages of chemical process (Yang et al., 2009; Salkin et al., 2000).

Factors	Advantages	Disadvantages
o Concerns for chemicals and temperature o pH o Chemical contact time o Waste and chemical mixing o Recirculation vs flow-through	👍 Unrecognizable waste 👍 Significant volume reduction 👍 Rapid processing 👍 PCDD/PCDFs emission free 👍 Waste deodorization	👎 Not suitable for all waste types 👎 Need for chemical storage 👎 Possible incomplete disinfection 👎 Moderate-high investment

Some systems combine heat with the chemicals to reduce the treatment cycle. An example of these systems is alkaline hydrolysis, which requires an aqueous solution with sodium hydroxide or potassium hydroxide at temperatures around 150 °C. This HCW treatment will be addressed in more detail in Chapter 3.

For an efficient disinfection is essential that: the disinfectant has the ability to act on all the key pathogen groups; the disinfectant is maintained in the waste at sufficient concentration or it is given enough time to achieve the required level of treatment for each of the key pathogen groups; and the treated waste (which may be highly absorbent) should not be rendered chemically hazardous due to the presence of residual disinfectant.

2.4 IRRADIATION PROCESSES

Gama irradiation, Cobalt-60, has been used for many years as a means of inactivating potential pathogens on the surfaces of many different products. Since the appropriate dose of radiation can be precisely calculated, it has been found to be an extremely reliable treatment system. A newer form of irradiation systems employs an electron beam generated by an accelerator to sterilize hazardous HCW. Electron beam irradiation uses a shower of high-energy electrons to destroy microorganisms by causing chemical dissociation and rupture of cell walls. Irradiation systems require extensive shielding to protect the workers. These processes can only treat relatively small quantities of waste. It does not alter the physical appearance of the material and would require a grinder or shredder to render the waste unrecognisable.

The types of waste that can be, or not, treated by irradiation are similar to those treated by autoclaving, dry heat and microwave. Table 2.9 summarizes the main advantages and disadvantages of irradiation process.

Table 2.9 – Advantages and disadvantages of irradiation process (Health Care Without Harm, 2004).

Advantages	Disadvantages
Air emissions free	Not reduce waste volume
Absence of liquid effluents	Personnel must be protected from radiation exposure
Low operating costs	Skilled operator needs
Well automated and requires little operator time	
Noiseless	

2.5 BIOLOGICAL PROCESSES

Biological processes are categorized as either aerobic or anaerobic treatments. Aerobic treatment requires oxygen and the microorganisms convert the organic components of the

hazardous waste into carbon dioxide and water. Anaerobic treatment occurs in absence of oxygen and the microorganisms convert the waste into methane and carbon dioxide.

In the biological processes, composting and vermiculture (digestion of organic wastes through the action of worms) have been used successfully to decompose hospital kitchen waste, as well as other organic digestible waste and placenta waste (Chartier et al., 2014). In the biological processes that use an enzyme mixture to decontaminate HCW, the resulting sludge is put through an extruder to remove water for sewage disposal. This process is also being used in the agricultural sector to break down animal waste. The natural decomposition of pathological waste through burial is another example of a biological process.

2.6 MECHANICAL PROCESSES

This technology is used with other types of equipment for the following reasons: 1) reduces the volume of waste; 2) removes or reduces physical hazards; and 3) renders the waste unrecognizable.

These technologies are commonly used for compaction and shredding/grinding HCW, due to their capability to reduce waste volume by about 60 – 80 % (Gupta et al., 2009; Diaz et al., 2005). The compaction process involves compressing the waste into containers to reduce its volume. The compaction is used in conjunction with other treatment methods, but it is normally less efficient than shredding or grinding and might generate infectious aerosols (Salkin et al., 2000). The purpose of shredding is to covert HCW into a more homogenous form that can be easily handled and efficiently sterilized. The shredding process includes granulation, grinding, pulping, etc. In these processes, HCW are physically broken into smaller particles in a container. The container is usually maintained at a negative pressure to ensure that no waste escapes from the device. The more advanced shredders are usually low-speed, high-torque, single-pass shredders with easily replaceable cutters and with discharge screens

to control the size of shredded waste. Nevertheless, shredding or grinding waste in a stand-alone unit, before disinfection, is not recommended because, although it increases the surface area of the waste and can make treatment easier, it can create an aerosol of infectious particles (Health Care Without Harm, 2014).

2.7 OTHER PROCESSES

Encapsulation is recommended as the easiest technology for the safe disposal of sharps. Sharps are collected in puncture-proof and leak-proof containers, such as high-density polyethylene boxes, metallic drums or barrels. When a container is three-quarters full, a material such as cement mortar, bituminous sand, plastic foam or clay is poured in until the container is completely filled. After this material has dried, the container is sealed and may be landfilled, stored, or buried inside the hospital premises. It is also possible to encapsulate chemical or pharmaceutical residues together with sharps. In Figure 2.7 is shown a schematic diagram of cement encapsulation. Several systems currently available provide waste containers which already contain chemical packets which, when activated through the addition of liquids, encapsulate the waste into solid clear or opaque blocks or cylinders. It is possible that the chemicals also treat the waste, but support documentation is limited.

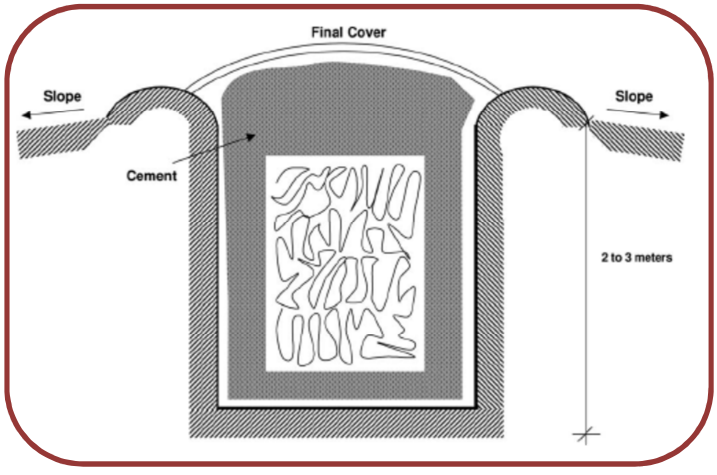

Figure 2.7 – Schematic diagram of cement encapsulation (source: Diaz et al., 2005).

The main advantages of the process are: simple and safe; low cost and also applicable to chemicals pharmaceuticals. Additionally, is effective to reduce the risk of scavengers gaining access to the hazardous HCW. Not recommended for non-sharp infectious waste.

Table 2.10 shows the main advantages and disadvantages of encapsulation process.

Table 2.10 – Advantages and disadvantages of encapsulation process

Advantages	Disadvantages
Simple, inexpensive and safe	To be regarded as a temporary solution
A solution that can be envisaged for sharps and pharmaceutical wastes	The quantities of waste treated are small
The risks for scavengers are reduced	The weight and volume of the waste is increased

Another treatment process, especially for pharmaceuticals waste, is the **inertization.** This involves mixing waste with cement and other substances before disposal to minimize the risk of toxic substances contained in the waste migrating into surface water or groundwater.

The typical proportions (by weight) for the mixture are: 65 % waste; 15 % lime; 15 % cement; 5 % water. The process is reasonably inexpensive and can be performed using relatively unsophisticated mixing equipment (Chartier et al., 2014).

2.8 ACKNOWLEDGMENTS

Figure 2.1, Figure 2.3, Figure 2.4 and Figure 2.8 are reprinted from Waste Management, vol 25, Diaz L.F., Savage G.M., Eggerth L.L., Alternatives for the treatment and disposal of healthcare wastes in developing countries, 626–637, 2005, with permission from Elsevier.

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CHAPTER 3 – ALKALINE HYDROLYSIS

3.1 INTRODUCTION

Alkaline hydrolysis is not a recent chemical process, given that it was patented in 1888 by Amos Herbert Hobson in the United States Patent Office, England. The main objective of this patent was the removal of nitrogenous materials from the bones to make a suitable fertilizer, and byproducts. In this work alkaline hydrolysis could have other benefits, such as a process to treat animal carcass materials. Only in the nineties this process was used to destroy tissue wastes, including anatomical parts, organs, placenta, blood, body fluids, specimens, human cadavers and animal carcasses (BREF, 2005). This technology is reported in full scale in the USA and Canada for disposal of transmissible spongiform encephalopathy (TSE) wastes. It is currently used in many institutions, laboratories, and animal disease diagnostic facilities to treat carcasses dispose and other forms of biological waste (BREF, 2005). Until 2009 no units were in operation in EU, due to the lack of approval of the technique, as required by Regulation (EC) No 1774/2002. Nevertheless, alkaline hydrolysis was considered an alternative method for the treatment of animal by-products when the Regulation (EC) No 1069/2009,

which defines the basic outline regulation covering the import of animal derived material not used on human or animal consumption, and the Regulation (EU) No 142/2011, that provides the implementing rules, were implemented. Together, these two documents address, both, animal by-products and animal derived products and cover all aspects for collection, processing and transport of these materials into and within the European Community.

Alkaline hydrolysis combines steam sterilization with tissue digestion using sodium hydroxide (NaOH) or potassium hydroxide (KOH). The process converts animal carcasses, human body parts and tissues in a sterile alkaline “soap” with a brownish coloration and high biochemical oxygen demand (BOD). The by-products generated by alkaline process are mineral constituents of the bones and teeth (which can be crushed and recovered as sterile bone meal) and a decontaminated aqueous solution of peptide chains, amino acids, sugars, soaps and salts.

In addition, the alkaline hydrolysis destroys fixatives in tissues and various hazardous chemicals, including formaldehyde, glutaraldehyde and many chemotherapeutic agents or cytotoxic agents (Health Care Without Harm, 2004). This process can also destroy pathogens including prion and, is effective in eliminating radioactively contaminated tissues (Thacker, 2004). Therefore, alkaline hydrolysis emerges, in some countries, as an alternative process for HCW treating, reported in USA and in UK (Health Care Without Harm, 2004).

This treatment has been shown to have significant advantages compared to other HCW treatments, because it sterilizes and destroys at once, and also reduces the total waste volume. It has also other important benefits, such as the absence of emissions releases into the atmosphere resulting in a minor odour production. For this reason, it is considered a green process. And, so, the treatment of infectious wastes and hazardous wastes by alkaline hydrolysis has been patented (US 5384092, US 20010053869, US 7910788, etc.). Table 3.1 shows the advantages and disadvantage of alkaline hydrolysis.

Table 3.1 – Advantages and disadvantages of alkaline hydrolysis (Thacker, 2004; USEPA, 2014).

Advantages	Disadvantages
Combination of sterilization and digestion into one operation	Potential issues regarding disposal of effluent
Reduction of waste volume and weight (> 97 %)	Limited capacity for destruction of large volumes
Complete destruction of pathogens, including prions	Not widely available
Elimination of radioactively contaminated tissues	Transportation concerns/costs
Production of limited odor or public nuisances	Residues require proper handling and management
	Public perception

Another attractive factor for this technology is the cost, which is smaller than for others technologies used to treat hazardous HCW. Thacker (2004) reported the estimated costs of \$320 ton^{-1}, including costs with steam, water, electricity, chemicals, labor, sanitary sewer and maintenance and repair. A similar value was obtained by Murphy et al. (2009) that calculated the cost of approximately \$260–\$310 ton^{-1} using alkaline hydrolysis to dispose of animal tissues and carcasses during their study of prion inactivation. However, these costs do not include the initial capital investment. Still, these authors considered as acceptable the financial costs of this technology and indicated alkaline hydrolysis as a valid alternative to incineration, landfill burial, and rendering for disposing of biological material potentially infected or contaminated with prion disease.

Alkaline hydrolysis technology can be of various sizes, from small to very large, with the installation of multiple units being appropriate for large scale operations. In addition, it can be used for on-site treatment, thereby saving on transport costs and decreasing environmental damage (BREF, 2005).

3.2 CHEMICAL FUNDAMENTALS

Hydrolysis is defined as a chemical transformation in which a molecule, *RX*, reacts with water, resulting the formation of a new covalent bond with OH and cleavage of the covalent bond with *X* in the original molecule (equation 3.1).

$$RX + H_2O \rightarrow ROH + X^- + H^+ \quad [3.1]$$

If occurs in the presence of acid or base it is called acid catalysis or base catalysis. Both catalysis can significantly accelerate hydrolysis kinetics, due the hydronium ion and hydroxide ion provide an alternative mechanism for hydrolysis that is energetically more favourable. In acid catalysis, hydronium ion provides a reaction pathway of lower energy by withdrawing electron density from the atom bearing the leaving group, *X*, thus making it more susceptible to nucleophilic attack by H_2O. Base catalysis occurs because OH^- is a much more reactive nucleophile than H_2O. Bases are usually aqueous solutions of alkali metal hydroxides, such as NaOH or KOH. The heat is used to accelerate the hydrolysis reactions.

Alkaline hydrolysis degrades the proteins, the major solid component of all animal cells and tissues, into salts of free amino acids. Proteins are linear polymers which consist of one or more long chains of amino acids. Amino acids are linked to each other by a peptide bond in which the carboxyl group of one amino acid is condensed to the amino group of another amino acid with elimination of water. Alkaline hydrolysis reverses the condensation of amino acids into proteins of the peptide bonds and the addition of water at the break. Moreover, under alkaline hydrolysis some amino acids such as arginine, asparagine, serine and glutamine are completely destroyed while others modify its configuration to structures of lower molecular weight (Fountoulakis and Lahm, 1998).

Due to its effectiveness in degrading the proteins, the alkaline hydrolysis of proteins recovered from slaughterhouse blood is used to obtain profitable peptides and free amino acids for animal feed. For this purpose, Álvarez et al. (2013) studied the use of NaOH to obtain useful peptides from porcine haemoglobin. They obtained an 80 % peptide recovery using 6 M NaOH at 50 °C during 24 hours. Lóki et al. (2010) examined the changes in D-amino acid content of slaughterhouse waste, and reported that alkaline hydrolysis at 135 °C for 2 hours with NaOH or KOH is sufficient for the entire destruction of the protein structure. The obtained product was unfit for being used as animal feed; however, the product resultant of the hydrolysis with KOH, after neutralization, may be used as nitrogen fertilizer in the soil.

Alkaline hydrolysis with the purpose to separate and recover chromium from tanned leather and collagen hydrolysate has also been investigated (Ferreira et al., 2008). Ferreira et al. (2014) investigated leather treatment at 150 °C for 1.5 hours with 4 M solution and found that under these conditions the quantity of leather and chromium dissolved was more than 98 % and 85 % respectively. The alkaline treatment (with lime) of chicken feather keratin to obtain a liquid product rich in amino acids and polypeptides, that can be used as an animal feed supplement, has also been studied (Coward-Kelly et al., 2006). They reported that at 150 °C, 80 % of feather keratin was solubilized within 25 min, whereas at 100 °C, a relatively longer reaction time (300 min) was needed to solubilize 80 % of keratin.

The alkaline hydrolysis is also used to quantify liberation of all amino acids of the substrate and the quantitative recovery of them in the hydrolyzate. This method is often applied in the quantification of phospho-amino acids, is applied in nucleic acid research and for the digestion of a wide variety of substrates in order to release products such as carbohydrate moieties, lipids, etc. (Fountoulakis and Lahm, 1998).

The animal fats, triglycerides, are esters composed of three fatty acids chains bound connected to a glycerol molecule. The triglycerides react with strong bases, as NaOH and KOH to form the carboxylate salts of fatty acids. Also, the phosphodiester bonds of nucleic acids are hydrolysed by alkaline hydrolysis into their constituent bases, sugars and phosphate. The carbohydrates are the most resistant compounds to alkaline hydrolysis. The glycogen, the most common large polymer of glucose in animals, requires much longer treatment time than it is required for other polymers. When broken down, the constituent monosaccharides are rapidly destroyed by the hot aqueous alkaline solution. Nevertheless, the alkaline hydrolysis is insufficient to destroy long hydrocarbon molecules, such as cellulose. Knill and Kennedy (2003) studied the cellulose degradation under alkaline hydrolysis. Their study reported that the production of acids with low molecular weight and the composition of the degradation products are influenced by some reaction parameters, such as temperature, type of the alkaline solution and concentration. The cellulose degradation products of low molecular weight, formic acid, acetic acid, glycolic acid, and lactic acid were detected at higher temperatures (approximately 280 °C). At lower temperatures the major alkaline degradation products observed were the glucoisosaccharinic acids and their lactones.

Some authors have been studying the degradation of some polymers by alkaline hydrolysis. Gu et al. (2001) evaluated the changes on polyester films using 3 M NaOH solutions at room temperature measuring both mass and total organic carbon losses, among other parameters. Their results showed an increase of mass and organic carbon losses with the increase of the exposure time; this fact was attributed to the hydrolysis of ester groups and the subsequent leaching of low molecular mass and water soluble fragments of the polyester material into the solution. Shin et al. (1998) studied the effect of NaOH concentration on the degree of polyvinyl chloride dehydrochlorination at high temperatures (between 150 °C and 250 °C) using 0 – 7 M NaOH solutions during 12 hours. They observed that the degree of dehydrochlorination

increased with increasing temperature, and reached about 100 % at 250 °C. The maximum rate of the dehydrochlorination was obtained using 3 M NaOH. Blazevska-Gilev and Spaseska (2007) described the alkaline dechlorination of polyvinyl chloride (PVC) in organic solvents at 30°C – 80 °C for 1 – 5 hours. The final products were polyvinyl alcohol with small chloride content and NaCl. Studies on the degradation of polyethylene terephthalate (PET) using alkaline solutions showed the depolymerizing of PET into small molecules as ethylene glycol and terephthalic salts (Kao et al., 1998; Karayannidis et al., 2002; Kumar and Guria, 2005).

3.3 DESTRUCTION/INACTIVATION OF DISEASE AGENTS

The successful inactivation of a Creutzfeldt–Jajob disease (CDJ) agent by a sequential process involving exposure to 1 M NaOH for 60 min, followed by autoclaving at 121 °C for 30 min (Taguchi et al., 1991), and the inactivation of 22A strain of scrapie agent by autoclaving at 121 °C for 30 min in the presence of 2 M NaOH (Taylor et al., 1997) has been proven. Taylor (2000) addressed the inactivation conditions of agents that cause TSE by some sterilization methods described in various studies. He reported that the hot solutions of NaOH appear to be completely effective in inactivation of these agents.

Bauman et al. (2006) studied the inactivation of prions by 0.1 M NaOH after pre-treatment with detergent. In the presence of detergent, prions become more accessible to NaOH, which can then inactivate prions by altering its structure.

Murphy et al. (2009) reported the inactivation of Prion–Positive Material by alkaline hydrolysis at 150 °C. More recently, the prion decontamination using 0.15 M NaOH at 25 °C for 1 h was shown to be partly effective with a prion reduction of 4 log10 (McDonnell at al., 2013).

The alkaline hydrolysis destroys all pathogens listed as index organisms by the STAATT (Table 2.1) which require a 6 log10 (99.9999 %) reduction in vegetative agents and a 4 log10 (99.99 %) reduction in spore forming agents (Thacker, 2004).

Kaye et al. (1998) evaluated the efficacy of alkaline hydrolysis by testing the destruction of the following microorganism cultures: *Staphylococcus aureus*, *Mycobacterium fortuitum*, *Candida albicans*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, *Mycobacterium bovis* BCG, MS-2 bacteriophage, and *Giardia muris*. The tests were carried out during digestion of animal carcasses in a digester at 110 – 120 °C and for 18 hours. The results showed the complete destruction of these cultures by alkaline hydrolysis.

Dixon et al. (2012) reported the use of alkaline hydrolysis at ambient temperature for the inactivation of the fish pathogens infectious salmon anaemia virus and *Lactococcus garvieae*.

3.4 PRINCIPLE OF OPERATION

According to the Commission Regulation (EU) No 142/2011, the alkaline hydrolysis must be carried out in a batch system and the material in the vessel must be constantly mixed in order to facilitate the digestion process until the tissues are dissolved and bones and teeth are softened. It requires either a NaOH or KOH solution (or a combination thereof), which must be used in an amount that assures approximate molar equivalency to the weight, type and composition of the animal by-products to be digested. The mixture must be heated to a core temperature of at least 150 °C and at a pressure of at least 4 bars.

The process is carried out in a tissue digester, which consists in a steam-jacketed, stainless-steel tank and a basket. After loading the waste in the basket (for the retention of bone remnants) and into the hermetically sealed tank, the alkali (sodium or potassium hydroxide) is added in amounts proportional to the quantity of waste in the tank, along with water. The contents are heated and stirred. Depending on the amount of alkali and temperature used, the time required is between three and eight hours. However, another important factor to consider in the processing time is the disease agents of concern present in waste. To treat bacterial and viral contaminated waste, the time required is three hours; nevertheless

according European Commission Scientific Steering Committee (2002), for material infected with TSE or potentially TSE-infected, the time recommended is six hours.

Figure 3.1 – Alkaline hydrolysis digester (source: Thacker, 2004).

The byproducts of the process consist in a solid and a liquid (effluent). The residue, correspondent to approximately 2 % of the initial weight and volume, the waste rich in mineral components of the bones and teeth is sterile and easily crushed into a power, which may be used as a soil additive. Some studies have been conducted to evaluate the recycling of by-products into animal feed supplement or fertilizer in soils for by alkaline hydrolysis (Kim and Patterson, 2003; Gousterona et al., 2003; Kalambura et al., 2011).

The effluent is characterized by the brownish color, high pH, normally around 10.3 to 11.5, high BOD (approximately 70 000 mg/L) and high COD (up to 100 000 mg/L) and can be discharged to the sewer after neutralization, however, subjected to the local or federal guidelines. The estimate quantity of effluent generated on the treatment of 907 kg of material is 2196 L of hydrolyzate and 4392 L of total effluent including hydrolyzate, cooling water, rinse water and coflush water. The sterile effluent resultant from the alkaline hydrolysis process can be a source of sustainable energy, in the form of the fertilizer generation, biodiesel fuels, and biogas from large-scale tissue digester units which generated large volumes of effluents.

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CHAPTER 4 – EFFECTS OF ALKALINE HYDROLYSIS AND AUTOCLAVING ON DISCARDED MEDICAL COMPONENTS PRESENT IN HEALTHCARE WASTE

This chapter is based on Pinho S. C.; Almeida M. F.; Nunes O. C. Effects of alkaline hydrolysis and autoclaving on inorganic components present in healthcare waste.
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ABSTRACT

In this work, samples of components usually present in healthcare waste, such as cotton, diapers, transfusion tubes, surgical gloves, examination gloves, adhesives, surgical masks, urine bag collectors, serum bottles and syringes, were subjected to alkaline hydrolysis or autoclaving and effects of these treatments were assessed. Both treatments were carried out at 135 °C and the weight loss and the carbon loss of the components as well as the total organic carbon and the chemical oxygen demand in the effluents were determined. The biodegradability of effluents was assessed by measuring the biochemical oxygen demand after 5 days.

Alkaline hydrolysis caused appreciable degradation in most of the components, with the adhesives and the diapers having the highest weight losses and carbon losses. Components made with low-density polyethylene, high-density polyethylene and polypropylene showed good chemical resistance with 2 M NaOH solution. The effluents obtained after alkaline treatment of

healthcare waste are hazardous due to their very high alkalinity. The effluent obtained after treatment of a mixture of all components using a 2 M NaOH solution was biodegradable with the following parameters: 6.5 g C/L of total organic carbon, 29.8 g O_2/L of chemical oxygen demand and 14.9 g O_2/L of biochemical oxygen demand after 5 days.

Although the autoclaving treatment degraded the components much less than alkaline hydrolysis, the effluents obtained from some components showed an appreciable organic load.

4.1 INTRODUCTION

The HCW is a heterogeneous mixture of wastes, consisting of paper products, textiles in the form of cotton and gauze and plastics. Plastics are the major fraction, around 30 % of initial weight.

The behaviour of some polymers such as PET (Kao et al., 1998; Karayannidis et al., 2002; Kumar and Guria, 2005), polyester (Gu et al., 2001), PVC (Blazevska-Gilev and Spaseska, 2007; Shin et al., 1998) and cellulose (Knill and Kennedy, 2003) have been studied when exposed to alkaline hydrolysis. Despite some achievements in degradation of materials with alkaline solutions, little is known about the interaction of materials under alkaline hydrolysis when digested together and the emissions resulting from this treatment. Also, the effects in common discarded medical components present in HCW during the autoclaving process as well as the final effluent composition and its treatability are not well known. This lack of knowledge makes authorities to have some reluctance in licensing autoclaving plants and in permitting their effluents to be discharged into domestic sewage.

In this work, the effect of a treatment similar to autoclaving in some discarded medical components present in HCW was studied as well as when they were treated with NaOH alkaline solutions. The effect on components was assessed by measuring weight loss (WL) and carbon loss (CL). In addition, thermogravimetric (TG) and differential scanning calorimetric (DSC) analyses were performed on samples before and after the treatments. The effluent

obtained after treating each component alone was characterised with respect to total organic carbon (TOC) and chemical oxygen demand (COD). Also, Principal Components Analysis (PCA) was used to establish differences the results obtained and to establish differences among the components and consequences of the two types of treatments.

Additionally, biochemical oxygen demand after five days (BOD_5) was determined in the effluents resulting from testing a mixture of all analysed components in order to assess its biodegradability and the possibility of being discharged in a domestic wastewater treatment plant.

4.2 MATERIALS AND METHODS

4.2.1 MATERIALS

HCW materials include textiles, glass, metals, anatomical waste and others, but mostly plastics and paper (Prüss et al., 1999). These materials are present in diapers, tubes for transfusion, surgical gloves, examination gloves, adhesives, surgical masks, bag collectors for urine, serum bottles and syringes, but also in some minor components, such as sensors for analysing characteristics from biological fluids (Gupta et al., 2003). Due to their clinical application, materials of these components are subjected to additional treatments during the manufacturing process, such as disinfection, application of anti-allergic substances, additives, and others. Samples of most of those HCW components, including cotton, were considered in this study.

Table 4.1 shows qualitative data on the HCW components used in the trials, according to the respective suppliers, as well as their total carbon (TC) determined in several samples.

Table 4.1 – Components of the HCW used in the experimental work, their materials and carbon content.

Material	Composition	C, %(1)
Serum bottle	Low-density polyethylene (LDPE)	84.76 ± 1.11
Syringe	Polypropylene (PP) and high-density polyethylene (HDPE)	89.01 ± 0.74
Transfusion tube	Polyvinyl chloride (PVC)	47.76 ± 1.38
Bag collector for urine	PVC	50.76 ± 1.34
Examination glove	PVC	53.27 ± 0.15
Surgical glove	> 90 % natural rubber	75.22 ± 1.63
Cotton	94 % cellulose	45.16 ± 0.53
Surgical mask	PP (outer and middle layer); polyester and pressed PE (inner layer); polyurethane (tapes)	84.54 ± 0.72
Diaper	Cellulose fibre and PP (70 – 80 %); PE film; thermoplastics adhesives; elastic threads; flocgel (5 –10 % of sodium polyacrylate)	43.27 ± 1.35
Adhesive	Non-woven polyester; synthetic adhesive from rubber	77.67 ± 0.05

(1) Mean of three determinations ± standard deviation (SD)

4.2.2 METHODS

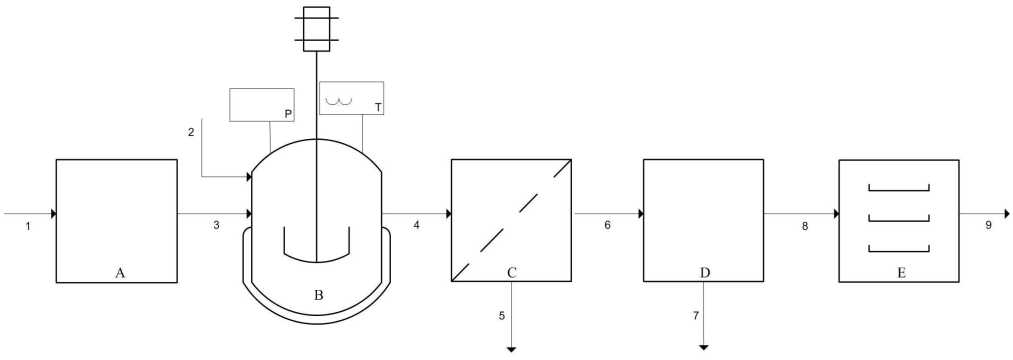
Alkaline hydrolysis and autoclaving-like treatments

The treatments were performed in a Parr batch reactor with a titanium vessel of 450 ml capacity under temperature control and with a pressure gauge. The reactor operated at 135 °C, with a heating rate of 10 °C/min up to 135 °C and holding time of 30 minutes. A liquid/solid ratio of 10:1 (w/w) was used in all the tests. The alkaline solutions tested consisted of 0.1 M, 1 M and 2 M, the last two being also studied by other authors (Taguchi et al., 1991; Taylor et al., 1997). The autoclave-like treatment was performed under the same conditions in the absence of NaOH; therefore, the solids were usually immersed in water and not only in a

water vapour saturated atmosphere. Despite that, it is believed that the consequences of this treatment are not much different than from those obtained from classic autoclaving. For simplicity, the autoclave-like treatment is herein referred to as autoclaving.

All the tests were made using samples with 2 g of each component; 5 g were used when a mixture was studied (0.5 g of each component). Since syringes were composed of two distinct materials, a sample with equal weight of each was taken. Except for cotton, the components were cut in fragments of approximately 1 cm^2 before the tests.

After cooling to ambient temperature, the resulting product was filtered, and, in the case of alkaline hydrolysis, the solid fraction was washed with distilled water in order to remove all of the sodium hydroxide. Subsequently, it was dried, held for 48 h in a desiccator at room temperature and finally weighted. Figure 4.1 shows a diagram of the process.



A – Fragmentation
B – Reaction
C – Filtration
D – Washing
E – Drying

1 – Untreated HCW
2 – NaOH solution
3 – Fragmented HCW
4 – Hydrolysed HCW + Effluent
5 – Effluent
6 – Hydrolysed HCW
7 – Effluent
8 – Washed hydrolysed HCW
9 – Treated HCW

Figure 4.1 – Diagram of the alkaline process.

The pH, TC, TOC and COD, as well as chlorides in case of the medical materials composed by PVC, were determined in the solutions resultant from the treatments. All the experiments were repeated three times and the results showed in the tables are the mean values of the 3 tests under the same conditions.

TC and TOC in solutions were determined with a Shimadzu TC analyser model TOC-VCSH, according to EN 1484 (1997). TC in the materials was determined with the same equipment using its solids module, according to EN 13137 (2001). Measurements of pH were made with a pH-meter model 632 of Metrohm. Chlorides were determined following 4500 B: Argentometric method. COD was determined following 5220 D: Closed reflux – colorimetric method; and BOD_5 following 5210 B: 5-Day BOD method as described by the Standard Methods for Examination of Water and Wastewater (APHA, 1998).

TG and DSC analyses

TG and DSC analyses on the selected components were performed using two equipments (Setaram, model 92-16.18 and model Labsys, respectively). Samples with almost the same volume, ranging from 9 to 40 mg due to their different densities, were placed in a platinum crucible (TG), or aluminium crucible (DSC), heated at a rate of 10 °C/min up to 135 °C and held 3600 s at that temperature; for syringes and surgical masks, composed by polyethylene (PE) and polypropylene (PP), the DSC analyses were prolonged by heating up to 200 °C at the same rate, in order to reach the end of melting peaks of both materials.

Blank tests were carried out for both TG and DSC analyses with unloaded crucibles using the same conditions. Thermal analysis was carried out on original components samples, in those from autoclaving and in those from alkaline hydrolysis with 1 M NaOH solutions. All the TG analyses were carried out in triplicate. The profiles shown are a mean of the 3 values of WL at the same temperature, corrected with the values obtained in the blank test.

Statistical analysis

The effect of autoclave-like and alkaline hydrolysis treatments on the tested components, based on WL, CL, TOC and COD parameters, was assessed through PCA using the software package CANOCO, version 4.5.

4.3 RESULTS AND DISCUSSION

4.3.1 HCW COMPONENTS TRANSFORMATIONS AND EFFLUENTS PRODUCED

Table 4.2 reports the WL values of the tested components after alkaline hydrolysis and autoclaving treatments, computed as the percentage of their initial weight. The low-density polyethylene (LDPE) serum bottle and the high-density polyethylene (HDPE) plus PP syringe samples had the smallest WL values not only after autoclaving but also at all the three NaOH concentrations tested. These results demonstrate the excellent chemical resistance of such materials to alkalis (Ehrenstein, 2001).

Table 4.2 – Weight losses (WL) in the samples of HCW components subjected to autoclaving and alkaline hydrolysis tests at 135 °C.

Material	WL, %[(1)]			
	Autoclaving	0.1 M NaOH	1 M NaOH	2 M NaOH
Serum bottle	N.D.	0.05 ± 0.01	0.03 ± 0.00	0.01 ± 0.00
Syringe	N.D.	0.07 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Transfusion tube	0.06 ± 0.01	0.60 ± 0.08	0.75 ± 0.18	1.12 ± 0.07
Bag collector for urine	0.18 ± 0.04	0.48 ± 0.07	1.35 ± 0.10	4.85 ± 0.45
Examination glove	1.41 ± 0.15	1.60 ± 0.08	5.73 ± 0.80	9.16 ± 1.20
Surgical glove	2.33 ± 0.17	2.42 ± 0.08	2.90 ± 0.00	3.58 ± 0.41
Cotton	1.59 ± 0.10	2.80 ± 0.29	5.36 ± 1.70	9.37 ± 0.96
Surgical mask	N.D.	1.82 ± 0.17	6.24 ± 0.84	8.14 ± 0.94
Diaper	9.40 ± 0,82	11.90 ± 0.73	21.70 ± 1.33	32.10 ± 0.00
Adhesive	0.28 ± 0.05	6.03 ± 0.60	45.87 ± 9.91	53.79 ± 8.50

(1) Mean of three experiments ± SD; N.D. – Not detected.

The surgical mask samples, composed of several materials (not exclusively PP), had non detectable WL under autoclaving but were degraded through alkaline hydrolysis. These treatments degraded the adhesives and diapers significantly, mainly when the 1 M and 2 M NaOH solutions were used. The WL obtained for diapers and adhesives with these two

solutions were above 20 %, being similar to or higher than the range of 20–35 % reported for autoclaving after HCW size reduction (Prüss et al., 1999). The assayed adhesive samples shrank drastically during the alkaline treatments, being kept agglomerated with part of its glue or any other binding agent. Most polyester present in composition was probably lost into the solution (Bendak, 1991).

Table 4.3 reports the CL values of the tested components after both treatments, calculated as the percentage of their initial carbon content, shown in Table 4.1. The values of CL followed the trend seen for the WL values (Table 4.2) i. e., the highest and lowest CL were observed on the components that had the highest and the lowest WL, respectively. CL is a relevant part of the WL and contributed significantly to the high organic load of the effluents resultant from alkaline hydrolysis treatment, particularly for adhesives and diapers (Tables 4.4 and 4.5).

Table 4.3 – Carbon losses (CL) in the samples of HCW components subjected to autoclaving and alkaline hydrolysis tests at 135 °C.

Material	**CL, %**			
	Autoclaving	**0.1 M NaOH**	**1 M NaOH**	**2 M NaOH**
Serum bottle	0.01	0.14	0.21	0.28
Syringe	0.01	0.16	0.21	0.22
Transfusion tube	0.04	1.05	1.07	1.13
Bag collector for urine	0.11	0.93	2.52	3.31
Examination glove	0.71	1.73	2.14	4.17
Surgical glove	0.35	1.05	1.77	1.65
Cotton	0.38	3.03	7.15	8.57
Surgical mask	0.04	1.61	6.42	6.47
Diaper	(1)	15.25	24.98	43.86
Adhesive	0.09	5.15	49.27	59.79

(1) CL was not determined because the diaper sample absorbed all the solution.

Table 4.4 – TOC, in mg C/L, and pH in the effluents resulting from autoclaving and alkaline hydrolysis of individual samples of HCW components.

Material	Autoclaving		0.1 M NaOH		1 M NaOH		2 M NaOH	
	TOC	pH	TOC	pH	TOC	pH	TOC	pH
Serum bottle	6	5.5	[21 – 46](2)	12.8	[37 – 52](2)	13.4	[33 – 73](2)	13.4
Syringe	13	7.7	40	12.7	45	13.1	78	13.9
Transfusion tube	23	7.6	124	12.7	339	13.3	473	13.7
Bag collector for urine	54	7.4	232	12.6	475	13.3	1517	13.8
Examination glove	380	7.3	814	12.8	1061	13.4	2129	13.8
Surgical glove	265	7.2	798	12.8	1071	13.4	[513 – 750](2)	13.9
Cotton	171	6.1	1051	12.7	2782	13.3	3649	13.8
Surgical mask	37	6.0	1487	12.7	4940	13.4	5311	13.9
Diaper	(1)	(1)	6381	12.6	7943	13.3	17325	13.7
Adhesive	68	5.5	3895	12.1	38133	13.0	45951	13.8

(1) Not determined because the diaper sample absorbed all the solution; (2) Due to the variability of the results, values are presented as its range.

As expected, all the effluents resultant from the alkaline hydrolysis assays showed high pH values, being approximately 12.7, 13.3 and 13.8 when 0.1 M, 1 M or 2 M NaOH were used, respectively. On the contrary, those from the autoclaving tests were close to neutrality. The alkaline effluents showed higher organic load than the autoclaved ones. In general, a positive correlation between the TOC and COD values and the NaOH concentration used was observed. TOC of the effluents resulting from the treatment of serum bottles and syringes were below 100 mg C/L under all the conditions tested. This result is overall agreement with the small WL obtained for such components. On the contrary, the effluents resulting from the treatment of diapers and adhesives showed the highest TOC and COD, which is also in agreement with the WL verified in both treatments. Chloride concentrations in the effluents from treatments of components made with PVC (such as the transfusion tubes, the examination gloves and the urine bag collectors) were below 10 mg/L (limit of detection for the method of analysis used). This fact confirms that there was a very slight PVC decomposition under the studied

conditions. Therefore, chlorides will not become a problem in the treatment of the respective effluents when these technologies are to be applied to treat HCW.

Table 4.5 – COD in the effluents from autoclaving and alkaline hydrolysis tests of samples of individual components.

Material	**COD, mg O_2/L**			
	Autoclaving	**0.1 M NaOH**	**1 M NaOH**	**2 M NaOH**
Serum bottle	90	[101 – 151](2)	[156 – 214](2)	[154 – 480](2)
Syringe	109	146	207	247
Transfusion tube	181	269	1242	1859
Bag collector for urine	423	553	1210	2437
Examination glove	563	3790	4909	11440
Surgical glove	1956	3827	5383	7357
Cotton	828	2943	8875	15107
Surgical mask	235	2517	16778	21850
Diaper	(1)	30646	34147	41971
Adhesive	337	14192	88192	143756

(1) Not determined because the diaper sample absorbed all the solution; (2) Due to the variability of the results values are presented as its range.

Table 4.6 shows TOC, COD and BOD_5 values of the effluents resultant from the treatments of a mixture of samples of all components. The estimates of TOC and COD values were calculated as a linear combination of the average values of these parameters in the effluents from individual tests of samples of each component. They were a reasonable first approximation to the TOC and COD values for the effluents resultant from the alkaline hydrolysis of a mixture of these components. Using the COD and BOD_5 values for calculating BOD_5/COD ratio, one obtains 0.44, 0.53 and 0.50, respectively, for the alkaline hydrolysis effluents and 0.35 for the autoclaving effluent. Thus, despite the fact that the effluents obtained from alkaline hydrolysis had a much higher organic load, they were more biodegradable than the ones from autoclaving. Since all ratio values are above 0.4, the alkaline effluents, after neutralization, could be accepted in a common domestic wastewater treatment plant.

Table 4.6 – TOC, as mg C/L, and COD and BOD_5, as mg O_2/L, in the effluents from autoclaving and alkaline hydrolysis tests of mixtures with samples of the components[(1)].

	Autoclaving	0.1 M NaOH	1 M NaOH	2 M NaOH
TOC	464 ± 42	1692 ± 245	5286 ± 900	6504 ± 83
$TOC_{estimate}$	(*)	1487 ± 7	5687 ± 30	7019 ± 21
COD	958 ± 53	5050 ± 900	12967 ± 1991	29779 ± 1212
$COD_{estimate}$	(*)	5725 ± 30	16074 ± 72	24682 ± 64
BOD_5	333 ± 32	2237 ± 366	6906 ± 1407	14875 ± 883

(1) Mean of four tests ± SD; (*) diaper effluent was all absorbed, thus there was no values for using in computation.

4.3.2 TG AND DSC ANALYSES

The changes in weight and heat flow detected in the blank tests of TG and DSC analyses were negligible. The WL values of the three TG replicate tests of a given component at a given temperature showed very low scatter with variation coefficients of less than 0.7 % in all temperatures ranges up to 135 °C, thus confirming the very good reliability of the analytical method.

All the average TG profiles of healthcare components after being subjected to autoclaving and alkaline hydrolysis were not differentiable from the original ones, except in case of diapers and cotton. These cellulose-containing components before the treatments had WL values of approximately 5.7 % and 6.2 %, respectively. In case of cotton (Figure 4.2), most of the WL is due to water evaporation (Deng et al., 2008). The diaper decomposed more in the alkaline hydrolysis treatment than in the TG analysis; alkaline hydrolysis increased the decomposition of part of the materials from diapers, mostly sodium polyacrylate from flocgel, keeping a residual material stable at least up to 135 °C.

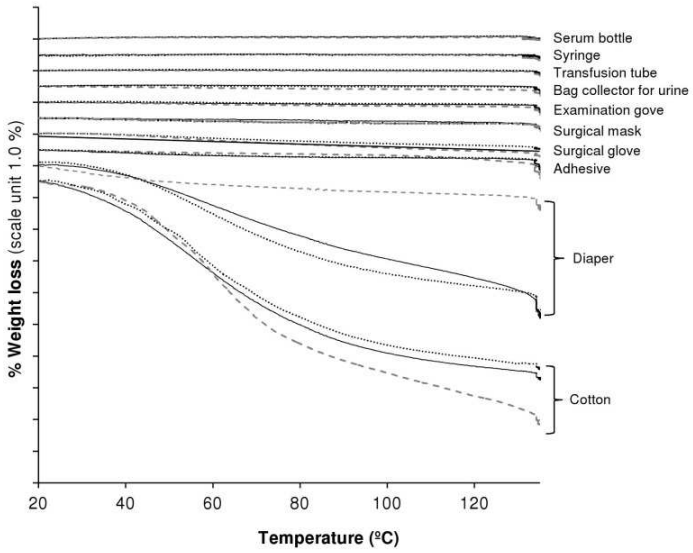


Figure 4.2 – TG profile of HCW components; (–) original; (---) hydrolysed; (····) autoclaved.

All the other components had higher WL after alkaline hydrolysis compared to the TG analysis, which means that they were chemically attacked and increasingly degraded by such solutions. Evaluated in the same way, autoclaving significantly affected the examination gloves and the surgical gloves, slightly affected the urine bag collectors and did not affect all the remaining sample components. Syringes, transfusion tubes, surgical masks and adhesives presented slightly less WL after autoclaving than in TG. As consequence, effluents resultant from treatment of examination gloves, surgical gloves and urine bag collector were those with the highest COD among all the effluents resulting from autoclaving, as shown in Table 4.5. The LDPE serum bottle and the HDPE plus PP syringe samples, both before and after the treatments showed negligible WL under the temperature cycle imposed, as shown in the Figure 4.2. This pattern is characteristic of medical components with very good chemical and thermal resistance in the range of conditions tested.

The TG profiles of the PVC components before the treatments were close to those observed in a previous study where similar conditions were used (Deng et al., 2008). Although being based in the same material (PVC), the urine bag collectors and the examination gloves had different

TG profiles and WL values after the alkaline hydrolysis when compared to the transfusion tubes. This behaviour is surely due to differences in its composition, namely the additives.

The surgical mask TG profiles showed the same trend of the other reasonably stable components with similar WL before and after the alkaline hydrolysis. TG analyses of adhesives and surgical gloves showed WL of the same magnitude, both for treated and untreated samples. Nevertheless, adhesives visibly decomposed more than surgical gloves when held at 135 °C for 3600 s. This behaviour was also found in the untreated diaper samples as shown in Figure 4.2. This instability was increased by the alkaline hydrolysis. Consequently, the effluents resulting from the adhesive and diaper treatment were the most contaminated of all.

The DSC profiles, in Figure 4.3, indicate that both autoclaving and 1 M NaOH alkaline hydrolysis caused sensible modifications in all the components. Except for serum bottles, the comparison of the profiles show that after the autoclaved and alkaline hydrolysis treatments the components required more specific energy for softening and melting than the untreated ones.

This was most probably due to the release of the less stable constituents during the treatments and to the resultant hydration that increases the amount water to be removed during the thermal cycle.

The profiles of the PVC components, i.e., transfusion tubes, urine bag collectors and examination gloves were similar. This is particularly true for the last two untreated components, which showed exothermic reactions above 105 °C whereas for transfusion tube was above 120 °C. In case of the untreated diapers, the exothermic reactions started near 50 °C and held up to the end of the thermal cycle, indicating the continuous degradation of this component. After alkaline hydrolysis treatment, the diaper substances responsible for the exothermic reactions were apparently eliminated.

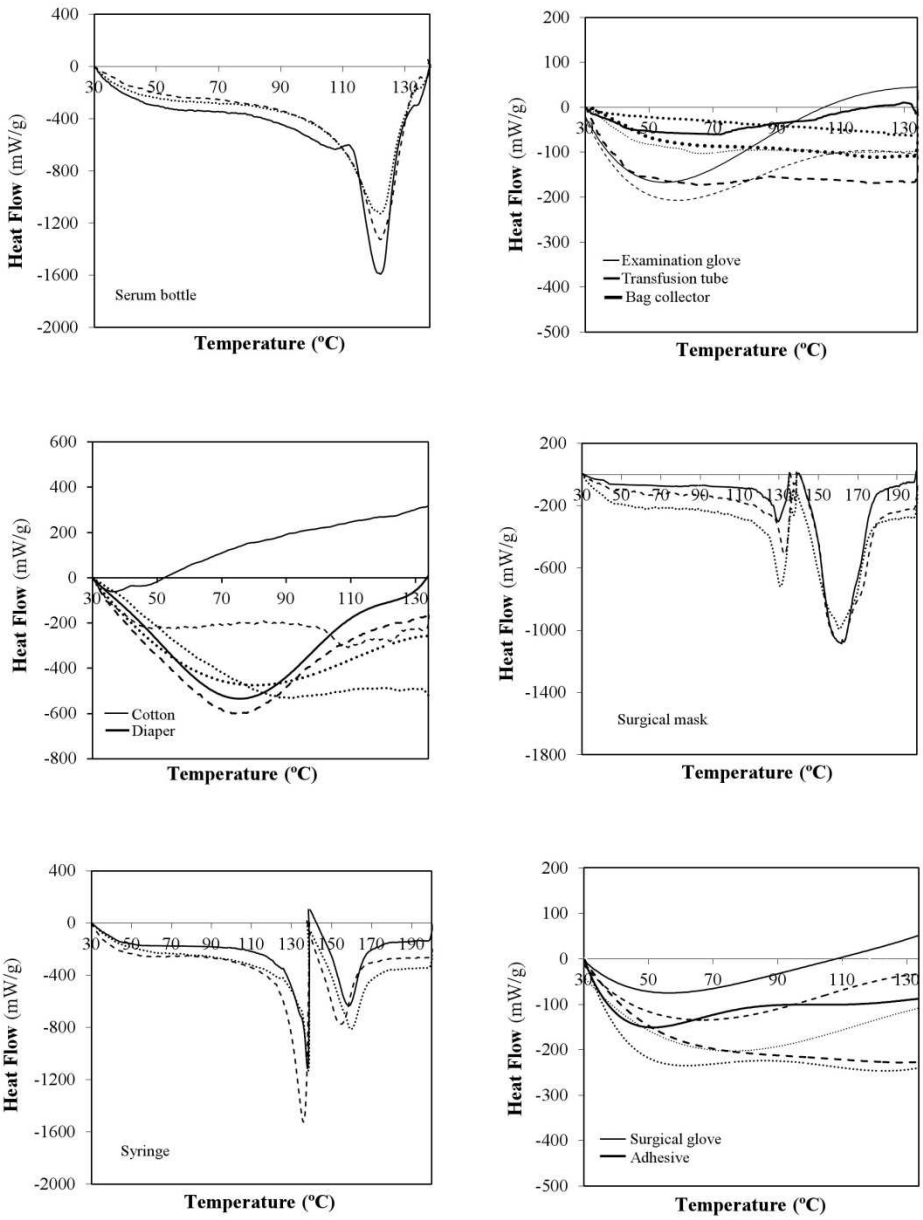


Figure 4.3 – DSC profile of HCW components, (–) original; (---) hydrolysed; (····) autoclaved. The DSC profiles of untreated and hydrolysed bag collector for urine coincide with DSC profiles of examination glove.

The same happened with the PVC components and the surgical glove. Syringe and surgical mask profiles show well defined patterns of endothermic transformations with two peaks and the serum bottle showed only one endothermic peak. No significant differences between the peak temperatures in the untreated and treated components occurred. Differences in the DSC

profiles of these three components are probably related to their composition. While syringe and surgical masks are multi-material components, serum bottles are composed by LDPE alone.

4.3.3 PRINCIPAL COMPONENTS ANALYSIS

Through multivariate analysis it was possible to conclude that the treatments promoted the degradation of all components, as measured by their WL and CL with consequent increase of the TOC and COD of their liquid effluents. The first two axes of the PCA, which could explain 99.4 % of the variation found among the components after the treatments. All the parameters used to characterize the components showed high eigenvalues and significant correlation values with axis 1, contributing to separate the samples with the highest WL, CL, TOC and COD values, i.e., those of diapers and adhesives treated with 1 M and 2 M NaOH solutions, from the others which clustered in group A in Figure 4.4 (a). These results indicate that among all the components and treatments tested, diapers and adhesives were the most prone to alkaline hydrolysis, and that the degree of their degradation correlated with the NaOH concentration used.

In an analysis excluding the diaper and adhesive samples, the 2 first orthogonal axes from PCA explained 97.9 % of total variance (Figure 4.4 (b)), and the samples clustered in 3 groups. Among this sub-set of components, samples of surgical masks after treatment with 1 M or 2 M NaOH, which had the highest WL and CL values and produced effluents with highest load of TOC and COD, clustered together. Among the remaining sub-set of components, also cotton treated with 1 M or 2 M NaOH and examination glove treated 2 M NaOH (group C) could be distinguished from the others (group B). These results indicate that after diapers and adhesives, surgical masks followed by cotton and examination gloves are the components more susceptible to alkaline hydrolysis, if NaOH concentrations higher than 1 M are used.

In order to assess the effect of the tested treatments on the less degradable components, multivariate analyses including the data of individual components were performed. Despite the small variation on the values of the analysed parameters after the different treatments, the PCA biplots obtained, herein exemplified with syringe data (Figure 4.4 (c)), indicated that the degree of degradation depended on the NaOH concentration. Therefore, autoclaving was the less aggressive treatment while 2 M NaOH promoted the highest deterioration of each individual component.

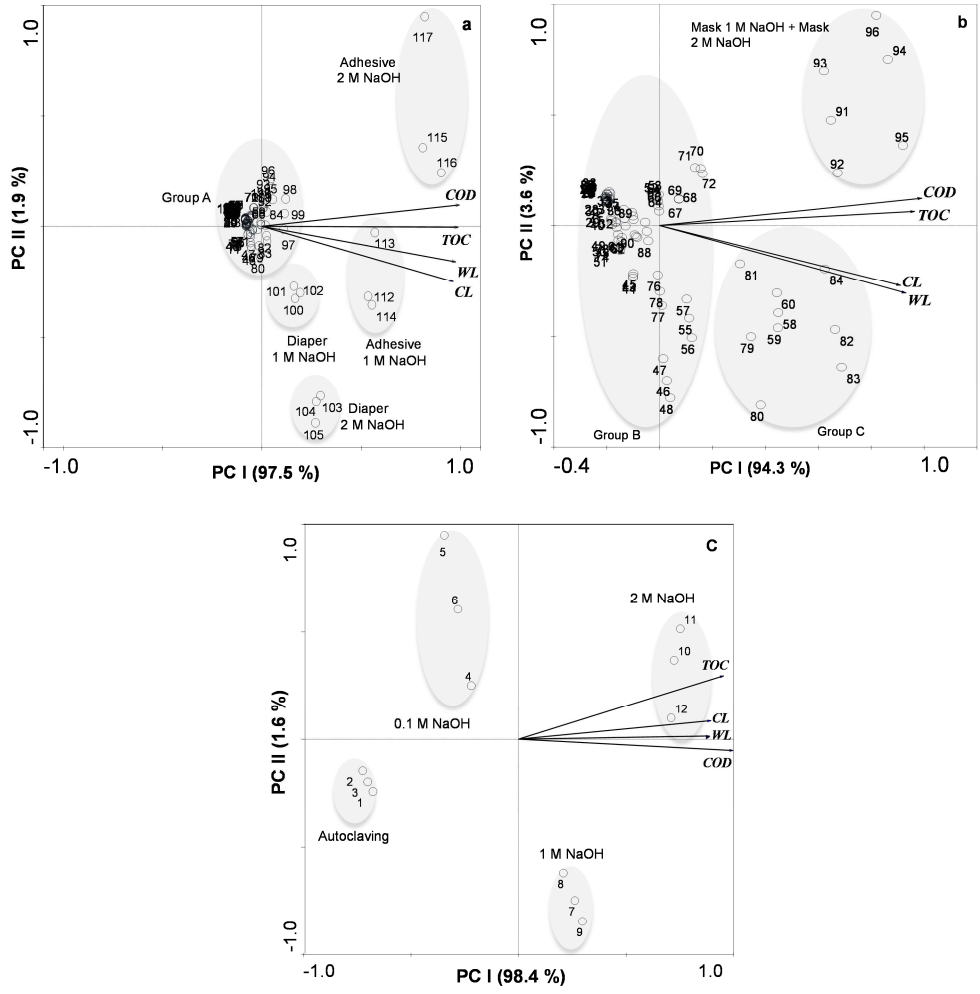


Figure 4.4 – Principal components analysis of the HCW treatments results and spatial distribution of the samples; arrows refer the parameters considered in the analysis (i.e., WL and CL of the sample and TOC and COD in the effluent produced): 3(a) – all the samples; 3(b) – all the samples except diaper and adhesive; 3(c) – syringe samples.

4.4 CONCLUSIONS

Common discarded medical components from healthcare waste subjected to autoclaving or alkaline hydrolysis degraded in a higher or lower degree according to the thermal resistance of the materials in their composition. Thus, components with LDPE, HDPE or PP, that are very stable up to 135 °C, showed good resistance to both treatments.

The components tested lost up to 10 % of their weight, which means that the treatments were not efficient in reducing the mass of waste, except in the case of diapers and adhesives where the reduction is appreciable as shown by a 30 to 50 % mass loss with the 2 M NaOH solution.

The composition of effluents resulting from the treatment depends on the degradation degree of the components and also on the solutions used. Those from alkaline treatment were hazardous due to their very high pH (> 12.5). On the contrary, the pH of effluents from autoclaving was close to neutrality or slightly acid. Alkaline effluents showed higher organic loads than those effluents obtained from autoclaving. Also, TOC and COD values increased as NaOH concentration increased in the solution used, reaching a COD of 42 and 144 g O_2/L for the 2 M NaOH solutions in the case of diapers and adhesives, respectively. Although with very high organic loads, the effluents produced in alkaline hydrolysis of a mixture of all the components were biodegradable after neutralization. Therefore, these effluents might be acceptable in a domestic wastewater treatment plant.

Autoclaving degraded components much less than alkaline hydrolysis. Therefore, the resulting effluents presented non-negligible organic loads, mainly from materials such as natural rubber, cellulose and polyvinyl chloride. Nevertheless, when treated under the same conditions, the effluents resulting from autoclaving were less biodegradable than those resulting from alkaline hydrolysis treatment, but showed values close to the limit of biodegradability.

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CHAPTER 5 – APPLICABILITY OF ALKALINE HYDROLYSIS TO DESTROY ANIMAL TISSUES PRESENT IN HEALTHCARE WASTE

This chapter is based on Pinho S. C.; Almeida M. F.; Nunes O. C. Applicability of alkaline hydrolysis to destroy organic components present in healthcare waste.
in 2nd International Conference WASTES: Solutions, treatments and opportunities.

ABSTRACT

In this study, the minimum conditions of temperature and NaOH concentration for the total destruction of animal tissues were evaluated, using pork or beef as surrogates. The alkaline hydrolysis trials were performed with 1 M and 2 M NaOH solutions at different temperatures and different hydrolysis times.

The pork and beef showed a similar behavior when subjected to alkaline hydrolysis. The destruction of meat was faster than the bone. The meat was totally hydrolyzed above 95 °C in less than 60 minutes. The effluents obtained after alkaline treatment are hazardous due to their very high pH. Although with very high organic load, the effluents produced in alkaline hydrolysis were biodegradable after neutralization. Therefore, it may be acceptable to discharge the neutralized effluents in a domestic wastewater treatment plant.

5.1 INTRODUCTION

One of fundamental policies for waste management is based on the waste quantities reduction. For this purpose the Best Environmental Practices (BEP) include source reduction, segregation, resource recovery and recycling. Likewise, the selection of the treatment system takes into account the waste characteristics, the volume and mass reduction obtained in the treatment, among many others (Chartier et al., 2014). Animal by-products is a type of waste that have been increasing over the last years due mainly to the increasing consumption of meat. Animal by-products are animal carcasses, parts of animals, or other materials which come from animals but are not meant for humans to eat. Of the 47 million tonnes of animals slaughtered for meat production in Europe every year, 17 million tonnes of by-products, such as minus hides, skins and bones for gelatin production are handled by the animal by-products industry. However, considerable numbers of carcasses are also left to rot or are illegally dumped (BREF, 2005). This illegal disposal poses a potential risk to the public health and to the environment.

In 2002, with the appearance of BSE emerged restrictions that have led to an increased proportion of solid material being disposed of to landfill and by incineration. The limits placed on the traditional uses for animal by-products have led to further alternative uses and to new methods for disposal.

Indeed, animal proteins have a very high biological value which opens wide possibilities for their use for generating of energy. Disposal by incineration and co-incineration is an advantageous energetic valorization process however, due to its high organic matter content, there is a great potential for anaerobic digestion. Nevertheless, this technology requires long process time and large facilities due to slow anaerobic processes involved (Ro et al., 2007).

Therefore, the alkaline hydrolysis emerges as a waste treatment option, mainly because it is able to significantly reduce the volume of animal wastes and produces sterile by-products

(Tracker, 2004) which can be used for soil fertilization (Kalambura et al., 2011). The efficacy of alkaline hydrolysis on the destruction of tissue wastes, including anatomical parts, organs, placenta, blood, body fluids, specimens, human cadavers and animal carcasses has been proven in some studies (Tracker, 2004). However, conditions for destruction are reported to be 150 °C with a time of contact between three and eight hours. In this work it is intended to study the minimal conditions for destruction of animal tissues. The behavior of animal tissues, such as pork and beef, was studied when treated by alkaline hydrolysis. The efficiency of the treatment was assessed by determination of weight losses on the materials and characterization of TOC, COD and BOD_5 in the effluents resulting from the alkaline hydrolysis treatment.

5.2 MATERIALS AND METHODS

The treatments were carried out in a Parr batch (Figure 5.1), described in Chapter 4, section 4.2, with a heating rate of 10 °C/min. The samples were heated at 80 °C, 90 °C, 95 °C, 100 °C and 110 °C, during 30 to 240 minutes. A liquid/solid ratio of 5:1 (w/w) was used in all the tests. The tests were made using samples of 20 to 30 g of pork meat including bones (5:1 or 4:1 of meat/bone). The alkaline solutions used were 1 M and 2 M NaOH. The selection of these concentrations was based on the results described in Chapter 4. To compare the behavior of pork and beef, both containing bone, tests were performed at 90 °C using 1 M or 2M NaOH solutions over 150 minutes.

To compare the hydrolysis efficiency of meat versus bone, pork meat or bone samples were hydrolized at 90 °C, with 1 M or 2 M NaOH at different times.

After cool down to ambient temperature, the resulting solid product was filtered, washed with distilled water in order to remove all of the sodium hydroxide, after which it was dried at room temperature, further held for 48 hours in a desiccator and finally weighted. The pH, TOC, COD and BOD_5 were determined in the solutions resultant from the treatments, according the

methods described in Chapter 4, section 4.2.2. Also, the main chemical compounds, in hydrolyzate at 110 °C and 1 M of NaOH solution, were analysed by gas chromatography with mass detector (GCMS) using an Agilent HP 6890/MSD 5793N from HP, 30 m × 0.25 mm I.D., 0.5 µm P/N 19091S-133 column; and using as carrier gas He at constant flux of 1.2 mL/min. Tests were carried out in the following conditions: split-less injector at 280 °C; oven 1 minute at 50 °C, followed by heating at 10 °C/min till 300 °C; transference line at 290 °C; and MSD scan mode. The separated compounds were identified using NIST 1998 library match.

Figure 5.1 – Reactor and temperature control.

Additionally, TG and DSC analyses were performed on the samples before and after alkaline hydrolysis with 1 M NaOH solution. The method used in TG and DSC analyses is described on Chapter 4, section 4.2.2. Briefly, samples of 40 mg were heated at a rate of 10 °C/min up to 135 °C and held 3600 s at this temperature; after they were heated up to 200 °C at the same rate.

5.3 RESULTS AND DISCUSSION

For the temperature and NaOH concentrations used in this work, it was verified that with temperature raise, the time required to hydrolyze pork and beef decreased. At 100 °C and 1 M

NaOH, 45 min was sufficient to destroy all the sample, however at 80 °C, 240 min was necessary. Accordingly, the percentage of solid residue decreased with the increase of the temperature (Table 5.1).

The increase of NaOH concentration, under specific conditions, namely at 90 °C and 95 °C for 1 M and 2 M NaOH, does not seem to influence the hydrolysis time. Indeed, small differences were found in all the analyzed parameters for tests carried out at 1 M and 2 M NaOH. Nevertheless, differences in TOC, COD and BOD_5 were observed, which may be due to variation on the proportion of meat and bone in each tested sample.

In the most aggressive conditions, temperature and NaOH concentration, the proteins were hydrolyzed and esterified as showed in chromatography analysis of hydrolyzate of 110 °C and 1 M NaOH solution. The molecular ions more frequently detected and with higher relative abundance had molecular mass of 28, 44, 72, 86 and 117. The main chemical structures identified include alkyl group and chains, amides and esters. NIST 1998 data base proposes the presence of propanamide, 4-methyl phenol, 4-methyl pentanamide, ethyl ester L-Isoleucine, indole, and triethyl phosphate.

Table 5.1 – Alkaline hydrolysis conditions of samples composed by pork meat including bones versus hydrolysis time, residue amount, TOC, COD and BOD_5 of the effluent produced.

Experimental conditions	Time (min)	Residue (w/w %)	Effluent		
			TOC (g/kg)	COD (g/kg)	BOD_5 (g/kg)
80 °C, 1 M NaOH	240	25.0	97.5	n.d.	n.d.
90 °C, 1 M NaOH	90	12.3	101.0	345.8	316.6
90 °C, 2 M NaOH	90	17.5	100.0	307.0	220.8
95 °C, 1 M NaOH	50	15.0	122.5	360.2	319.2
95 °C, 2 M NaOH	50	4.9	101.6	312.0	271.2
100 °C, 1 M NaOH	45	3.2	110.6	351.5	302.2
100 °C, 2 M NaOH	40	3.6	83.0	287.4	204.7
110 °C, 1 M	35	3.8	115.7	356.9	260.9
110 °C, 2 M	35	3.1	116.1	345.6	230.3

n.d.- not determined

When bovine and pork were compared, both containing meat and bone, it was observed small differences only for short times of contact (up to 60 min). For higher times of hydrolysis, the TOC values obtained for both type of samples were similar, independently of the NaOH concentration (1 M or 2 M) as shown in Figure 5.2.

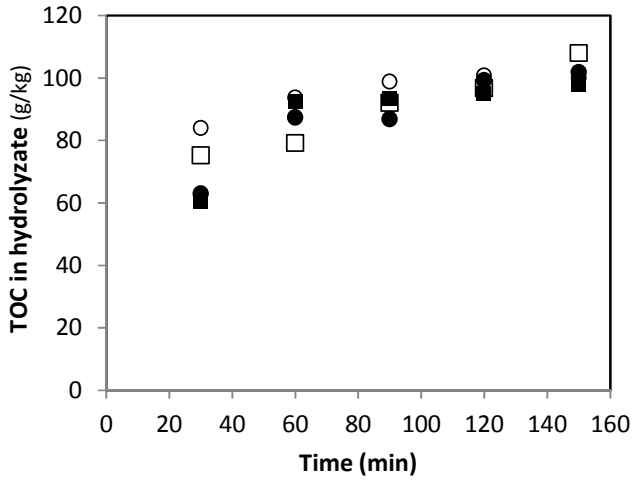

Figure 5.2 – Alkaline hydrolysis of pork ((□) 1 M NaOH; (■) 2 M NaOH) and bovine ((○) 1 M (●); 2 M NaOH) meat at 90 °C over time.

When meat and bone were hydrolyzed separately at 90 °C, it was observed that meat requires less time of contact than bone, as expected due to their composition. The meat is composed mainly by water followed by proteins and lipids, which are easily hydrolyzed. The bone is composed predominantly by mineral component (carbonated hydroxyapatite), organic component (mainly type I collagen, 22 % by weight and 36 % by volume) and water (Collins et al., 2002; Figueiredo et al., 2012).

For the same temperature and NaOH concentration, higher TOC values and lower solid residue values increased with time of hydrolysis, i.e., the treatment efficiency increased for both type of samples (Table 5.2). As expected, for the same time of contact and temperature, treatment with 2 M NaOH showed a better efficiency, for both type of samples (Table 5.2).

Even with under the harsher conditions tested (90 °C, 2 M NaOH and 60 minutes), it was not possible to completely destroy the bone samples, since solid residues constituted 20 % of the initial weight. This resultant solid residue could be crushed with reduced pressure to a powder sized fragments, probably because hydrolysis promotes the digestion of the structural collagen, which is needed to strengthen the bone structure (Collins et al., 2002) but not the mineral fraction, mainly constituted by calcium phosphate (Kaye et al., 1998) (Fig.5.3). In contrast, 90 °C, 1 M NaOH and 45 minutes were sufficient to achieve complete meat hydrolysis as shown in Table 5.2.

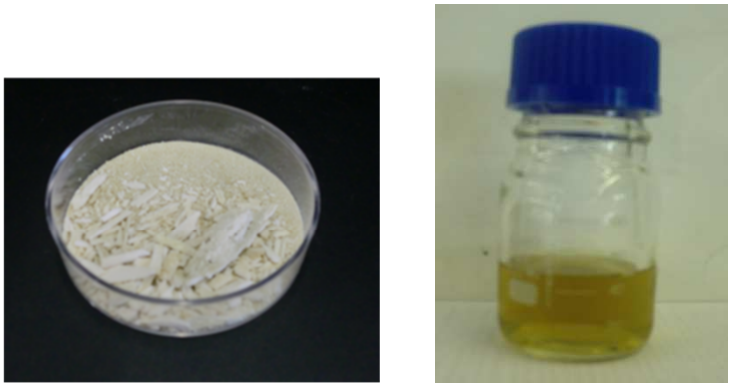

Figure 5.3 – Hydrolyzed bone and hydrolyzate.

Table 5.2 – Alkaline hydrolysis conditions versus hydrolysis time and residue amount of the meat and bone.

	Experimental conditions	**Time (min)**	**Residue (%)**	**TOC (in hydrolyzate) (g/kg)**
Meat	90 °C, 1 M NaOH	30	6.7	90.2
		60	0.0	94.0
	90 °C, 2 M NaOH	30	1.0	89.1
		45	0.0	92.0
Bone	90 °C, 1 M NaOH	30	66.5	77.9
		45	38.2	85.6
		60	18.2	104.4
	90 °C, 2 M NaOH	30	52.7	90.0
		45	23.4	92.0

The analysis of the TG profiles of bone showed that the highest weight loss occurred during the first heating step, up to 135 °C (Figure 5.4). Untreated bone showed a weight loss of 5 % due, essentially, to water loss. In opposition, TG profiles of hydrolyzed bone at 80 °C, 90 °C and 100 °C with 1 M NaOH showed weight losses of 10 %. Most probably, these weight losses were due to the increased amount of water absorbed by the matrix during the treatment. The fact that hydrolyzed bone showed similar TG profiles independently of the temperature, suggest that this parameter does not seem to influence the hydrolysis of the bone inorganic matrix.

Similar conclusions were obtained through the analysis of the DSC profiles. These profiles showed an endothermic peak corresponding to the evaporation of water for both untreated and hydrolyzed bone, which occurred at a lower temperature for the hydrolyzed ones.

The TG analysis of untreated meat showed a high weight loss of 74 % due essentially due to water evaporation, the main component of meat (data not shown). As expected, it was not possible to obtain TG profiles of treated meat, since no residues were obtained.

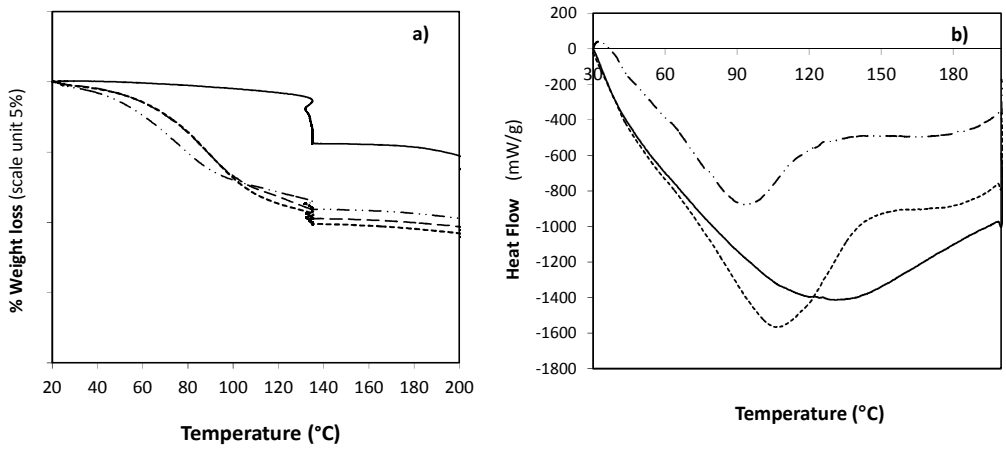

Figure 5.4 – TG (a) and DSC (b) profiles of bone; (–) untreated; (–···–) alkaline treated at 100 °C (---) alkaline treated at 90 °C; (····) alkaline treated at 80 °C.

The effluents obtained from alkaline hydrolysis of animal tissues showed a brownish color and gelatinous, suggesting that the collagen was solubilized (Collins et al., 2002). The effluents showed also a higher value of TOC, COD and BOD_5 with average values of about 20 000 mg/L, 70 000 mg/L and 50 000 mg/L, respectively. Although with very high pH, approximately 13, and organic load, the effluents produced in alkaline hydrolysis are, presumably, biodegradable, since a BOD_5/COD ratio above 0.70 was obtained after neutralization. Therefore, these effluents might be discharged and treated in a wastewater treatment plant.

5.4 CONCLUSIONS

Higher NaOH concentration and longer times of contact favored the hydrolysis of animal tissues. When tested separately, the meat was hydrolyzed faster than the bone when subjected at same treatment conditions. Meat hydrolysis was complete while bone generated a solid residue, which was easily crushed into a powder. Temperature at which hydrolysis was carried out influenced considerably the time required to destroy animal tissues as well as the solid residue obtained.

Effluents resultant from alkaline hydrolysis of animal tissues, although with very high pH and organic load, were, presumably, biodegradable after neutralization.

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CHAPTER 6 – INACTIVATION OF *Geobacillus stearothermophilus* SPORES BY ALKALINE TREATMENT

This chapter is based on Pinho S. C., Nunes O. C., Lobo-da-Cunha A., Almeida M. F.
Inactivation of *Geobacillus stearothermophilus* spores by alkaline treatment.
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ABSTRACT

This study reports alkaline treatment as an alternative disinfection/sterilization method for healthcare waste. The effects of this treatment on the resistance of *Geobacillus stearothermophilus* spores were investigated and the influence of temperature and NaOH concentration was evaluated. The alkaline conditions were performed using 0.1 M, 0.25 M, 0.5 M, 0.75 M or 1 M NaOH at 80 °C, 100 °C and 110 °C. In addition, spore inactivation in the presence of animal tissues and discarded medical components, used as surrogate of healthcare waste, was also assessed. The effectiveness of the alkaline treatment was carried out by determination of survival curves and D–values.

No significant differences were seen between D–values obtained at 80 °C and 100 °C. The D–values obtained at 110 °C (2.3 – 0.5 min) were approximately 3 times lower than those at 100 °C (8.8 – 1.6 min). The alkaline treatment may be used in future as a disinfection or sterilization alternative method for contaminated waste.

6.1 INTRODUCTION

A large number of methods are available to inactivate microorganisms. Most of them use the same fundamental principle of heat, chemicals, irradiation or combinations of these. Several methods are currently used for the sterilization, defined as a process that destroys all forms of life including dormant. These methods include plasma, vapour-phase hydrogen peroxide, ozone, chloride dioxide, autoclaving, ethylene oxide and radiation. The selection of the method depends on the type of material being treated as well as the intended purpose. For instance, the last three methods are the most widely used for the sterilization of medical instruments. Each of these methods has advantages and disadvantages. Autoclaving is usually employed to kill bacteria, viable spores including endospores and virus in heat resistant materials. At 121 °C or higher, sterilization is achieved. When temperature below 121 °C is used a disinfection process occurs, which may kill vegetative forms of microorganisms, such as pathogens or other harmful organisms but do not inactivate bacterial endospores (Russell, 2001).

Autoclaving is extremely time-consuming and is not adequate to treat heat sensitive materials. Exposure to ethylene oxide is highly efficient due to its penetrative properties. Therefore, it is considered one of the most suitable sterilization processes for thermo sensitive materials. However, ethylene oxide is extremely toxic and presents risks associated with handling a flammable (Mendes et al., 2007). Radiation by gamma rays or electron beam are also very effective sterilization methods, but can affect product integrity and can degrade polymers and rubbers. Additionally, their utilization requires high capital investment (Haji-Saeid et al., 2007). Plasma technology has been studied as an alternative to conventional sterilization methods (Kylián et al., 2006; Yardimci and Setlow, 2010). This method has some advantages over others, such as low energy consumption, absence of residuals and toxic emissions, safety and low capital and operational costs (Yardimci and Setlow, 2010). Nevertheless, it has a particular

limitation, namely its incompatibility with some polymeric materials (Lerouge et al., 2002). Sterilization processes are not only necessary for high added-value materials. Indeed, tonnes of HCW are produced per year (Lee and Huffman, 1996; Diaz et al., 2008) and must be treated to eliminate the infectious potential prior to disposal. Autoclaving and incineration are the main processes used for treating HCW (Lee and Huffman 1996, Sukandar et al., 2006). However, these processes demand high investment and exploration costs and it is not appropriate to treat small quantities of HCW. In this context, it is essential to develop effective low cost alternative sterilization processes.

Various microorganisms, including pathogens, produce dormant forms, which permit their survival under stress conditions, such as high temperature, irradiation or chemical damage. Amongst these structures, the endospores, herein further designated as spores, produced by some low G+C Gram-positive bacteria, are the most resistant to harsh conditions. Several spore traits have been described to be involved on resistance against physical and chemical antimicrobial agents. The low water content in the spore core seems to be the most important factor of a spore wet heat resistance. Indeed, the wet heat resistance correlates negatively with the core water content (Setlow, 2006). The high core mineralization also confers wet heat resistance; ions such as Ca^{2+} ensure a higher wet heat protection than Mg^{2+}, Mn^{2+}, Na^{+} and K^{+}. Another essential factor to the spore resistance is the high quantity of small acid-soluble spore proteins (SASPs) that protect the spore DNA by its saturation with α/β–type SASP and DNA repair systems (Setlow, 2006; Leggett et al., 2012).

Geobacillus stearothermophilus comprise low G+C Gram-positive, thermophilic non-pathogenic organisms, and their spores are one of the most heat and chemical agents resistant. Indeed, the low water content in the core and the intrinsic thermostability of proteins confers to spores of thermophilic species a higher resistant to wet heat than to those of mesophiles (Guizelini et al., 2012). Therefore, the spores of this organism are often used as

a biological indicator to assess the effectiveness of sterilization methods (López et al., 1997; Watanabe et al., 2003; Wood et al., 2010).

This study reports the alkaline treatment as disinfection and sterilization alternative methods for waste contaminated with infectious agents. The successful inactivation of a Creutzfeldt–Jajob disease (CDJ) agent by a sequential process involving exposure to 1 M NaOH, followed autoclaving at 121 °C for 30 min (Taguchi et al., 1991), the inactivation of 22A strain of scrapie agent by autoclaving at 121 °C for 30 min in the presence of 2 M NaOH (Taylor et al., 1997) and the inactivation of prions by 0.1 M NaOH after pre-treatment with detergent (Bauman et al., 2006) has been proven. More recently, the prion decontamination using 0.15 M NaOH at 25 °C for 1 h was shown to be partly effective with a prion reduction of 4 log10 (McDonnell at al., 2013).

In the present study the effect of alkaline treatment on the degree of *G. stearothermophilus* spores inactivation, in terms of decimal reduction times (D–value), at three temperatures (80 °C, 100 °C and 110 °C) and different sodium hydroxide concentrations was assessed. In addition, dipicolinic acid (DPA) released from endospores after the alkaline treatment was detected by the terbium dipicolinate fluorescence method.

6.2 MATERIALS AND METHODS

6.2.1 PREPARATION OF *Geobacillus stearothermophilus* SPORES

Strain *G. stearothermophilus* 22^T was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). *G. stearothermophilus* was grown in Nutrient Agar (Liofilchem) at 55 °C for 4 days. After incubation, the biomass was scraped from the agar surface and washed with sterile distilled water. The resulting suspension was incubated at 80 °C for 15 min. After cooling down, the suspension was centrifuged at 1000×g for 30 min at 5 °C. The supernatant was decanted, and the biomass was washed in chilled sterile distilled water and re-

centrifuged. This step was repeated twice. After re-suspension in water, the suspension was incubated at 37 °C for 60 min in the presence of lysozyme (100 µg/mL) for peptidoglycan breakdown. After washing with sterile distilled water for three times, and centrifugation at 1000×g for 20 min at 5 °C, the suspension was incubated with sodium dodecyl sulphate (SDS) at 2.5 % and incubated at 60 °C for 15 min, to increase the membrane fragmentation. After, the spores were washed with sterile distilled water for three times. Confirmation of the integrity of cells and spores after each step was carried out through transmission electron microscopy analysis (Figure 6.1A). The final suspension of spores was serially diluted with sterile distilled water to obtain approximately 10^7 colony-forming units per ml (CFU/mL) and stored at 4 °C.

6.2.2 ALKALINE TREATMENT

The experiments were carried out in a Parr batch reactor with a titanium vessel of 450 mL capacity under temperature control and with pressure reading. Five milliliters of spore suspension at 10^7 CFU/mL was mixed with 45 mL of NaOH solution at different concentrations (0.1 M, 0.25 M, 0.5 M, 0.75 M or 1 M). The batch reactor was heated at temperatures of 80 °C, 100 °C or 110 °C with heating rates of 5 °C/min. When the temperature stabilized, samples of 1.5 to 2 mL were taken, at regular time intervals up to 30 min. A control was made by heating the spore suspension at 100 °C without NaOH.

To evaluate the behaviour of spores in the presence of materials usually present in HCW, experiments with animal tissues (pork meat and pork bone) and a mix of discarded medical components (cotton, diapers, tubes for transfusion, surgical gloves, examination gloves, adhesives, surgical masks, bag collectors for urine, serum bottles and syringes) were performed. Except for cotton, the discarded medical components were cut in fragments of approximately 1 cm^2 and all the assays were carried out using samples with 1 g of each

component. The experiments performed with those components were carried out at the same conditions that performed in their absence. Approximately 10 g of material (animal tissues or discarded medical components) was added to the spore suspension (10^7 CFU/mL) with 50 mL of 0.5 M NaOH solution. All experiments were carried out in triplicate.

6.2.3 INCUBATION AND SURVIVAL COUNTS

The number of surviving spores was determined by the viable plate count method. Samples of heated spore suspensions (1.5 – 2 mL) were cooled in ice-water and neutralized with an HCl solution to pH 7. Samples were serially diluted in saline solution (0.85 % NaCl, w:v) and 0.1 mL were spread on triplicate nutrient agar plates and incubated at 55 °C for 24 h, 48 h, 72 h, 96 h and 120 h. It was verified an increase in the cell counts over time, stabilizing the cell growth at 96 h. Thus, the D–values were calculated using data obtained after 96 h of incubation. A positive control consisting on the enumeration of the total cell counts (CFU/mL) of the spore suspension used in each assay was performed in parallel.

6.2.4 FLUORIMETRIC DETECTION OF DPA

The DPA released by a 10^6 CFU/mL spore suspension after autoclaving at 121 °C for 30 min and after the hydrolysis at 110 °C, with 1 M NaOH was determined through a fluorimetric method, as previously described (Navarro et al., 2008; Rosen et al., 1997). Briefly, a 1 000 µL aliquot of suspension was added into 1 cm quartz cuvette with 40 µL of 10 nM $TbCl_3$ and 800 µL water distilled. The photoluminescence was measured at 270 nm excitation and 546 nm emission wavelengths in a spectrofluorometer. A calibration curve was prepared with DPA (2,6 pyridinedicarboxylic by Sigma-Aldrich) concentrations ranging from 0 up to 10 nM. As control, a standard DPA solution at 10 nM was quantified after the aforementioned

autoclaving and alkaline treatments. Five independent replicates were carried out for each condition.

6.2.5 TRANSMISSION ELECTRON MICROSCOPY

Bacterial and spore suspensions were fixed for 4 h with 2.5 % glutaraldehyde and 4 % formaldehyde (obtained from hydrolysis of paraformaldehyde) diluted in 0.1 M cacodylate buffer (pH 7.2), post-fixed overnight with 2 % OsO_4 in cacodylate buffer, stained in bloc with 1 % uranyl acetate, dehydrated with ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate before being observed in a JEOL 100CXII transmission electron microscope.

6.3 RESULTS AND DISCUSSION

Preliminary assays with vegetative cells of *G. stearothermophilus* demonstrated that they were very sensitive to alkaline solutions, since 0.1 M NaOH at 100 °C killed 99.9999% of the initial 10^6 cells/mL after 30 min of contact (data not shown). On the other hand, under the same conditions, only 99.9 % of the *G. stearothermophilus* spores were inactivated. In opposition, the spores were not inactivated at 100 °C in the absence of NaOH (Figure 6.2). Spore morphology was not affected by treatment with lysozyme (Figure 6.1B) or SDS (Figure 6.1C).

The survival curves, obtained when the *G*. *stearothermophilus* spores were exposed to alkaline conditions, exhibited biphasic curves with a slope tailing, as shown in the Figure 6.2 and Figure 6.3. There are several models that describe the inactivation of microorganisms (Chick, 1908; Kamau et al., 1990; Cole et al., 1993). Cerf (1977) proposed a model for populations constituted by two-fraction with a constant inactivation rate for each fraction. In this model it

is assumed that inactivation of both fractions is independent and irreversible, each following first order kinetics:

$$\frac{N(t)}{N_0} = f e^{-k_1 t} + (1-f) e^{-k_2 t}$$

where $N(t)/N_o$ is the proportion of surviving spores, t is the exposition time (min), k_1 and k_2 ($k_1 > k_2 \geq 0$) are the death rate constants for first fraction and second fraction, respectively, f and (1-f) are the initial proportion in first fraction and second fraction, respectively, and e is the Naperian base. The first and second fractions describe the death of the less and the more resistant spores, respectively (Xiong et al., 1999).

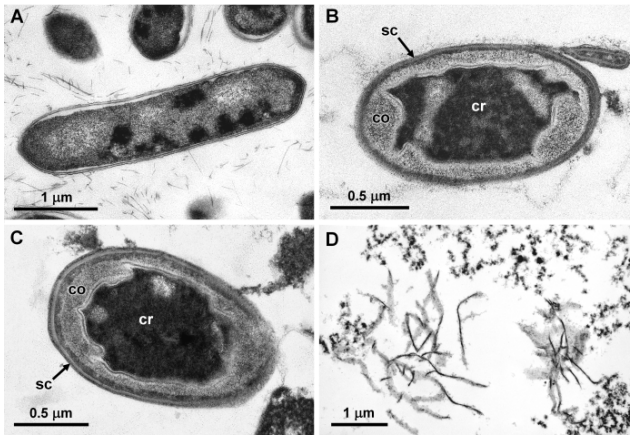

Figure 6.1 – TEM images of *Geobacillus stearothermophilus.* Vegetative cells suspension (A), spores suspension after addition of lysozyme (B), after addition of SDS (C) and spore debris after alkaline treatment (D). *sc*, spore coat; *co*, cortex; *cr,* core.

Given the biphasic behaviour of the survival curves of the *G. stearothermophilus* spores, the D–values, at specified conditions, were determined by estimating the parameter values of the Cerf`s model. Table 6.1 reports the D–values, 6 log10, f, k_1, and k_2.

The differences between the D–values at 80 °C and 100 °C were not significant for NaOH concentrations of 0.5 M and 0.75 M; the 6 log10 reduction times also were similar. Given that both incubation at 80 °C and 100 °C are currently used to isolate spores of low G+C content Gram positive bacteria (Gerhardt, 1994), at these two temperatures spore inactivation was solely due to the presence of the alkaline solution. Indeed, no spore inactivation was observed in the controls performed at 100 °C in the absence of NaOH, as shown in Figure 6.2. At 100 °C, 6 log10 reductions varied between 66.1 and 19.7 min in the presence of 0.1 and 0.75 M NaOH, respectively (Table 6.1).

As expected, for each temperature tested, the calculated D–values decreased with the increase of the NaOH concentration. At 100 °C, the lowest D–value (0.9 min), obtained with 0.75 M, was about ten times lower than that obtained with 0.1 M NaOH (8.8 min).

Table 6.1 – Decimal time reduction (D–value), 6 log10, estimates of the model parameters and standard derivation values for the Cerf model. Data presented are the mean of three independent experiences with standard deviation.

Conditions	**D–value** (min)	**6 log10** (min)	f	k_1 (min^{-1})	k_2 (min^{-1})
80 °C, 0.5 M	1.9 ± 0.2	28.4± 2.7	0.9919±0.0219	1.2168±0.0777	0.3176±0.1179
80 °C, 0.75 M	1.0 ± 0.0	20.2±0.0	0.9993±0.0000	2.3189±0.2117	0.3909±0.0280
100 °C, 0.1 M	8.8 ± 0.8	66.1± 1.5	0.4144±0.0866	3.0538±0.0000	0.2010±0.0029
100 °C, 0.25 M	2.5 ± 0.2	29.8± 3.6	0.7170±0.0577	5.9338±0.1111	0.4218±0.0531
100 °C, 0.5 M	1.6 ± 0.2	24.3±.3.2	0.9967±0.0026	1.5322±0.2923	0.3296±0.1792
100 °C, 0.75 M	0.9 ± 0.0	19.7± 1.1	0.9977±0.0008	2.4692±0.0604	0.3940±0.0343
100 °C, 1 M	0.8 ± 0.0	10.8± 0.1	0.9691±0.0369	3.1831±0.1160	0.9500±0.0168
110 °C, 0.1 M	2.3 ± 0.1	40.8± 6.0	0.9990±0.0029	1.0075±0.0752	0.0872±0.0806
110 °C, 0.25 M	1.8 ± 0.3	29.0± 2.2	0.9993±0.0005	1.1392±0.0287	0.2148±0.0197
110 °C, 0.5 M	0.5 ± 0.0	24.0± 0.1	0.9998±0.0004	5.2480±0.1350	0.2274±0.0523

On the other hand, for each NaOH concentration tested, the calculated D–values decreased with the increase of temperature. For NaOH concentrations of 0.1 M, 0.25 M and 0.5 M, the D–values obtained at 110 °C (2.3 – 0.5 min) were approximately 3 times lower than those at 100 °C (8.8 – 1.6 min). The combined effect of high temperature (110 °C) and NaOH (1 M) led to the complete inactivation of spores (6 log10 reduction) after 5 min.

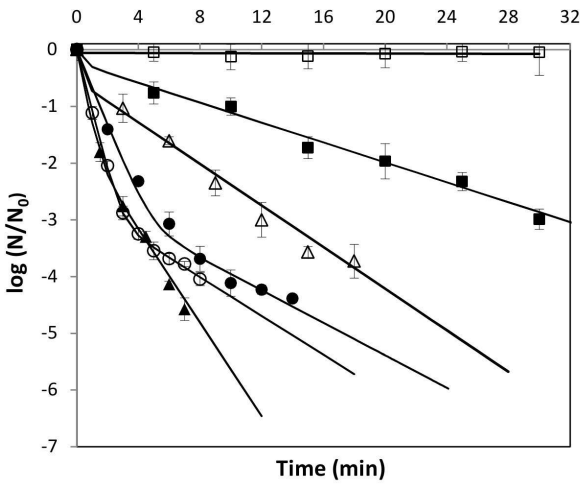

Figure 6.2 – Survival curves for the *Geobacillus stearothermophilus* exposed to alkaline treatment at 100 °C with various NaOH concentrations; 0 M NaOH (□); 0.1 M NaOH (■); 0.25 M NaOH (Δ); 0.5 M NaOH (●); 0.75 M NaOH (○); 1 M NaOH (▲) and predicted by the Cerf model (—). Vertical bars represent standard deviations of the means.

To confirm spores inactivation, DPA released after alkaline treatment (110 °C, 1 M NaOH, 30 min) was quantified, and compared to that released after autoclaving (121 °C, 30 min). The concentration of DPA after autoclaving (1.8 nM) was approximately 3.5 times higher than that quantified after the alkaline treatment (0.5 nM) (Table 6.2). Given this unexpected result, the effect of temperature and NaOH on the DPA determination was carried out, using a 10 nM standard solution of this compound. It was verified that the presence of NaOH interfere with the DPA quantification, since after the alkaline treatment the concentration of this organic acid was about 3 times lower than that after heating at 110 °C for 30 min (Table 6.2). Confirmation

of spores destruction after the alkaline treatment was given by TEM analysis. No spores were observed after the alkaline treatment (Figure 6.1D).

Table 6.2 – Concentration of 10 nM standard DPA, and DPA released from endospores after autoclaving and alkaline treatment.

Conditions	**DPA released (nM)**
DPA standard (110 °C, 30 min)	9.8±0.0
DPA standard (110 °C, 1 M NaOH, 30 min)	3.3±0.2
Autoclaving (121 °C, 30 min)	1.8±0.1
Alkaline treatment (110 °C, 1 M NaOH, 30 min)	0.5±0.1

Given the importance of medical waste sterilization, the behaviour of spores in the presence of components usually present in medical waste was assessed at 110 °C and 0.5 M NaOH. In first minutes (1 – 2 min), the rate of spores inactivation in the presence of discarded medical components and animal tissues was similar to that in the absence of materials, as shown in the Figure 6.3. However, after that period, there was a greater heat and alkaline resistance of those spores comparably to the ones solely in NaOH solution. Such differences can be explained by diffusion mechanism that occurred with sodium hydroxide and materials. In addition, NaOH consumption in hydrolysis of the materials occurred. Indeed, under the conditions tested, the animal tissues were almost destroyed. Nevertheless, the time required for the complete inactivation of spores in the presence of discarded medical components and animal tissues (6 log10 of 25 min and 26 min, respectively) was not much longer than that needed in their absence (6 log10 of 24 min).

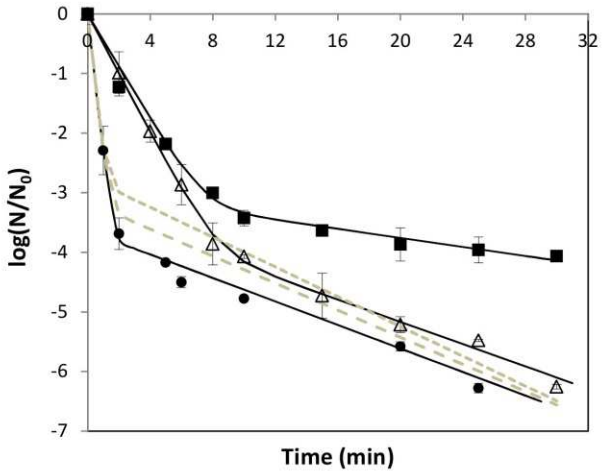

Figure 6.3 – Survival curves for the *Geobacillus stearothermophilus* exposed to alkaline treatment at 110 °C with various NaOH concentrations; 0.1 M NaOH (■); 0.25 M NaOH (Δ); 0.5 M NaOH (●); and predicted data by the Cerf model in the absence of components (—); discarded medical components (–––) and animal tissues (---). Vertical bars represent standard deviations of the means.

The survival curves of *G*. *stearothermophilus* spores after being subjected to alkaline treatment are typical of a mixture of two fractions or sub-populations with different resistance to stressful conditions, such as heat (Abraham et al., 1990). This difference in heat resistance has been attributed to different physiological states in the spore population (Iciek et al., 2006). A dormant spore transits to a vegetative cell by activation, germination and outgrowth. The activation is a reversible process; only when the germination phase starts the spore can no longer return to its dormant state (Leggett et al., 2012). Hence, spore suspensions may contain sub-populations of activated and dormant ones. Spores in the activated state are described as more sensitive to stressful conditions than in the dormant state. Thus, in the present study the spores were inactivated in two stages: the first corresponds, most probably, to the inactivation of the less resistant spores and the second of the more resistant ones.

The decline observed at 110 °C in spore heat resistance can be explained by an increase in the core water content. Although the mechanism of spore inactivation by wet heat is not entirely clear yet, it is partially due to the rupture of the spore inner membrane permeability barrier, which causes an increase in the core water content (Setlow, 2006). The spores inactivation by alkaline treatment seems to involve the removal of alkali-soluble coat proteins with consequent inactivation of the lytic enzymes essential for cortex hydrolysis and spore germination (Duncan et al., 1972). Treatment efficiency was proved by the release of DPA to the suspension after the alkaline treatment and TEM observations.

It has been previously reported that inactivation of *G. stearothermophilus* at low temperatures (< 100 °C) can be achieved using chemicals agents (Mazzola el al., 2003; Rogers et al., 2007; Unger-Bimczok et al., 2008), high–pressure carbon dioxide (Watanabe el al., 2003) and supercritical carbon dioxide with added hydrogen peroxide (Hemmer et al., 2006) but the time required to inactivate spores is high. As shown in Table 6.3, the D–values found in literature for inactivation assays carried out at temperature ≤100 ºC were higher than those obtained in this work, except when using high pressure treatments. The D–values herein obtained at 100 °C were even lower than those found in studies using thermal inactivation at temperatures above 100 °C. Except in the experiments carried out with 0.1 M NaOH, the highest D–value obtained was 2.3 min (Table 6.1), In contrast, at 120 °C, López et al. (1997) reported D–values ranging from 1.32 to 2.84 min and at 121 °C, Feeherry et al. (1987) and Guizelini et al. (2012) reported D–values from 1.3 to 5.4 min. Nevertheless, the time required to complete inactivation of *G. stearothermophilus* spores in the present study was probably, different from those obtained in abovementioned studies. Indeed, at 121 °C the thermal inactivation of spores generally follows a first order linear kinetics while under alkaline treatment, as described above, inactivation curves were non-linear.

Table 6.3 – Decimal time reduction (D–value) described in literature for *G.stearothermophilus* spores under different treatments.

Treatments	**D–value(min)**	**Reference**
Rapid decompression		Hayakawa et al. (1998)
200 MPa, 95 °C	6	
200 MPa , 85 °C	11	
High – Pressure Carbon Dioxide		Watanabe el al. (2003)
30 MPa, 95 °C	29.9	
30 MPa, 85 °C	130	
30 MPa, 75 °C	179	
High – Pressure		Patazca et al. (2006)
700 MPa, 100 °C	0.29	
500 MPa, 100 °C	1.3	
700 MPa, 92 °C	0.49	
500 MPa, 92 °C	1.81	
Chemical agents (25 °C)		Mazzola et al. (2003)
Sodium hypochlorite, 0.05%	9.4	
Sodium hypochlorite, 0.1%	3.5	
Glutaraldehyde, 2.0%	25	
Formaldehyde, 0.5%	10.9	
Chlorhexidine, 2.0%	9.1	

6.4 CONCLUSIONS

Given the low temperature values needed to achieve sterilization, the results herein obtained suggest that alkaline treatment may be implemented in the future as a disinfection or sterilization alternative method for contaminated HCW. The time required for total inactivation of spores in the presence of the tested animal tissues and discarded medical components, identical to those commonly found in HCW, was similar to that obtained in their absence.

The major disadvantage of this treatment is the production of an effluent with high alkalinity, which adds to the process one additional neutralization step before discharge. Nevertheless, the low cost of alkali and acid solutions when compared with the energy required to achieve sterilization when wet heat is the only antimicrobial agent, may be a stimulus for the implementation of this kind of waste treatment in future.

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CHAPTER 7 – CHARACTERIZATION OF EFFLUENTS RESULTANT FROM ALKALINE HYDROLYSIS TREATMENT

ABSTRACT

This work aims to characterize the effluents from alkaline hydrolysis tests with samples of components usually present in healthcare waste, such as cotton, diapers, transfusion tubes, surgical gloves, examination gloves, adhesives, surgical masks, urine bag collectors, serum bottles and syringes, animal tissues when subjected to a temperature of 110 °C and 1 M NaOH solution. Some of the parameters imposed by the Portuguese legislation with regard to discharge of effluents were determined; also, tests for aerobic or anaerobic biodegradation of those effluents were carried out. The effluents from alkaline hydrolysis tests showed values lower than discharge limit values for almost all the parameters except pH, total nitrogen, TOC, COD and BOD_5. Due to organic load the last three parameters showed very high values.

Despite these effluents presented high total nitrogen, TOC, COD and BOD_5 they showed a coefficient of total aerobic biological degradation of 50.5 % ± 2.5 % and 52.9 % ± 3.7 %, for the alkaline hydrolysis tests with discarded medical components and animal tissues, respectively. The toxicity of these effluents was 2 % and 22 % to discarded medical components and animal tissues, respectively. The anaerobic biodegradability obtained for effluents from alkaline hydrolysis tests with discarded medical components were 22.3 % ± 4.2 and 42.2 % ± 6.5 %, for animal tissues.

7.1 INTRODUCTION

The autoclaving and incineration are the main processes for treating HCW (Sukandar et al., 2006). These technologies have advantages and disadvantages vis-à-vis other alternatives of treatment, some of them not well clarified, yet. So, autoclaving and incineration may be advantageously substituted by alkaline hydrolysis to treat some types of HCW offering an interesting basis of decentralised treatment for reducing the risks of infection from handling and transporting HCW. The choice of HCW treatment is a task that involves not only treatment efficiency, itself, but also environmental factors. For this reason the aim of this work was to characterize the effluents from alkaline hydrolysis of some components usually present in HCW and, also, evaluate their biodegradability under aerobic or anaerobic conditions.

In aerobic biodegradation occurs the breakdown of organic compounds by microorganisms in the presence of oxygen. In anaerobic processes, the microorganisms convert organic matter into biogas in the absence of oxygen. The biogas consists, essentially, of methane (CH_4) and carbon dioxide (CO_2) (Angelidaki and Sanders, 2004). The ratio between CH_4 and CO_2 depends on the oxidation state of the carbon present in the organic material, i.e. the more reduced the organic carbon content is, the more CH_4 will be produced (Angelidaki and Sanders, 2004).

There are several factors that affect biodegradation of organic compounds such as: temperature; pH; oxygen concentration; moisture; salinity. Temperature is a major factor in the biodegradation process. In case of anaerobic digestion the optimum temperature may vary depending on feedstock composition and type of digester, but in most anaerobic digestion processes it should be maintained relatively constant to sustain the gas production rate.

7.2 MATERIALS AND METHODS

7.2.1 MATERIALS

The samples studied were from representative components commonly present in HCW, including cotton, diapers, tubes for transfusion, surgical gloves, examination gloves, adhesives, surgical masks, bag collectors for urine, serum bottles and syringes. These components are described in chapter 4, section 4.2.1. Pork meat was also used in the tests to simulate the pathological waste content of HCW. For simplicity, the first components are herein referred to as discarded medical components and the second ones (pathological wastes) as animal tissues.

7.2.2 ALKALINE HYDROLYSIS TESTS

The preparation of discarded medical components was carried out according to the procedure described in Chapter 4, section 4.2.1. The reactor with the sample tested, described in the previous Chapter 4, was heated at the heating rate of 10 °C/min up to 110 °C and held for 35 minutes. The selection of temperature, time and alkaline solution concentration was based in the results presented in Chapter 5 and Chapter 6. The temperature of 110 °C and the concentration of 1 M for the NaOH solution were chosen because they have been the conditions under which the complete inactivation of *Geobacillus stearothermophillus* was achieved in less time. The time selected was that needed for the complete destruction of the animal tissues in the conditions of temperature and concentration above referred.

A liquid/solid ratio of 5:1 (w/w) was used in the discarded medical components hydrolysis tests and 10:1 (w/w) for the animal tissues ones. In the tests, 20 g of the sample (2 g of each component in case of the mixture of discarded medical components) were mixed with either 100 mL or 200 mL of 1 M NaOH aqueous solution.

After cooling to room temperature, the obtained mixture was filtered through glass funnels using Whatman n. 1 filter paper, by gravity or under vacuum conditions. The solid fraction was washed with distilled water in order to remove all of the adherent sodium hydroxide. Following, it was dried and finally weighted. The liquid fraction – the effluent – was immediately characterized or frozen for analysis.

All the experiments were repeated three times and the results showed in the tables are the mean values of the 3 tests carried out under the same conditions.

7.2.3 EFFLUENTS CHARACTERIZATION METHODS

The effluent was characterized according the methods described in Chapter 4, section 4.2.2, namely TOC, COD and BOD_5; the remaining parameters were determined following the methods listed in Table 7.1. Metals were determined by Atomic Absorption Spectroscopy (AAS), with an Unicam model 969 equipment, the cyanides with Merck spectroquart, model NOVA 60, nitrates and sulfates with Dionex Ion Chromatograph, model ICS-2100, and total nitrogen was measured in Shimadzu TC analyser model TOC-VCSH.

7.1 – Chemical methods to characterize effluents

Parameter	Methods
pH	4500 B – Electrometric Method (a)
Ammonia	4500 D – Selective Electrode Method (a)
Cyanides	EPA 335.2; ISO 6703
Nitrate and sulfate	4110 B – Ion Chromatography (a)
Phosphorous	4500 P – Ascorbic Acid Method (a)
Total metal: Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb	US EPA 7000B:2007 and AAS
Oil and Grease	5520 D – Soxhlet Extraction Method (a)

(a) Standard Methods for Examination of Waters and Waste Water

7.2.4 AEROBIC BIODEGRADATION TESTS OF EFFLUENTS

The liquid fractions from alkaline hydrolysis of discarded medical components and animal tissues were subjected to aerobic biodegradation tests using the respirometry method in a BM-Advance Respirometer, according to the method described in Annex 2. The sludge needed in the method were collected from the aerobic waste water treatment plants of Freixo or Ponte de Moreira, about 3 km or 10 km of laboratory. In the tests, 700 mL of sludge with 5 mL of effluent or acetate with the same COD of the effluent were used. They were carried out at least in triplicate at the controlled temperature of 20 °C using a range of 2.6 % to 2.8 % of total solid sludge.

7.2.5 ANAEROBIC BIODEGRADATION TESTS OF EFFLUENTS

The liquid fractions from hydrolysis tests with the discarded medical components and animal tissues were subjected to anaerobic biodegradation tests based on the ISO11734:1995 standard, according to the method described in Annex 3. The inoculum was collected from the anaerobic waste water treatment plant of the Freixo. The tests were carried out in vessels of 125 mL, with 60 mL of medium, using approximately 2 g/L total solid of inoculum sludge and 5 mL of effluent. In the control tests, cellulose and gelatine was used. The vessels were incubated at 35 °C during 60 days. All the tests were done at least in triplicate. The generated methane was measured by gas chromatography (GC) using Shimadzu GC, model GC – 2014.

7.3 RESULTS AND DISCUSSION

7.3.1 EFFLUENTS CHARACTERIZATION

The parameters characterized were those recommended by Portuguese regulation, Decree Law No 236/98, which establishes their maximum admissible values for effluent discharge.

Table 7.2 reports the parameters, respective values and threshold values for the effluents from alkaline hydrolysis of discarded medical components. Almost all the determined parameters are below the threshold values, except pH, total nitrogen, TOC, COD and BOD_5. The last three were much above the threshold values, and total nitrogen, mostly as nitrate, is four times the allowable value for effluent discharge. To these values of parameters determined in the effluents corresponds a significant degradation of components during the hydrolysis tests, since there was a loss of approximately 10 % of their initial weight.

Table 7.2 – Characterization of effluent from alkaline hydrolysis of discarded medical components.

Parameter	Effluent	Emission limit value
pH	13.2 ± 0.3	6.0 – 9.0
Total nitrogen, mg N/L	62 ± 2	15
Ammonia, mg NH_4/L	4.0 ± 1.2	10
Nitrate, mg NO_3/L	49 ± 7	50
Sulfate, mg SO_4/L	5.9 ± 2.1	2000
Total phosphorus, mg P/L	4.1 ± 1.8	10
Cyanides, mg/L	<0.01	0.5
Al, mg/L	1.6 ± 0.0	10
Cu, mg/L	<0.04	1.0
Fe, mg/L	<0.06	2.0
Cd, mg/L	<0.03	0.2
Mn, mg/L	<0.03	2.0
Pb, mg/L	0.2 ± 0.0	1.0
Ni, mg/L	0.1 ± 0.0	2.0
Cr, mg/L	<0.1	2.0
As, mg/L	<0.001	1.0
Hg, mg/L	<0.001	0.05
TOC, mg C/L	6073 ± 182	(*)
COD, mg O_2/L	19117 ± 476	150
BOD_5, mg O_2/L	8616 ± 927	40

(*) parameter not considered in Decree Law No. 236/98

The effluents from alkaline hydrolysis tests with animal tissues showed low values for all the metals analysed as well as for sulfate and cyanides. The remaining parameters were above the threshold values, as shown in Table 7.3. These effluents present very high organic loads, with TOC, COD and BOD_5 values of approximately 14 g/L, 51 g/L and 25 g/L, respectively. Also, the obtained values for total nitrogen and ammonia are 200 and 20, respectively, times higher than the allowed by current Portuguese regulation. Nitrogen released corresponds to organic nitrogen, mainly in the form of proteins and amino acids, and its values as well as TOC, COD and BOD_5 are the result of the total destruction of the biological material.

Table 7.3 – Characterization of effluent from alkaline hydrolysis of animal tissues.

Parameter	Effluent	Emission limit value
pH	12	6.0 – 9.0
Total nitrogen, mg N/L	3030 ± 200	15
Ammonia, mg NH_4/L	274 ± 32	10
Nitrate, mg NO_3/L	63 ± 7	50
Sulfate, mg SO_4/L	4.0 ± 0.1	2000
Total phosphorous, mg P/L	58 ± 4	10
Cyanides, mg/L	<0.01	0.5
Al, mg/L	3.1 ± 0.2	10
Cu, mg/L	<0.04	1.0
Fe, mg/L	0.7 ± 0.1	2.0
Cd, mg/L	<0.03	0.2
Mn, mg/L	<0.03	2.0
Pb, mg/L	0.2 ± 0.0	1.0
Ni, mg/L	0.6 ± 0.0	2.0
Cr, mg/L	<0.1	2.0
As, mg/L	<0.001	1.0
Hg, mg/L	<0.001	0.05
Oil and Grease, mg/L	760 ± 310	15
TOC, mg C/L	14378 ± 1100	(*)
COD, mg O_2/L	51467 ± 4856	150
BOD_5, mg O_2/L	25269 ± 3350	40

(*) parameter not considered in Decree Law No. 236/98

7.3.2 AEROBIC BIODEGRADATION OF EFFLUENTS

Under the test conditions used, the effluent resultant from the alkaline hydrolysis tests with discarded medical components showed a coefficient of total aerobic biological degradation of 50.5 % ± 2.5 % and a toxicity value of 2 %. The value for coefficient of total aerobic biological degradation of animal tissues was 52.9 % ± 3.7 % and the toxicity was 22 %.

Based on these results, the aerobic biological process can be a good option of treatment for these effluents.

7.3.3 ANAEROBIC BIODEGRADATION OF EFFLUENTS

In the test conditions studied, the effluents from alkaline hydrolysis of discarded medical components showed a coefficient of total anaerobic biological degradation of 22.3 % ± 4.2 %. The same coefficient for animal tissues was 42.2 % ± 6.5 %, value close to the limit of biodegradability, thus it may be advantageous to treat these effluents by an anaerobic process. Therefore, they may be utilized as an energy source for biogas production, for example, adding them to an anaerobic co-digestion plant.

7.4 CONCLUSIONS

Although effluents from alkaline hydrolysis tests of both discarded medical components and animal tissues do not comply the admissible maximum values for all parameters considered by Portuguese regulation, namely total nitrogen, TOC, COD and BOD_5, they gave aerobic biodegradability values above 50 %. It means that these effluents can be treated by an aerobic biological process, as those common in the domestic wastewater treatment plants, after neutralization. The effluents from the alkaline hydrolysis tests with animal tissues can also be treated by an anaerobic biological process, therefore being a source of biogas and working as a

feedstock of anaerobic digesters. On contrary, the discarded medical components subjected to alkaline hydrolysis generated effluents with low anaerobic biodegradability.

The effluents from alkaline hydrolysis of animal tissues showed a very high organic load and were rich in nitrogen, which leads to opening some interest for applications such as liquid fertilizer.

7.5 REFERENCES

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CHAPTER 8 – SCALE UP EXPERIMENTS

ABSTRACT

This work aims to present some results related to a scaled up test of alkaline hydrolysis treatment using a real sample of infectious HCW, namely the characteristics of the effluents obtained when using a 1 M NaOH aqueous solution and temperature of 110 °C during 35 min. Some of the parameters imposed by the Portuguese legislation with regard to discharge of effluents were determined and the disinfection effectiveness was assessed. The effluents from alkaline hydrolysis treatment showed higher values than discharge limits for all the parameters analysed: pH, total nitrogen, ammonia, TOC, COD and BOD_5. The disinfection efficiency obtained with this alkaline treatment was confirmed.

8.1 INTRODUCTION

In the alkaline treatment tests described in the previous chapters, small amounts of synthetic samples of HCW and laboratory conditions were always used. However, since the real HCW is a heterogeneous mixture of components with coarser sizes, just by increasing the sample size it'll be possible to have a better representativeness of the HCW produced and respective results from alkaline treatments. Therefore, in order to gain additional confidence in the effectiveness of the alkaline treatment, it will be desirable to test it under the previous identified suitable conditions with higher amounts of HCW. Thus, first of all a new reactor having 70 L of useful volume was conceived and designed with the purpose of complying with the requirements of treatment from infectious HCW generated in many of the small and medium services in the private and public healthcare Portuguese system. Second, a real sample of infectious waste from Group III, according Portuguese classification, was obtained in a public hospital following the sound practice of infectious waste collection, storage and transport. Third, this sample was characterized and after being sub-sampled it was the basis of the scale up experiments here described. With this study, the effluents from two scale-up alkaline tests using the conditions of treatment defined in the laboratorial trials were characterized. Also, the disinfection effectiveness of the alkaline treatment was assessed.

8.2 MATERIALS AND METHODS

8.2.1 PILOT SCALE REACTOR

A reactor with an internal volume of 70 L was built in 316 AISI stainless steel. It was designed to work at least under a pressure of water vapour in equilibrium at 200 °C. It has controlling devices to guarantee safety operation, namely temperature controller and discharge vapour safety valve regulated to release vapour at 130 °C. In addition, in order to increase operation

efficiency, it was designed to have an oscillating movement around its horizontal axis for mixing the solid and liquid feed, as well as to facilitate discharge and cleaning steps, in these cases assuming appropriate static positions. The pilot scale reactor is shown in Figure 8.1.

Figure 8.1– Pilot reactor and real infectious HCW used in the alkaline hydrolysis tests.

8.2.2 MATERIALS

The infectious HCW was kindly provided by Hospital S. João, in Porto. It was a heterogeneous mixture of different components, which, in the perspective of characterisation, were separated in four types, respectively, paper, plastics, absorbents and liquids. The plastic fraction contained: syringes, gloves, tubes, serum bottles and bag collectors for urine. The absorbents fraction contained: adhesives, gauzes, compresses and diapers. Table 8.1 shows the composition of infectious HCW sample obtained for the experimental work.

Table 8.1 – Composition of infectious HCW sample used in the tests.

Fraction	% (w/w)
Paper	3.2
Plastic	30.4
Absorbents	9.4
Liquids	57.0

8.2.3 ALKALINE HYDROLYSIS TESTS

The selection of temperature, time and alkaline solution concentration was based in the results presented in Chapter 5 and Chapter 6. A liquid/solid ratio of 30:4 (w/w) was used; 4 kg of HCW sample was mixed with 30 L of 1 M NaOH aqueous solution. The sample was heated up to 110 °C and held 35 minutes at this temperature.

After cooling, the liquid fraction (the effluent) was immediately characterized, or frozen for further analysis, following the methods described in Chapter 4, section 4.2.2 and Chapter 7, section 7.2.2. Two independent assays were performed.

8.2.4 ASSESSMENT OF DISINFECTION EFFICIENCY

The total number of heterotrophic mesophilic bacteria in HCW (CFU/kg) was determined by the viable plate count method. To assess the microbial load, 100 g of solid fraction infectious HCW sample was mixed with 750 mL of sterile saline solution (0.85 % NaCl, w:v) during 30 min. The resultant suspension was further serially diluted with saline solution up to 10^{-7}, and 0.1 mL of each dilution were spread on triplicate PCA plates and incubated at 30 °C for 48 h. To assess the disinfection efficiency, samples of effluent (10 mL) were cooled down and neutralized with a highly concentrated HCl solution to pH 7 to avoid sample dilution; 0.1 mL of the neutralized effluent were spread on triplicate PCA plates and incubated at 30 °C for 48 h.

8.3 RESULTS AND DISCUSSION

Due to aggressiveness of caustic soda, alkaline hydrolysis enhances the degradation of some the components typically present in HCW of group III. The effluent resultant from the alkaline

hydrolysis treatment of real infectious HCW showed a brownish color and ammonia odor (Figure 8.2).

Figure 8.2 – Effluent from the scaled-up alkaline hydrolysis treatment of real infectious HCW.

The parameters determined on the effluent were those referred in Chapter 7 as having higher values than the threshold values for effluent discharge established by Portuguese regulation, Decree Law No. 236/98. Table 8.2 reports those parameters, the respective average values obtained from two independent tests of alkaline hydrolysis with infectious HCW samples and the respective threshold values for the effluents discharge.

Table 8.2 – Characterization of the effluent from the scaled up alkaline hydrolysis treatment of real infectious HCW.

Parameter	Effluent	Emission limit value
pH	12.9	6.0 – 9.0
Total nitrogen, mg/L	308	15
Ammonia, mg/L	138	10
TOC, mg C/L	1422	(*)
COD, mg O_2/L	4862	150
BOD_5, mg O_2/L	1797	40

(*) parameter not considered in Decree Law No. 236/98

As expected, all the determined parameters (pH, total nitrogen, ammonia, TOC, COD and BOD_5) were higher than the threshold values. These effluents presented high organic loads, with TOC, COD and BOD_5 values of approximately 1.4 g/L, 4.9 g/L and 1.8 g/L, respectively.

These high values are, most probably, the result of the partial destruction of the some material, such as absorbents, paper and the hydrolysis of the liquid fraction. In visual evaluation it was possible to see that the bag collectors for urine burst, the serum bottles were shrunken and paper and absorbers were partially destroyed, which led to a volume decrease, estimated of about 30 %. The infectious HCW after alkaline hydrolysis treatment is shown in Figure 8.3.

The total nitrogen and ammonia were, respectively, 20 and 10 times higher than the levels allowed by the current Portuguese regulation. The nitrogen present in effluent was essentially composes by ammonia (about 45 %). This high value of ammonia is, probably, due to the hydrolysis of the liquid fraction of HCW, in particular from bursting of the bag collectors for urine.

Figure 8.3 – Real infectious HCW after alkaline hydrolysis treatment.

Concerning to the microbial load, the real infectious HCW contained $8 – 9 \times 10^6$ CFU/kg of total heterotrophic mesophilic bacteria. This value may be under-evaluated because it was only the solid fraction and not the whole of the real infectious HCW sample that was analyzed as a precaution to avoid possible contamination of the operator. The results obtained to assess disinfection efficiency, confirmed that the alkaline hydrolysis treatment was effective in bacteria inactivation. Indeed, none of the three suspensions of neutralized effluent tested formed any CFU/kg on the plates corresponding to microbial load in effluent of

$<7.5 \times 10^4$ CFU/kg. In future work would be useful to study the application of other methods to assess disinfection in order to reduce the detection limit of the method used.

8.4 CONCLUSIONS

The results obtained with a real sample of infectious HCW at pilot scale corroborate those obtained in the tests carried out in the laboratorial study.

The alkaline effluents from infectious HCW treatment showed high organic loads, high nitrogen values and a strong alkaline pH. The alkaline hydrolysis allowed a reduction of the total heterotrophs of at least 2 log. Therefore, alkaline hydrolysis may be used as an alternative process to treat infectious HCW reducing its volume; thus, it can be further disposed of as municipal waste.

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CHAPTER 9 – CONCLUSIONS AND FUTURE WORK

9.1 CONCLUSIONS

Degradation of the components usually present in healthcare waste, when subjected to autoclaving or alkaline hydrolysis at the same conditions, occur at a higher or lower degree according to the thermal resistance of the materials in their composition. Despite autoclaving degrade materials much less than alkaline hydrolysis, the effluents generated have an appreciable organic load and showed values of biodegradability close to the limit. Alkaline hydrolysis degrades significantly almost of the components, particularly adhesive and diaper; on contrary, components constituted by LDPE, HDPE or PP show good resistance to the treatment. The alkaline hydrolysis is not efficient in reducing the mass of waste because the materials tested have lost only up to 10 % of their weight, except in case of diapers and adhesives for which the reduction is 30 % to 50 %, respectively. The effluents resultant from alkaline treatment show higher organic loads than autoclaving and are biodegradable after

neutralization. Under conditions commonly used in autoclaving, the application of alkaline hydrolysis treatment to a HCW essentially constituted by polymers does not present any relevant advantage.

However, the application of alkaline hydrolysis treatment in milder conditions may be a valuable option of treatment to HCW because it proved to be able to sterilize and destroy animal tissues at temperatures lower than those used in common processes of heat sterilization.

The results obtained showed that the inactivation of *Geobacillus stearothermophilus* was achieved under milder conditions than those described in the literature, due to the combination of alkaline conditions and heat.

Although this treatment has the disadvantage of generating alkaline effluents with very high organic load, they can be treated by an aerobic biological process, as those commonly found in the domestic wastewater treatment plants, after neutralization. The effluents from the alkaline hydrolysis of animal tissues with very high organic loads can also be treated by an anaerobic biological process, being, therefore, a source of biogas and working as a feedstock of anaerobic digesters.

The results obtained with a real sample of infectious HCW of group III at pilot scale corroborate those obtained in laboratorial tests. The resultant effluents showed high organic loads, high nitrogen values, high pH and disinfection effectiveness was verified.

9.2 FUTURE WORK

The perspective is to address the treatment in a more comprehensive way, i.e. from the components behavior, gaseous emissions and resultant effluents, including answers to all the

environmental effects in a life cycle point of view. Thus, the main subjects that are considered important to be developed in the future are:

- study the transformations that may occur in alkaline hydrolysis of other components which have not been studied at this work and may be present in HCW;
- look into the gaseous emissions resulting from the alkaline hydrolysis of HCW in the tested conditions, particularly to the presence of organic halogen compounds;
- better characterize the hydrolyzate from alkaline hydrolysis in order to define alternatives of its application, for example the possibility of treating animal tissues, which by having a high protein content, would be further used in agriculture, as organic fertilizer;
- assess autoclaving followed by sanitary landfilling as well as alkaline hydrolysis with landfilling from a life cycle perspective of HCW management.

ANNEX 1 – CHEMICAL TEST METHODS DESCRIPTION

A.1.1 TOTAL ORGANIC CARBON (TOC)

Total organic carbon was determined according EN 13137:2001 and EN 1484:1997 which proposed two methods. In the indirect method, TOC is obtained by the difference between the results of the measurements of total carbon (TC) and total inorganic carbon (TIC). TC is converted to carbon dioxide by combustion (at approximately 700 °C) in an oxygen-containing gas flow. The amount of carbon dioxide released is measured by infrared spectrometry. TIC is determined separately from another subsample by means of acidification (with ortophosphoric acid at 200 °C).

In the direct method, the carbonates present in the sample are previously removed by acidification. The carbon dioxide released is measured by infrared spectrometry and it indicates the amount of TOC directly.

In the present study, the indirect method was used and TC and TOC in solutions were determined with a Shimadzu TC analyser model TOC-VCSH (Figure A.1.1), according to EN 1484 (1997). TC in the materials was determined with the same equipment using its solids module, according to EN 13137 (2001).

Figure A.1.1 – TC analyser used in TOC and TC determination.

A.1.2 CHEMICAL OXYGEN DEMAND (COD)

The chemical oxygen demand (COD) method determines the amount of oxygen required to oxidize the organic matter in a sample, under specific conditions of oxidizing agent, temperature and time. COD was determined following the 5220 D: Closed Reflux - Colorimetric Method by the Standard Methods for Examination of Water and Wastewater (1998).

In this method, the sample was refluxed in a strongly sulphuric acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). The sample (of 2.5 mL) and reagents (Table A.1.1) were added in the digestion vessel (culture tubes of 16 × 100 mm). After digestion (2 hours of reflux time at 150 °C), the consumed oxygen was measured against standards at 600 nm using a Shimadzu spectrophotometer, model UVmini-1240 (Figure A.1.2).

Five standards of potassium hydrogen phthalate with COD from 100 to 900 mg O_2/L were prepared.

Table A.1.1 – Reagents used in COD determination.

Reagents/volume	
Digestion solution/1.5 mL	10.216 g of $K_2Cr_2O_7$ 167 mL of conc. H_2SO_4 33.3 g of $HgSO_4$ in 1000 mL of distilled water
Sulphuric acid reagent/3.5 mL	5.5 g of $AgSO_4$/kg H_2SO_4
Potassium hydrogen phthalate standard (KHP)	425 mg of KHP in 500 mL distilled water

Figure A.1.2 – Digester and spectrophotometer used in COD determination.

A.1.3 BIOCHEMICAL OXYGEN DEMAND (BOD)

The biochemical oxygen demand (BOD) method measures the quantity of oxygen required for the biochemical degradation of organic matter plus the oxygen used to oxidize inorganic matter, such as sulphides and ferrous iron. It also may measure the oxygen used to oxidize reduced forms of nitrogen, unless their oxidation is prevented by an inhibitor.

The BOD after five days was determined following the 5210 B: 5-Day BOD Method as described by the Standard Methods for Examination of Water and Wastewater (1998).

The method consists of filling with diluted sample, to overflowing, an airtight bottle and incubating the bottle at 20 °C for five days. The dilution water contains the inoculum and the following reagents (Table A.1.3).

Table A.1.3. – Reagents used in dilution water of BOD determination.

Reagents	
Phosphate buffer solution	8.5 g of KH_2PO_4
	21.75 g of K_2HPO_4
	33.4 g of $Na_2HPO_4.7H_2O$
	1.7 g of NH_4Cl
	in 1000 mL of distilled water
Magnesium sulphate solution	22.5 g of $MgSO_4.7H_2O$
	in 1000 mL of distilled water
Calcium chloride solution	27.5 g of $CaCl_2$
	in 1000 mL of distilled water
Ferric chloride solution	0.25 g of $FeCl_3.6H_2O$
	in 1000 mL of distilled water

The dissolved oxygen (DO) was measured initially and after incubation, using an Orion DO meter, model 850. The BOD was computed from the difference between the initial and final DO. The BOD_5 in the sample (in mg/L) is given by the equation:

$$BOD_5 = \frac{(D_1 - D_2) - (B_1 - B_2) \times f}{P}$$

Where: D_1 – DO of diluted sample before incubation (mg/L); D_2 – DO of diluted sample after incubation at 20 °C for five days (mg/L); B_1 – DO of seed control before incubation (mg/L); B_2 – DO of seed control after incubation (mg/L); f – ratio of seed in sample to seed in control; P – decimal volumetric fraction of sample used.

Figure A.1.3 – DO meter used in BOD_5 determination.

A.1.4 TOTAL NITROGEN AND AMMONIA

Total nitrogen was measured using the principles of oxidative combustion chemiluminescence. The sample was injected into the high temperature furnace where it was catalytically combusted at 720 °C in a carrier/oxygen atmosphere. The nitrogen oxide formed was measured in a Shimadzu TC analyser model TOC-VCSH (Figure A1). The calibration curve was obtained using five standards from 20 to 100 mg/L.

In this work the ammonia was determined according to 4500 F: Ammonia-Selective Electrode Method, as described by the Standard Methods for Examination of Water and Wastewater (1998).

The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride. During the test, dissolved ammonia (NH_3(aq) and NH_4^+) is converted to NH_3(aq) by raising pH to above 11 with NaOH. NH_3(aq) diffuses through the membrane and changes the internal solution pH that is sensed by a pH electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion-selective electrode that serves as the reference electrode. Potentiometric measurements were made with a pH meter having an expanded millivolt scale. The method is applicable to the measurement of 0.03 to 1400 mg NH_3-N/L.

For calibration curve the standards used were 1000, 100, 10, 1, 0.1 mg NH_3-N/L. The standard solutions were prepared with NH_4Cl in 1000 mL of distilled water. The standard solutions or the samples were stirred (magnetic bar), the electrode introduced and the NaOH added to raise the pH above 11. After 5 minutes readings of millivolt for standards and samples were performed. A tenfold change of NH_3-N concentration produces a potential change of about 59 mV.

The standard curve was done using semi logarithmic graph paper, plotting ammonia concentration in mg NH_3-N/L on the log axis versus potential in millivolts on the linear axis.

ANNEX 2 – AEROBIC RESPIROMETRIC TEST

A.2.1 INTRODUCTION

The aerobic respirometric test is used to estimate the biodegradability of the waste/water. It is performed under aerobic conditions and measured the oxygen consumption and/ or the amount of CO_2 released. This oxygen consumption, or respiration, is proportional to the COD biodegradable fraction where more substrate available to the bacteria, more oxygen is consumed. Deviations in oxygen uptake can occur in the presence of a toxicant or any inhibitory condition that specially affects the biomass.

These tests can be performed in a few days, hours or even minutes. The measurements can be carried out in "closed bottles", where no aerobic takes place, or "dynamic", where oxygen is allowed to flow during the test.

The activated sludge is the "reagent" and, for that reason, its preparation and condition deserves special care so, after collecting the sludge must leaving aerated along 24 hours from the day before; but in case of extended aeration process type with long hydraulic retention time and efficient performance, normally it is only necessary a couple of hours of aeration.

The final sludge volume that the analyzer normally need is 1 liter. For that, it is convenient to get at least 2 liters at the time of collecting the sample.

A.2.2 PRINCIPLE

The aerobic test was based on the oxygen uptake by the microorganisms contained in the activated sludge from biological reactor in one wastewater treatment plant.

The tests were carried out in a closed circuit batch reactor glass, by means of continuous dissolved oxygen measurements from the activated sludge, mixed liquor and mixing formed with sludge and sample to be analyzed. The measured dissolved oxygen is resultant effect of the microorganisms respiration in the activated sludge, from the biological oxidation of substrate (organic matter or ammonium) and from its own survival consumption (endogenous respiration).

Figure A.2.1 – Respirometer used in the aerobic tests.

A.2.3 DETERMINATION OF AEROBIC BIODEGRADATION

The determination of aerobic biodegradation is given by the ratio of the curves slopes of oxygen consumption during the sample and acetate tests.

The Figure A.2.2 shows the results obtained with one of the three tests performed with effluents resultant from alkaline hydrolysis of discarded medical components.

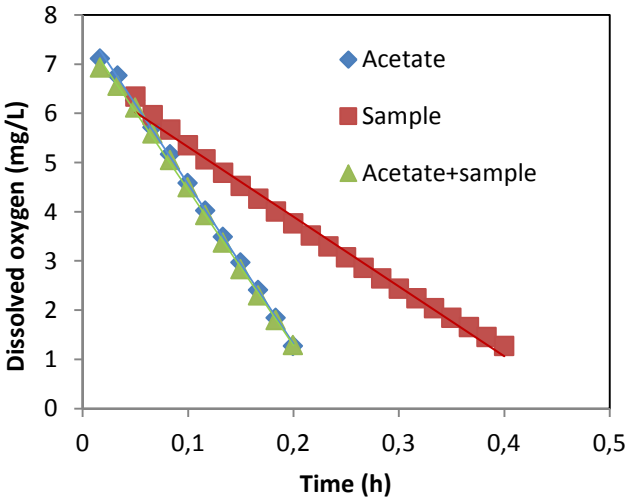

Figure A.2.2 – Dissolved oxygen versus time for sample and acetate.

ANNEX 3 – ANAEROBIC BIODEGRADABILITY TEST

A.3.1 INTRODUCTION

The anaerobic biodegradation is a naturally occurring process of decomposition and decay, by which organic matter is broken down in the absence of oxygen.

The anaerobic digestion involves a complex processes of degradation, which are described by the following steps: Hydrolysis; Acidogenesis; Acetogenesis ; Methanogenesis.

In hydrolysis large polymers such as lipids, polysaccharides and proteins, are broken down into soluble monomers, such as, fatty acids, sugars and amino acids (Angelidaki et al., 2009). Hydrolysis of different compounds occurs in different time frames. For instance, the hydrolysis of carbohydrates lasts within a few hours, of lipids and proteins is done in a few days and cellulose and lignin are broken down only at a slow rate and incompletely.

In acidogenesis, the monomers from hydrolysis are fermented by acidogenic bacteria into carbon dioxide, hydrogen, ammonia, and organic acids.

Acetogenesis is the breakdown of organic acids (volatile fatty acids) to acetate, carbon dioxide and hydrogen.

Methanogenesis converts acetate, formaldehyde, hydrogen and carbon dioxide into methane and water. The methane is formed in strictly anaerobic conditions.

Many factors affect the rate of methane generation such as, temperature, pH, moisture content, nutrient content and concentration of toxic substances.

Temperature is the most important variable in controlling the rate of microbial metabolism in anaerobic conditions. Higher temperatures increase microbial activity, with activity roughly doubling for every 10 °C increase within the optimal range. Anaerobic process can be designed for temperatures appropriate for mesophilic bacteria (25 – 40 °C) or thermophilic bacteria (45 – 60 °C). The effect of temperature on anaerobic process only influences the degradation rates and not the ultimate biodegradability of a compound (Angelidaki and Sanders, 2004).

The optimal pH values for the acidogenesis and methanogenesis steps are different. Low pH can inhibit acidogenesis while high pH leads to an increase in free ammonia, which is toxic for the methanogenic population (Lesteur et al., 2009). An optimal pH range for all the metabolic processes is between 6.4 and 7.2.

The relationship between the amount of carbon and nitrogen present in organic materials is represented by the C:N ratio. Optimum C:N ratios in anaerobic digesters are between 20 and 30. A high C:N ration is an indication of a rapid consumption of nitrogen by the methanogens and results in low gas production. On the other hand, a lower C: N ratio causes ammonia ion accumulation with consequent pH values exceeding 8.5, which is toxic to methanogens. Optimum C:N ratio of the feedstock materials can be achieved by mixing waste of low and high C:N ratio, such as organic solid waste mixed with sewage or animal manure.

High levels of ammonia, soluble sulfides, soluble salts of metals, and alkali and alkaline-earth metal salts in solution (e.g. those of sodium, potassium, calcium, or magnesium) can be toxic to methanogens.

The anaerobic biodegradability tests are based on the measurement of one or more products involved in the biological reaction or measurement of substrate depletion. The methods to characterize the anaerobic process based on product formation consist in the determination of the biogas (end product) or intermediates products. Since the biogas is the fundamental end product of anaerobic process, most methods are based on monitoring the biogas production.

The biogas production can be measured using volumetric methods, manometric methods or measurement of methane and carbon dioxide by gas chromatography. Volumetric and manometric methods can use manual or automated devices. In gas chromatography methods the determination of biogas may be by: thermal conductivity detector where both methane and carbon dioxide are measured; flame ionization detector, where only methane is measured (Angelidaki and Sanders, 2004).

Methods based on substrate depletion, determine some parameters such as TOC, COD, dissolved organic carbon, volatile solids, etc., or direct analysis of the compound that is being used as substrate (Angelidaki and Sanders, 2004).

Methane potential can be expressed specifically per amount of waste (L CH_4/kg-waste), per volume of waste (L CH_4/L-waste), per mass volatile solids added (L CH_4/kg-VS) or COD added (L CH_4/kg-COD). The volume is usually expressed in standard pressure (1 atm) and temperature (0 °C) conditions (STP conditions) (Angelidaki et al., 2009).

A.3.2 PRINCIPLE

To verify the anaerobic biodegradability of effluents resultants from alkaline hydrolysis of organic and inorganic wastes a laboratory method based in ISO 11734: Water quality – Evaluation of the “ultimate” anaerobic biodegradability of organic compound in digested sludge – Method by measurement of the biogas production was used.

This method consists in an aqueous biodegradation test at a mesophilic temperature (35 °C ± 2 °C) in sealed vessels with a synthetic growth medium with a mixed microbial population derived normally from a waste water treatment facility. The sludge is diluted with a mineral salts medium. The increase in headspace pressure in the test vessels resulting from the production of CO_2 and CH_4 is measured. The dissolved CO_2 (inorganic carbon) is measured at the end of the test. The method uses as inoculum washed of domestic sewage or laboratory grown. To ensure the anaerobic conditions pure N_2 is used as purge gas. Incubation takes normally up to 60 days or till a plateau phase is reached, in the dark, with stirring or shaking.

A.3.3 REAGENTS

Medium

The medium contains the constituents below referred and, in order to remove oxygen, it must be purged with nitrogen for about 20 min immediately before use.

Inoculum sludge

The sludge was collected from a digester at a sewage treatment plant. The sludge was incubated at 35 °C ± 2 °C for up to 7 days. After digestion the sludge was wash to reduce the inorganic carbon. The final concentration of total solids in the test vessels was in the range of 1 g/L to 3 g/L.

Reference substances

Cellulose or gelatin were the substances used in control vessels and prepared solutions were expected to have a degradation degree higher than 60 %.

Medium	
Anhydrous potassium dihydrogenphosphate (KH_2PO_4)	0.27 g
Disodium hydrogenphosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	1.12 g
Ammonium chloride (NH_4Cl)	0.53 g
Calcium chloride dihydrate ($CaCl_2.2H_2O$)	0.075 g
Magnesium chloride hexahydrate ($MgCl_2.6\ H_2O$)	0.10 g
Iron(II) chloride tetrahydrate ($FeCl_2.4H_2O$)	0.02 g
Resazurin (oxygen indicator)	0.001 g
Sodium sulfide nonahydrate ($Na_2S.9H_2O$)	0.1 g
Stock solution of trace elements	10 mL
De-oxygenated water	to 1 L
Stock solution of trace elements	
Manganese chloride tetrahydrate ($MnCl_2.4H_2O$)	0.05 g
Boric acid (H_3BO_3)	0.005 g
Zinc chloride ($ZnCl_2$)	0.005 g
Copper(II) chloride ($CuCl_2$)	0.003 g
Disodium molybdate dihydrate ($Na_2MoO_4.2H_2O$)	0.001 g
Cobalt chloride hexahydrate ($CoCl_2.6H_2O$)	0.1 g
Nickel chloride hexahydrate ($NiCl_2.6H_2O$)	0.01 g
Disodium selenite (Na_2SeO_3)	0.005 g
Water	to 1 L

A.3.4 TEST PROCEDURE

To ensure the anoxic conditions the tests were carried out in plastic bags fitted with gloves. The samples, blank and controls were prepared at least in triplicate, in 125 mL vessels (Figure A.3.1). The same volume of inoculum and medium was added to the vessels with samples, blanks and controls. Following, the pH was measured and adjusted. The prepared vessels were incubated at 35 °C ± 2 °C for up to 60 days, in the dark, ensuring that all vessels were maintained at the digestion temperature.

In this work, the biogas production (methane) was measured by gas chromatography with a flame ionization detector using the gas conditions described in Table A.3.1. The gas chromatograph used was a Shimadzu, model GC-2014.

The measurement was compared with five standard with known methane content. For the calibration curve methane was added to the vessels with same headspace volume used in sample, blank and control vessels. The methane production from inoculum was subtracted from the methane production of the samples.

Figure A.3.1 – The samples, blank and controls vessels used in anaerobic tests.

A.3.5 DETERMINATION OF ANAEROBIC BIODEGRADATION

The coefficient of total degradation was calculated using the equation:

$$D_t = CH_4(STP)/(350 \times COD_{sample}) \times 100) \quad \text{(Eq. A.2.1)}$$

Where:

D_t is the total biodegradation, expressed as a percentage;

CH_4 is the volume of methane produced, in mL, expressed in the Standard Temperature and Pressure (STP), respectively 0 °C and 1 atm.

COD_{Sample} is the chemical oxygen demand of sample (g).

It is admitted that 1 g of COD produces about 350 mL of CH_4. Although, bacterial growth uses part of the organic matter that is consumed during methane production, in biodegradability determination this value was not accounted.

The Figure A.3.2 shows the results obtained with one of the three tests performed with effluents resultant from alkaline hydrolysis of discarded medical components and animal tissues.

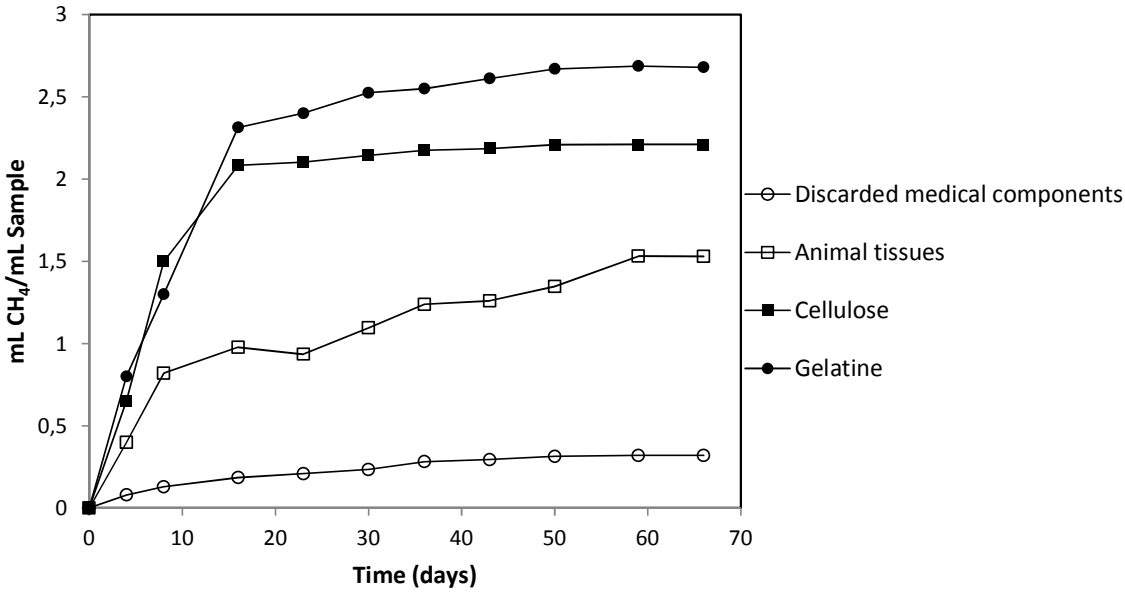

Figure A.3.2 – Cumulative methane production (at 0 °C and 1 atm) of samples and controls.

Table A.3.1 – Gas chromatography conditions.

Gas chromatography conditions	
Column temperature	175 °C
Oven temperature	200 °C
L column flow	30/30 (mL/min)
R column flow	40/40 (mL/min)

Figure A.3.3 – Gas chromatograph.

A.3.6 REFERENCES

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