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Characteristics of effluents from healthcare waste treatment with alkaline hydrolysis

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Abstract

Although alkaline hydrolysis emerges as an alternative process to treat healthcare waste (HCW), information about its emissions is scarce, namely as regards effluents production. This work aims to characterize the effluents from alkaline hydrolysis tests with samples of components usually present in HCW, under a temperature of 110ºC and with 1 M NaOH aqueous solutions. Some of the regulatory parameters for discharging effluents were determined; also, tests for assessing aerobic and anaerobic biodegradation of those effluents were carried out. The effluents showed values lower than threshold values for almost all the parameters except pH, total nitrogen, TOC, COD and BOD_5. Although with high organic load, the effluents from discarded medical components (DMC) and animal tissues (AT) showed a percentage of aerobic biodegradation of 50.5 and 52.9%, respectively. The anaerobic biodegradability obtained for the effluents from DMC were 22.3 and 42.2% for those with AT.

Keywords

aerobic biodegradation; anaerobic biodegradation; hospital waste; medical waste; sodium hydroxide.

Introduction

Autoclaving and incineration are the main processes used for treating the hazardous fraction of healthcare waste (HCW) (Lee & Huffman 1996; Sukandar *et al*. 2006). These technologies have advantages and disadvantages (Yang *et al*. 2009; Windfeld & Brooks 2015) when compared with other alternatives of treatment. The major drawback of incineration process is the possible issue of a wide range of hazardous pollutants including dioxins, furans, heavy metals, fine dust particles and other pollutants resultant of incomplete combustion (Alvim-Ferraz & Afonso 2005; Sukandar *et al*. 2006; Singh & Prakash 2007; Insa *et al*. 2010, Verma 2014). Autoclaving is not suitable for all waste types and it requires a mechanical process to reduce the waste volume, which increases the operating costs. Additionally, due to the low mass reduction achieved in this treatment, there is a considerable mass of waste that is disposed of in landfills (Windfeld & Brooks 2015).

In this context, alkaline hydrolysis may be an alternative treatment process for HCW in

some countries (Health Care Without Harm 2004). The successful decontamination of the prion (Murphy *et al*. 2009; McDonnell *et al*. 2013) and inactivation of potentially infectious agents including virus, bacteria, fungi and protozoa (Kaye *et al*. 1998; Neyens *et al*. 2003; Murphy *et al*. 2007; Dixon *et al*. 2012; Pinho *et al*. 2015a) have been proved. Thereby, this treatment has been shown to have significant advantages when compared to other treatments, because it sterilizes and destroys at once and reduces the total waste volume. Additionally, alkaline hydrolysis has a range of application larger than autoclaving, since it can also accept organic tissues.

The alkaline hydrolysis may also be a waste treatment option for animal by-products, mainly because it is able to significantly reduce the volume of animal wastes and produces sterile by-products (Thacker 2004; Franke-Whittle & Insam 2013). In 2002, with the appearance of BSE, the European Parliament and the Council published Regulations (EC 1774/2002; EC 1069/2009; EU 142/2011) imposing restrictions on the use and disposal of animal by-products, which has led to an increased proportion of solid material being disposed of to landfill or incinerated. Furthermore, the disposal in landfill is contradictory with the European Union polities that set specific targets for disposing the biodegradable waste.

The alkaline hydrolysis is not as efficient in mass reduction of waste constituted essentially by polymers as it is with animal tissues (AT), which are completely destroyed (Kaye *et al*. 1998; Pinho *et al*. 2015b). However, after alkaline treatment the wastes are disinfected (Kaye *et al*. 1998; Franke-Whittle & Insam 2013) and they may be disposed of in municipal landfills in the same manner as non-infectious waste. The drawback of this treatment is the production of a liquid effluent with high pH value and high organic load resultant of the total destruction of the biological material (Thacker 2004; Franke-Whittle & Insam 2013). Its discharge directly into a sanitary sewer can potentially pose problems.

The biological treatments carried out in wastewater treatment plants (WWTP) are the most commonly used to treat waters with high organic loads before discharge in the environment, because these treatments are cost effective when compared with those based on physic-chemical processes. Hence, most of the urban WWTP, which were primarily designed to treat sewage, may receive industrial effluents, as long as they are compatible with the characteristics of the incoming sewage.

In this context, the choice of HCW treatment is a task that involves not only the 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 generally applied in WWTP flowsheets. And thus, assess if their composition was compatible with their discharge in the sanitary sewer, and further treated through a biological process in the receiving urban WWTP.

Materials and methods

Alkaline treatment

The experiments were carried out in a batch reactor model 4842 Parr, with a titanium vessel of 450 mL capacity under temperature control and with pressure reading.

Two samples representative of HCW were prepared. The first included cotton, diapers,

tubes for transfusion, surgical gloves, examination gloves, adhesives, surgical masks, bag collectors for urine, serum bottles and syringes (Table 1), and are herein referred to as discarded medical components (DMC). The second sample was composed by pork meat to simulate the pathological waste content of HCW, which is referred to as AT. Except for cotton, the DMC were cut in fragments of approximately 1 cm^2. Liquid/solid ratios of 5 : 1 (w/w) and 10 : 1 (w/w) were used in the DMC and AT hydrolysis tests, respectively. In the tests, 20 g of the sample (2 g of each component in case of the mixture of DMC) were mixed with either 100 or 200 mL of 1 M NaOH aqueous solution. The samples were heated up to 110ºC with a heating rate of 10ºC/min and held for 35 min. The selection of these conditions, that is temperature, NaOH concentration and time of contact, was based on previous studies (Pinho *et al*. 2015a,b) showing to be possible the complete inactivation of *Geobacillus stearothermophillus* and the complete destruction of the AT.

After cooling to room temperature, the obtained hydrolizates were filtered through glass funnels using Whatman n. 1 filter paper, by gravity or under vacuum conditions. The liquid fraction – the effluent – was immediately characterized or frozen at 220ºC. All the experiments were repeated three times and the results are the mean values of the three tests carried out under the same conditions.

Effluents characterization

The chemical characterization of the effluents was carried out according to the standards listed in Table 2. In addition, the main chemical compounds of the liquid effluent resultant from the alkaline hydrolysis of AT was analysed by gas chromatography with mass detector (GCMS) using an Agilent HP 6890/MSD 5793N from HP, 30 m 3 0.25 mm I.D., 0.5 ㎜ P/N 19091S-133 column; and using helium as carrier gas at constant flux of 1.2 mL/min. Analyses were carried out in the following conditions: split-less injector at 280ºC; oven 1 min at 50ºC, followed by heating at 10ºC/min till 300ºC; transference line at 290ºC; and MSD scan mode. The separated com- pounds were identified using the NIST 1998 library match.

Aerobic and anaerobic biodegradation tests

The effluents from alkaline hydrolysis of DMC and AT were subjected to aerobic or anaerobic biodegradation tests. The respirometric assays were carried out using a BM-Advance Respirometer. The aerobic sludge needed in this method was collected from the activated sludge tank of a WWTP at about 3 km of the laboratory. Following, the sludge was aerated for 24 h and then used for the analysis. In the tests, 700 mL of sludge with 5 mL of alkaline hydrolysis effluent (after neutralization with 5 M H_2SO_4) were used. An acetate solution with the same COD of the effluent was used as control. Tests were carried out at least in triplicate at a controlled temperature of 20ºC using 2.6–2.8% of total solid sludge. The determination of aerobic biodegradation was calculated by integration of the obtained respirometric curve of oxygen uptake rate versus time.

The same equipment was employed in toxicity tests based on oxygen uptake rate inhibition, according to the test OECD 209. The toxicity was calculated by integration curves of oxygen uptake rate versus time between sample and acetate.

The anaerobic biodegradation tests were based on the ISO11734:1995 standard. The inoculum was collected from an anaerobic digestion process at about 10 km of the laboratory. To ensure the anaerobic conditions, the tests were carried out in plastic bags

fitted with gloves. All the samples, blank and controls were prepared at least in triplicate, in closed 125 mL vessels, with 60 mL of the recommended medium, using approximately 2 g/L total solid of inoculum sludge and 5 mL of alkaline hydrolysis effluent. Following, the pH was measured and adjusted to 7. In the control tests, standard cellulose and gelatine with the same TOC of the effluents were used (Angelidaki *et al*. 2009). The vessels were incubated at 35ºC±2ºC for up to 60 days, in the dark, ensuring that all them were maintained at the digestion temperature. The generated methane was collected from the headspace and measured by gas chromatography (GC, Shimadzu, model GC-2014) with a flame ionization detector using the gas under the following conditions: column temperature at 175ºC; oven temperature at 200ºC; L column flow at 30/30 (mL/min) and R column flow at 40/40 (mL/min).

The measurement was compared with five standards with known methane content. For the calibration curve, methane was added to the vessels with the same headspace volume used in sample, blank and control vessels. The methane production from inoculum was subtracted from the methane production of the samples. The coefficient of total biodegradation was calculated using the equation:

$$D_t = CH_4(STP)/(350 \times COD_{sample}) \times 100$$

Where: Dt 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 0ºC and 1 atm; COD_{sample} is the chemical oxygen demand of sample in grams. 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.

Results and discussion

Effluents characterization

After alkaline hydrolysis treatment of the DCM sample, a solid and a liquid fraction were obtained. Most of the components of the solid fraction were not destroyed, remaining without apparent modification of weight and volume. Only the serum bottles were shrunken and the absorbers were partially destroyed which led to a total volume and mass decrease of about 10%. In opposition, in the AT sample no solid fraction was obtained because the pork meat was completely destroyed. These results were similar to those obtained in others studies (Kaye *et al*. 1998; Thacker 2004; Pinho *et al*. 2015b). According to previous studies, the solid fraction has no infectious character (Kaye *et al*. 1998; Franke-Whittle & Insam 2013; Pinho *et al*. 2015a) and, thus, can be further disposed of in municipal solid waste landfills.

Nevertheless, the alkaline hydrolysis treatment generates an effluent with high pH, and depending on the type of wastes treated, it is expected to contain different types and concentrations of organic compounds.

The parameters chosen to characterize the alkaline hydrolysis effluents were those

recommended by the Portuguese regulation, Decree Law No 236/98, which establishes their maximum admissible values for effluent discharge. The COD and BOD_5 threshold values for discharge from urban WWTP recommended by Council Directive 91/271/CEE, are slightly lower, that is 125 g/L for COD and 25 mg/L for BOD_5. Table 3 reports these parameters, the threshold values and the respective values for the effluents from alkaline hydrolysis of DMC. As expected, due to their composition and refractory nature of polymers, almost all the determined parameters were below the threshold values, except pH, total nitrogen, TOC, COD and BOD_5. The last three parameters were much above the threshold values, and total nitrogen, mostly as nitrate, was four times the allowable value for effluents dis- charge. The values of these parameters result from some degradation of components during the hydrolysis tests, since there was a loss of mass of approximately 10% of their initial weight.

The effluents from alkaline hydrolysis with AT showed low values for all the metals analysed as well as for sulfate and cyanides, as expected for this type of sample which is essentially of proteic nature. The remaining parameters were above the threshold values, as shown in Table 4. These effluents presented very high organic loads, with TOC, COD and BOD_5 values of approximately 14, 51 and 25 g/L, respectively, similar those obtained by Thacker (2004), who treated animal carcasses by alkaline hydrolysis. Also, the obtained values for total nitrogen and ammonia were respectively 200 and 20 times higher than those allowed by regulation for effluents discharge. These very high values were the result of the total destruction of the biological material. Since, the AT sample is composed mainly by water followed by proteins and lipids, which are easily hydrolyzed.

Alkaline hydrolysis degrades the proteins, the major solid component of all animal cells and tissues, into salts of free amino acids. In proteins, amino acids are linked to each other by peptide bonds 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. 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 & Lahm 1998).

According to NIST 1998 data base used to identify the compounds detected in the GCMS analyses, the nitrogen released from the AT samples was mainly organic nitrogen, in the form of alkyl groups and chains, amides and esters, namely ethyl ester L-isoleucine, indole, 4-methyl pentanamide, 3-methyl butanamide and propanamide (molecular mass of rv160, 117, 115, 101, 73, respectively). The degradation of the amino acids produced also organic compounds such as 4-methyl phenol, acetaldehyde and nitrogen gas (molecular mass of rv108, 44 and 28, respectively).

Aerobic and anaerobic biodegradation of effluents

Under the test conditions, the effluents resultant from the alkaline hydrolysis with DMC and AT showed a percentage of aerobic biodegradation above 50%. However, the toxicity of the DCM and AT effluents was quite different as shown in Table 5. The toxicity value for effluents from AT was 10 times higher than for those from DMC, probably due to the high values of total nitrogen and ammonium present in the AT effluents. Despite the fact that these effluents showed higher toxicity than DMC effluents, their aerobic biodegradability

was slightly higher, and, for sure, it could be improved with a previous denitrification stage. On contrary, the refractory nature of compounds resulting from polymeric degradation of DMC materials makes aerobic degradation difficult even with a more equilibrate C/N ratio (Pinho *et al*. 2015c). In a certain way, the tests of aerobic biodegradability corroborate with BOD_5/COD ratio values obtained of 0.45, and 0.49 for DMC effluents and AT effluents, respectively. This ratio is also used to assess the biodegradability of an effluent; and ratios above 0.4 have been reported to indicate high biodegradability (Amat *et al*. 2009).

In anaerobic biodegradation tests, the percentage of biodegradation for both effluents was below 50%. This is not an unexpected result, since anaerobic processes require more quantity of 'digestible' carbon and energy sources than aerobic processes because they are less efficient in terms of energy; also, large quantities of ammonia have been recognized as being toxic to the methanogenic population of microorganisms responsible for an essential step of the overall process (Lesteur *et al*. 2009). Thus the insufficient organic (short chain, digestible) nutrients in the DCM effluent and the large quantity of ammonium present in the AT effluents cloud explain low anaerobic biodegradability of both effluents, respectively, 22.3 and 42.2%.

Anyway, the results obtained suggest that, after a neutralization step, liquid effluents resulting from alkaline hydrolysis may be treated by a biological process – aerobic, anaerobic or both – along with other effluents generated in hospitals and others, namely those from urban sewage. The issue seems to be having or not a dedicated WWTP; and, in terms of achieving higher efficiency and lower costs of treatment, the option tends to be of using existing urban WWTP and discharging controlled pre-neutralized alkaline treatment volumes of effluents that do not cause any disturbances in their treatment performance. Note that because the hospital effluents have, beside high organic loads, high loads of potential dangerous microorganisms, a chlorination step is sometimes required before entering into an urban WWTP (Mohee 2005; Tsakona *et al*. 2007; Verlicchi *et al*. 2010). According to these results alkaline hydrolysis seems to have no reasons for be considered as an option to treat some types of HCW. It may be used as an alternative process, particularly for small producers, to whom it offers an interesting basis of decentralised treatment reducing the risks of infection from handling and transporting HCW. Indeed, the small producers of HCW, due to issues such 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.

Another attractive factor for this treatment is the cost, which is smaller than for others technologies used to treat HCW. Thacker (2004) reported the estimated costs of \$320 ton^{21}, 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^{21} using alkaline hydrolysis to dispose of AT 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 alter- native to other treatments.

Conclusions

This study showed that the liquid effluents generated by the alkaline hydrolysis of medical waste cannot be discharged without previous treatment, even after neutralization. Both DCM and AT alkaline hydrolysis effluents showed high values of total nitrogen, TOC, COD and BOD_5. Nevertheless, aerobic biodegradability values of both type of effluents was above 50%, suggesting that after neutralization they may be treated by aerobic biological processes, as those commonly used in the domestic WWTP. The percentage of anaerobic biodegradation of the AT effluent was close to the 50% limit of biodegradability, suggesting that it may also be treated by an anaerobic biological process.

Alkaline hydrolysis when coupled with a biological treatment of the effluents may be a valuable option of treatment of HCW, because it sterilizes and destroy AT at temperatures lower than those used in common processes of heat sterilization.

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Conflict of interest

No conflict of interest declared.

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Table 1 Components of the HCW used in the experimental work and their materials

Material	Composition
Serum bottle	Low-density polyethylene (LDPE)
Syringe	Polypropylene (PP) and high-density polyethylene (HDPE)
Transfusion tube	Polyvinyl chloride (PVC)
Bag collector for urine	PVC
Examination glove	PVC
Surgical glove	>90% natural rubber
Cotton	94% cellulose
Surgical mask	PP (outer and middle layer); polyester (PE) and pressed PP (inner layer); polyurethane (tapes)
Diaper	Cellulose fibre and PP (70–80%); PE film; thermoplastics adhesives; elastic threads; flocgel (5–10% of sodium polyacrylate)
Adhesive	Non-woven polyester; synthetic adhesive from rubber

Table 2 Chemical methods to characterize effluents

Parameter	Methods
pH	4500 B – Electrometric Method (APHA 1998)
Ammonia	4500 D – Selective Electrode Method (APHA 1998)
Cyanides	EPA 335.2; ISO 6703
Nitrate and sulfate	4110 B – Ion Chromatography (APHA 1998)
Phosphorous	4500 P – Ascorbic Acid Method (APHA 1998)
Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb	US EPA 7000B:2007 and AAS
Oil and Grease	5520 D – Soxhlet Extraction Method (APHA 1998)
Total Organic Carbon	EN 1484 (APHA 1998)
Chemical Oxygen Demand	5220 D: Closed reflux – colorimetric method (APHA 1998)
Biochemical Oxygen Demand	5210 B: 5-Day BOD method (APHA 1998)

Table 3 Characterization of the effluents generated by the 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

*According to Decree Law No. 236/98.

Table 4 Characterization of the effluents generated by the 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

*According to Decree Law No. 236/98.

Table 5 Aerobic biodegradation, anaerobic biodegradation and toxicity for effluents

Effluent	% Aerobic biodegradation	% Anaerobic biodegradation	% Toxicity
Discarded medical components	50.5 ± 2.5	22.3 ± 4.2	2
Animal tissues	52.9 ± 3.7	42.2 ± 6.5	22