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Bioremediation and Biodegradation

Evaluation of the biodegradability of hazardous industrial solid waste: Study of key parameters

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Abstract

The biological stability of solid waste is one of the main problems related to the environmental impact of landfills and their long-term emission potential. Current European legislation (European Landfill Directive, EC/99/31) introduced the need to reduce biodegradable organic compounds deposited in landfills; however, it set neither official parameters nor methods to define the stability of such a waste. In Spain, biodegradability is generally evaluated using the biological oxygen demand/chemical oxygen demand (BOD_5/COD) ratio, measuring it on the leachate, thus not considering the non-soluble fraction and therefore creating false negatives. To solve this problem, the biodegradability of hazardous industrial waste has been determined by measuring its respirometric activity (AT_4). Our results show that the measure of the AT_4 is independent of the enrichment with a microbial inoculum, and a sample size no higher than 20 g could be a reasonable value for a sensitive biodegradability determination. The highest respirometric index is obtained in waste with pH values between 6.5 and 10.5. Furthermore, respirometric biodegradability values are independent of traditional parameters of organic matter characterization such as BOD_5/COD ratio, volatile content, and total and dissolved organic carbon. Consequently, the AT_4 parameter provides new information on the composition and stability of organic matter in hazardous industrial waste. Its incorporation into pre-disposal waste characterization protocols allows to identify waste that exceeds recommended biodegradability thresholds. This approach ensures that only waste meeting specified biodegradability standards is deposited, avoiding landfill emissions and related environmental impacts, and thereby improving the overall effectiveness and sustainability of waste management practices.

Abbreviations: AT_4, respiration activity; BOD_5, biological oxygen demand at 5 days; COD, chemical oxygen demand; DM, dry matter; DOC, dissolved organic carbon; GHG, greenhouse gases; LOI, loss on ignition; TOC, total organic carbon.

1 | INTRODUCTION

Nowadays, global warming and climate change remain among the most significant environmental issues facing our planet. Waste management represents the fourth largest anthropogenic greenhouse gas (GHG) emission source, accounting for approximately 5% of the GHG emissions (IPCC, 2007), considered as emerging air pollutants, that even in small amounts have a great contribution to global warming (Allen, 2014; Olof, 2010). In European countries, Landfill Directive 1999/31/EC (European Commission, 1999) and Waste Framework Directive 2008/98/EC (European Commission, 2008) introduced strict requirements for preventing the negative impacts of waste on environment urging to reduce the amount of biodegradable materials disposed in landfill.

According to the UNE-CEN/TR 14980 (2009) standard, a biodegradable material is the one capable of undergoing biological decomposition under the natural conditions present in the biosphere. A waste is considered unstable if it contains a high proportion of biodegradable organic matter allowing a high microbial activity. Hence, in order to deposit a waste in a landfill it is necessary that the material has reached an elevated level of stability to reduce its environmental impact (emission of GHG, generation of odor, self-combustion, generation of leachate, production of volatile organic compounds, presence of vectors—insects, rodents, birds—and growth of pathogens). Therefore, in order to develop sustainable waste management strategies, a proper standard for waste biodegradability assessment is needed.

In the field of wastewater, the use of the 5-day biological oxygen demand parameter (BOD_5) is fully accepted for the determination of biodegradable organic matter. In the field of solid waste, a parameter usually used to evaluate the biodegradability is the relationship between BOD_5 and the chemical oxygen demand (COD) of the waste leachate (Cossu & Raga, 2008; Cossu et al., 2012). However, this parameter is only representative of the biodegradability of the water-soluble part and, therefore, does not take into account other components of the waste, such as oils, fats, and hydrocarbons, which for the most part are retained in the solid waste (Wodzinski & Bertolini, 1972; Wodzinski & Coyle, 1974).

The Council Decision of December 19, 2002, establishing the criteria and procedures for the admission of waste to landfills, according to Article 16 and Annex II of Directive 1999/31/EC (European Commission, 1999), does not establish any specific criteria for the control of waste biodegradability; however, it is indicated that the Member States may establish additional criteria. Some European countries have adopted respirometric techniques to characterize their solid waste. In this sense, Ireland (Environmental Protection Agency, 2009), Germany (German Federal Government, 2001), Austria (OE-NORM S2027-4, 2012), Poland (Jęrczak & Szpadt, 2008), and Slovenia (Republic of Slovenia, 2013) have established the AT_4 parameter to indicate the degree of stabilization of the screen fraction of mixed municipal waste after mechanical-biological treatment (MBT). Normally, the biological stabilization of organic matter in MBT plants is conducted via anaerobic digestion or composting. Likewise, the European Union (2001), in its draft proposal on biological waste treatment, also recommends the respirometric measure AT_4 (Table 1). This table shows that there is no consensus on the biological stability limit values and their consequent acceptance to landfill, being Germany the country that establishes the lowest value for the stability of the residues. In addition, the AT_4 indicated by Germany, Austria, and Ireland is lower (5 and 7 mg O_2 g^{-1} DM, where DM is dry matter, respectively) than the draft proposal of the European Union (10 mg O2 g^{-1} DM). In this sense, the European Union states that: "Stabilization" means the reduction of the decomposition properties of the waste until reaching a respirometric activity after 4 days (AT_4) of <10 mg O_2 g^{-1} DM (European Commission, 2001).

Core Ideas

- Respirometric tests assessed the biodegradability of hazardous industrial waste.
- Biodegradability is independent of the enrichment with a microbial inoculum.
- Sample mass size of 20 g offers an adequate biodegradability determination.
- Higher biodegradability is obtained in samples with 6.5–10.5 pH values.
- Biodegradability is independent of organic matter characterization parameters.

TABLE 1 Admission values for the established waste characterization method respiration activity (AT_4) parameter in different European legislations.

Country	Admission limit	Standard
Ireland	7 mg O_2 g^{-1} DM	Environmental Protection Agency (2009)
Germany	5 mg O_2 g^{-1} DM	German Federal Government (2001)
Austria	7 mg O_2 g^{-1} DM	OE-NORM S2027-4 (2012)
Slovenia	10 mg O_2 g^{-1} DM	Republic of Slovenia (2013)
Polonia	10 mg O_2 g^{-1} DM	Jęrczak and Szpadt (2008)
European Union	10 mg O_2 g^{-1} DM	European Commission (2001)

Abbreviation: DM, dry matter.

TABLE 2 Percentage of each specific type of waste analyzed in this study and its European Waste Code (EWC) for the classification of waste ($N = 146$).

Specific waste	%	EWC
Partly stabilized hazardous waste	64.4	19 03 04
Sludges from physico/chemical treatment containing hazardous substances	14.4	19 02 05
Sludges from on-site effluent treatment containing hazardous substances	8.2	07 07 11
Exhausted resins used for chloride retention	1.4	07 07 10
Sludge from manufacturing of wire and metal wire	2.1	12 01 99
Machining sludges containing hazardous substances	1.4	12 01 14
Spent activated carbon	1.4	15 02 02
Solid waste from gas treatment	0.7	10 02 07
Sludges from biological treatment of industrial wastewater	1.4	19 08 11
Extraction powder from the manufacture of brake pads	2.1	16 0.3 03
Sludges from urban wastewater treatment	0.7	19 08 05
Waste from mechanical treatment of waste containing hazardous substances	1.4	19 12 11
Ashes and dust generated in blasting and pyrolysis processes of pitted metal parts	0.7	08 01 17

Hazardous industrial waste originates in an industrial or manufacturing process and displays some of the properties contained in Annex III of the Waste Frame Directive, such as explosive, flammable, corrosive, toxic, mutagenic, etc. Among the residues generated in the EU in 2020, 95.5 million metric tons (4.4% of the total) were classified as hazardous waste (Eurostat Database, 2021). From the location where the hazardous waste is generated, it is transported to treatment facilities in order to recycle, treat, or dispose of the waste. The treatment stabilizes the waste organic fraction in order to comply with the Directive 1999/31/EC (European Commission, 1999) and Directive 2018/850/EC (European Commission, 2018), which regulate the disposal of waste. In the present study, the evaluation of the biological stability of solid hazardous industrial waste has been conducted using an adjusted aerobic respirometric technique (AT_4). The respirometric AT_4 index is one commonly used method for waste stability measurement, and it has seen much interest in the specialized literature (Cossu & Raga, 2008; Bernat et al., 2022). The choice of this parameter is due to its simple methodology, relatively short duration and its frequent citation in European legislation. The aim was, first, to adjust the method to hazardous industrial waste since these samples have, apparently, low respiratory activity and potential toxicity (Kumar et al., 2023; Ruiz et al., 2011). In this sense, hazardous waste containing heavy metals can be harmful to the microbial process. Therefore, the influence of the amount of sample and the addition of supplemental industrial microbial inoculum have been evaluated. One hypothesis was that the sample size affects the biodegradability determination since larger weight samples would improve the measurement of low biodegradability values ensuring the reliability of the biodegradability assessment (Tchobanoglous et al., 1993). The other hypothesis was that the supply of supplemental biomass to the samples might accelerate the degradation of biodegradable organic matter because of the likely inhibition of native indigenous bacteria by the toxic waste. That is why we tested with and without supplemental bacterial consortium from industrial active sludge. Second, the goal was to find out if the AT_4 parameter provides new information that cannot be obtained from the traditional chemical indicators commonly used to assess organic matter, with particular emphasis on whether the values of the AT_4 present a correlation with the BOD_5/COD ratio. The AT_4 procedure is based on the manometric measurement of the oxygen consumption necessary to degrade the biodegradable organic material. The results obtained for AT_4 were compared with the BOD_5/COD ratio in the leaching test eluate and with the traditional key physicochemical parameters for the determination of the organic matter content. The relationship between the analyzed variables was examined and explained by the use of correlation analysis.

2 | MATERIALS AND METHODS

2.1 | Industrial waste samples

Samples of industrial hazardous solid waste were collected from the trucks arriving at the hazardous landfill managed by ATLAS Gestión Medioambiental, following UNE-EN, 14899 (2007) and UNE-CEN/TR, 15310 (2008) standards. A total of 146 samples were analyzed, which included partially stabilized and inert hazardous waste, fly ash from industrial and municipal waste, and sludge with hazardous compounds (Table 2). All samples had a stability corresponding to the allowed Spanish landfill disposal limit (BOD_5/COD ratio lower than 0.5). Upon arrival at the laboratory, waste samples were dumped on plastic trays, manually ground to size <10 mm, and homogenized by mixing. Afterward, they were deposited into plastic bottles and stored at 4˚C for further analysis.

2.2 | Initial characterization of the waste

The initial characterization of the waste included analyses of moisture content, loss on ignition (LOI), pH, total organic carbon (TOC), dissolved organic carbon (DOC), COD, and BOD_5. The moisture content, LOI and TOC were measured directly on the solid waste. The moisture content was measured by drying the samples at 105˚C to a constant weight following the Spanish standard DIN EN 14346:2007-03 (German Institute for Normalization, 2007). The LOI (volatile solids) was quantified by incineration at 550˚C (UNE-EN 15169; 2007) and the TOC was performed according to UNE-EN 13137; 2002). Following the analytical standards, the pH, DOC, COD, and BOD_5 were measured on the waste aqueous eluate that was obtained by water extraction of solid waste using distillate water (1:10 ratio of solid/liquid) for 24 h under shaking (UNE-EN 12457; 2002). The pH, DOC, COD, and BOD_5 were determined according to UNE-EN 12506 (2004), UNE 77004 (2002), and UNE-EN 1899-1 (1998), respectively. Analyses were performed in triplicate.

2.3 | Mineral medium and inoculum

The mineral medium used in the biodegradability tests (respirometry) was made up according to OECD Test Guideline 301B. It contained the following nutrients per liter of distilled water: 85 mg KH_2PO_4, 217.5 mg K_2HPO_4, 334 mg $Na_2HPO_4 \cdot 2H_2O$, 5 mg NH_4Cl, 27.5 mg $CaCl_2$, 22.5 mg $MgSO_4 \cdot 7H_2O$, and 0.25 mg $FeCl_3 \cdot 6H_2O$ (OECD, 1992). Note that 1 mL of this solution was diluted in 1 L of distilled water adding 20 drops of allylthiourea (5 g L^{-1}) as nitrification inhibitor. Then the solution was aerated for 1 h at 20˚C. The microbial inoculum was the active sludge supernatant from an industrial wastewater treatment plant. The inoculated mineral solution was prepared by adding 20 mL of inoculum to the aerated mineral solution and making up to a final volume of a liter.

2.4 | Acclimation

A volume of inoculated mineral solution was added to different amounts of sample until the water holding capacity of the material was reached. For the determination of the water retention capacity the Austrian standard OE-NORM S2027-4 was followed. Afterward, the samples were pre-incubated in 1–2 cm thick layers at 20˚C for 5–7 h to acclimatize the microbial community to the waste. To avoid an excessive drying the samples were visually controlled periodically and, if necessary, some additional water was added after the pre-aeration period.

2.5 | Respirometric activity test

Respiratory activity was determined with static manometric respirometers (OxiTop-C WTW, OxiTop System). Each respirometer consisted of a 960-mL watertight glass reaction vessel, a pressure sensor, a head for data recording, and a reservoir for 50 mL of 2 M NaOH solution. The acclimatized samples were placed in hermetically sealed flasks with fixing clamps and incubated for 7 days at a constant temperature of 20˚C in the absence of light. A hermetical flask containing only the inoculated mineral solution was also incubated as a blank. To assess the influence of the addition of inoculum, some samples were supplemented only with mineral solution (no inoculum) until the water holding capacity was reached. In these cases, only mineral solution was incubated as a blank. To validate the inoculated mineral solution, a standard test was performed using a calibration solution of glucose and glutamic acid (GGA) as the primary carbon source (150 mg L^{-1} glucose and 150 mg L^{-1} glutamic acid) according to ISO, 5815-1:2003.

As oxygen was consumed, the produced CO_2 was absorbed by the NaOH solution, causing a pressure drop directly related to oxygen consumption. The reaction jars were aerated regularly by unscrewing the OxiTop heads, allowing air to enter the headspace in order to never achieve complete oxygen depletion in the vessel during the measurement. A controller (OxiTop OC 110 WTW) was used to collect data from the pressure sensor loggers. A software (Achat OC, PC communication software version 2.03) was used to download the data from the controller to a spreadsheet. Oxygen consumption was determined from the pressure drop, using the principles of the ideal gas law, through the following relationship:

$$\mathrm{BA} = \frac{\mathrm{Mr}\left(\mathrm{O}_2\right)}{R \times T} \times \frac{V\,Fr}{m\,Bt} \times \Delta p$$

where BA corresponds to the respiration of the solid sample in mg O_2 kg^{-1} of dry sample, Mr is the molar mass of oxygen (32,000 mg mol^{-1}), V_{Fr} is the free volume of the container in L, R is the constant of the ideal gases (83.14 L mbar mol^{-1} K^{-1}), T is the measurement temperature in Kelvin (293.15 K), m_{Bt} is the mass of the dry solid sample in kg, and Δp is the pressure difference obtained between the blank and the sample.

TABLE 3 Variables included in the data set.

Abbreviation	Varia
$MASS_{SAMPLE}$	Mass of sample
$MASS_{LOSS105}$	Mass loss at 105˚C
AT_4	Respirometric activity
VOL_INOCUL	Volume of inoculum
COD	Chemical oxygen demand
BOD_5	% Day biological oxygen demand
BOD_5/COD	BOD_5/COD % ratio
LOI	Loss of ignition
DOC	Dissolved organic carbon

The reaction vessels were placed in a constant temperature of 20˚C because changes in the temperature would led to changes in pressure, giving incorrect measurements of oxygen consumption. The AT_4 parameter refers to the 4-day demand for oxygen by microorganisms; however, the test itself must be carried out longer—typically for 7 days. This is related to the potential occurrence of the *adaptation* or *lag-phase*, that is, the time in which microorganisms adapt to the new environment before the logarithmic growth phase. This acclimation period could represent enzyme induction, gene transfer or mutation, or, where the necessary enzymes are available, growth in population of the responsible microorganisms (Howard, 1984). An assumption has been made that the lag-phase ends when the mean oxygen demand after 3 h reaches 25% of the 3-h mean values, occurring at the time of a maximum increase in the oxygen demand, determined for the first 4 days. The oxygen mass used during the lag-phase is deducted from the oxygen mass taken up during the whole test (lag-phase + 4 days).

2.6 | Data analysis

Data from the analyses of 146 collected samples of industrial solid waste includes the variables described in Table 3. Additionally, $MASS_{DRY}$ is calculated from $MASS_{SAMPLE}$ and $MASS_{LOSS105}$ according to:

$$MASS_{DRY} = \frac{MASS_{SAMPLE}\left(100 - MASS_{LOSS_{105}}\right)}{100}$$

Descriptive exploratory data analyses were run on the variables. There was a limited number of missing data: observations with missing data were not removed in general, but they were excluded from the analysis where a missing datum was involved. No outliers were detected according to the standard boxplot criteria. Correlograms, using Spearman correlation coefficients, were used for visualizing relation among variables. Normality of data was assessed with Anderson–Darling tests, and non-parametric tests were used for inference (i.e., Spearman correlation test for relationship between variables and Mann–Whitney test to compare group locations). The relevance of adding an inoculum in the AT_4 determination was studied by comparing the results obtained with and without inoculum using boxplots and a Mann–Whitney test.

TABLE 4 Composition of industrial solid waste: Respiratory activity and physicochemical parameters (N = 146).

	Median	Average	Min	Max
AT_4 (mg O_2 g^{-1}) DM	1.6	6.5	0.1	67.2
LOI (%) DM	20.6	24.1	1.31	67.3
TOC (%) DM	11.9	14.2	1.3	91.2
COD (mg L^{-1})	1866	4105	34	46,000
BOD_5 (mg L^{-1})	277.5	575.2	1	3120
BOD_5/COD (%)	12.9	19.1	1.5	51.0
DOC (mg kg^{-1}) DM	2900	5721	94	45,500
pH	9.37	9.72	4.94	13.03

Abbreviations: AT_4, respiration activity; BOD_5, biological oxygen demand at 5 days; COD, chemical oxygen demand; DM, dry matter; DOC, dissolved organic carbon; LOI, loss on ignition; TOC, total organic carbon.

3 | RESULTS AND DISCUSSION

3.1 | Relationship between AT_4 and parameters related to organic matter

The 146 hazardous industrial solid waste samples were analyzed for the aerobic respiration activity (AT_4) and for the physicochemical parameters related to the characterization of the organic matter (Table 4). The values of AT_4, LOI, and TOC ranged from 0.1 to 67.2 mg O_2 g^{-1} DM, 1.3% to 67.3%, and 1.3% to 91.2%, respectively. The AT_4 results reported in this study were similar to those observed by Binner et al. (2012) for landfill site samples and for biostabilized municipal material after anaerobic digestion but remarkably lower than those obtained by Colón (2017) from municipal mechanical-biological biostabilized material after composting.

A potential relation among the biodegradation index (AT_4) and the physicochemical variables of hazardous industrial solid waste was sought. A correlation matrix among each pair of analyzed indicators is presented in Table 5, including microbial respirometric index (AT_4) and measures related to organic matter content. Pearson's correlation coefficients between the selected indicators were measured with a significance level set at $p < 0.1$. Table 5 shows that AT_4 is independent of the key chemical and physical parameters, in particular LOI, TOC, COD, and BOD_5. Inference test on the Spearman correlation for AT_4 and the rest of the variables

TABLE 5 Correlation matrix between respiration activity (AT_4) and different characterization parameters.

	LOI	TOC	COD	BOD_5	BOD_5/ COD	DOC
AT_4	0.16	0.06	−0.15	−0.09	0.01	−0.36**
LOI	1	0.50**	0.04	0.08	0.05	0.09
TOC		1	0.01	0.03	0.15	0.29**
COD			1	0.82**	−0.35**	0.61**
BOD_5				1	0.17	0.46*
BOD_5/COD					1	−0.11
DOC						1

Abbreviations: BOD_5, biological oxygen demand at 5 days; COD, chemical oxygen demand; DOC, dissolved organic carbon; LOI, loss on ignition; TOC, total organic carbon.
**Correlation is significant at the 0.1 level.

do not allow to reject the independence of the variables (at 95% confidence level), so independence is assumed. This means that the biological respirometric AT_4 parameter provides information, which cannot be obtained through the chemical indicators commonly used to assess organic matter. Other works have found similar results when correlating respiration indices with chemical parameters (Scaglia & Adani, 2008; Zach et al., 2000; Bayard et al., 2018). These authors concluded that physicochemical analytical variables were not appropriate to predict waste stability.

The lack of correlation can be explained because most of the physicochemical parameters do not distinguish between biodegradable organic matter, which is resistant to decomposition, since their analytical determination methods are based on material combustion at high temperatures or strong chemical oxidation, where the whole organic content is destroyed without distinction between labile or inert organic material (Barrena et al., 2009). In any case, it seems evident that these indicators are not representative of the actual polluting potential of solid waste in landfills related to the emissions of greenhouse gases (especially methane).

A strong positive correlation between biochemical oxygen demand (BOD_5) and COD is inferred from the data compiled in our study ($r = 0.82$). Both parameters are closely related, and it is generally accepted that BOD_5 and COD share an empirical relationship under certain conditions depending on the characteristics of the waste (Russell, 2006).

One of the goals of the study was to observe whether a low microbial respiration (AT_4) leads to a low BOD_5/COD ratio and vice versa. This relationship is important because in Spain, a non-official way to evaluate the biodegradability of solid waste uses the BOD_5/COD ratio, based on the European Regulation 1272/2008 (European Commission, 2008b) for classification of organic substances. According to this regulation, a substance with a $BOD_5/COD \geq 0.5$ is considered likely to biodegrade rapidly in the aquatic environment and thus this value has been taken as the waste stability limit for landfill disposal. According to our results, no correlation was observed between BOD_5/COD ratio and respirometric biodegradation value ($r = 0.01$). These findings are in agreement with various studies supported by APAT (2003) and Cossu and Raga (2008) on the solid phase of excavated waste in a closed landfill. In Cossu and Raga's (2008) study, the ratio BOD_5/COD showed no correlation with the respirometric index. The authors detailed that their results were due to the high degree of heterogeneity of the landfill samples and that no information on the extent of stabilization can be expected from the ratio BOD_5/COD in samples of varied composition coming from different origins. In general, no information on the extent of the stabilization process can be expected from the comparison of the BOD_5/COD in samples of different composition. The discrepancies between the biodegradability values obtained from AT_4 and the BOD_5/ COD ratio in the tested hazardous samples might result not only from the high degree of diversity and complexity of industrial waste but also from the differences among the analyzed material phase. While AT_4 is measured directly from the solid waste, the analysis of BOD_5 and COD is carried out from the soluble leaching eluate of the sample. To obtain this leachate, only a part of the organic compounds is mobilized from the solid to the liquid phase. Our results show that the leaching eluate would not be representative of the totality of organic compounds present in the sample solid waste. The eluate will only represent the easily mobilizable water-soluble organic fraction, and it might underestimate the biodegradable poorly soluble compounds, which for the most part stay retained in the solid waste (D'Imporzano & Adani, 2007). There exist a whole range of compounds that are easily biodegradable but poorly water soluble. For instance, anthraquinone is used as a reference standard in biodegradability tests according to ISO 10634 ISO (2018) because it is readily biodegradable but water insoluble (62% biodegradation at day 28, according to EC Test Guideline C.4.E.). Isodecyl neopentanoate, used as an emollient, also has very low water solubility and moderate biodegradability (Sweetlove et al., 2016). Other examples of water insoluble chemicals with high biodegradability are lubricant base oils, especially ester-based oils, with greater inherent biodegradation due to the presence of carboxylic acid groups that bacteria can readily utilize. The first step in their degradation is the enzyme-catalyzed cleavage of the ester bond. The enzymes that catalyze this biodegradation reaction include esterases and lipases that are synthesized by a wide range of microorganisms (Broekhuizen et al., 2003). Additional labile materials are waxes, whose main components (long chain n- and iso-alkanes) are readily degraded by numerous species of bacteria despite their extremely low water solubility under different environmental conditions (Hanstveit, 1992; M'rassi et al., 2015). Until now, solubility has not been recognized as a key variable in biodegradation testing, but it may partly

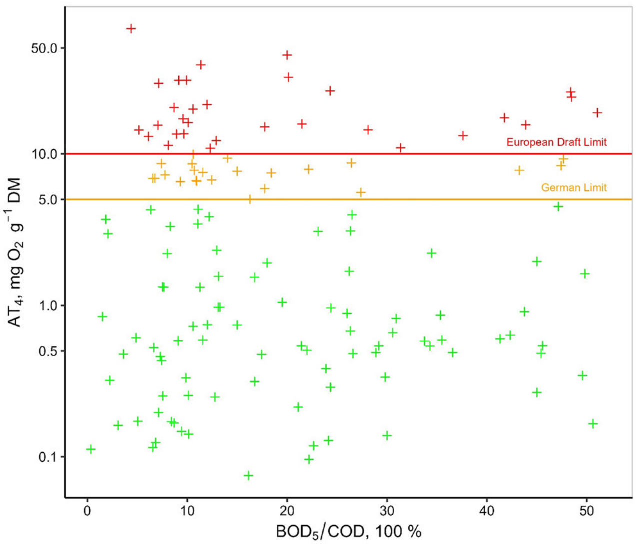

FIGURE 1 Relationship between the biological oxygen demand at 5 days/chemical oxygen demand (BOD_5/COD) ratio and the oxygen uptake at 4 days (AT_4) of the tested samples. German legislation and European Draft limit are shown.

explain the sometimes inconsistent results of past testing values of the BOD_5/COD ratio in industrial waste. In this context, Száraz et al. (2003) and Cossu et al. (2012) already stated that aquatic tests were not suitable for the biodegradation assessment of solid water-insoluble material. Likewise, Liu et al. (2015) indicated that the water-soluble organic fraction was poorly correlated to aerobic biodegradability of biomass residues.

Figure 1 depicts the relationship between the total oxygen consumption after 4 days (AT_4) and the BOD_5/COD ratio of the tested samples. Almost all solid waste presented ratio values <50% corresponding to the allowed disposal landfill limit. The graph reveals that 35% of the hazardous waste considered non-biodegradable according to current Spanish stability limit (BOD_5/COD ratio <50% in the abscissa axis) would be considered biodegradable according to German legislation limit (AT_4 >5 mg O_2 g^{-1} DM, Table 1) and prohibited from being disposed of in landfill. In addition, up to 20% of this waste would be considered biodegradable and non-stable according to the second draft of the working document on the biological treatment of biowaste proposed by the European Union, 2001 (AT_4 value higher than 10 mg O2 g-1 DM, Table 1), posing a risk to health and the environment.

3.2 | AT_4 dependence on the pH and sample dry mass

To further explain the variability, AT_4 was modeled against the available sample data, dry mass, pH, volume of inoculum, and the different measures of organic matter. Table 6

TABLE 6 Fitted model for the natural log of respiration activity (AT4) ($R^2 = 0.66$, $F = 92$, $p = 2 \times 10^{-16}$, $n = 145$). ***$p < 0.001$.

	Coefficient	Error	*T*	*p*-value
Intercept	−3.26567	2.21695	−1.473	0.142966
pH	1.83527	0.45881	4.000	0.000102***
pH^2	−0.10940	0.02385	−4.587	9.82×10^{-6}***
log(MASS_DRY)	−0.82327	0.09410	−8.749	5.90×10^{-15}***

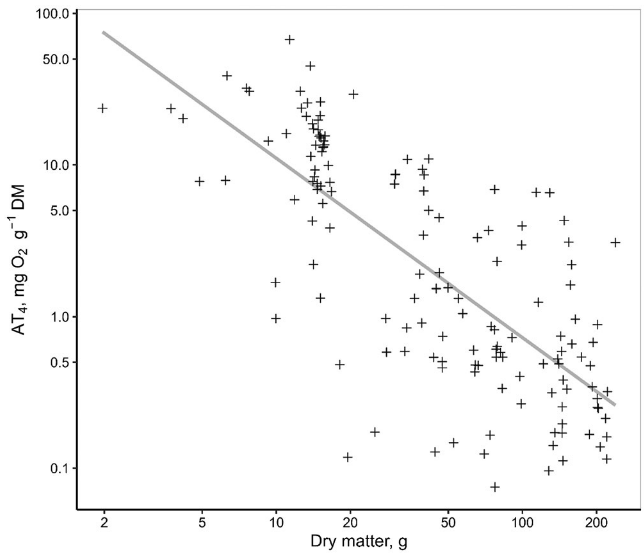

FIGURE 2 Respiration activity (AT_4) respiration index versus sample dry mass.

summarizes the coefficients of the model and their errors. Residuals have been checked to be homoscedastic and normally distributed. Our results show that the sample dry mass is an important factor that affects the biodegradability determination.

Figure 2 shows the AT_4 as a function of the dry matter. Our results evidence that as sample dry mass increases, the magnitude of the respiration diminishes. These findings agree with Komilis and Kletsas (2012), who confirmed that sample size significantly affects oxygen consumption. Apparently, as the sample mass within the respirometer increases, oxygen diffusion to the interior of the material is limited leading to a slow organic matter degradation and reduced respiration activity of the solid sample.

However, Figure 2 also shows that the behavior may be different for samples with smaller and larger dry matter content. Figure 3 shows independent fits for the samples with dry matter below 17.5 g and samples with dry matter above 17.5 g. Fitted models are summarized in Table 7. Below 17.5 g and for the available data, the dependence on the dry matter and the pH is not significant. Above 17.5 g, the fitted model and the coefficients are significant.

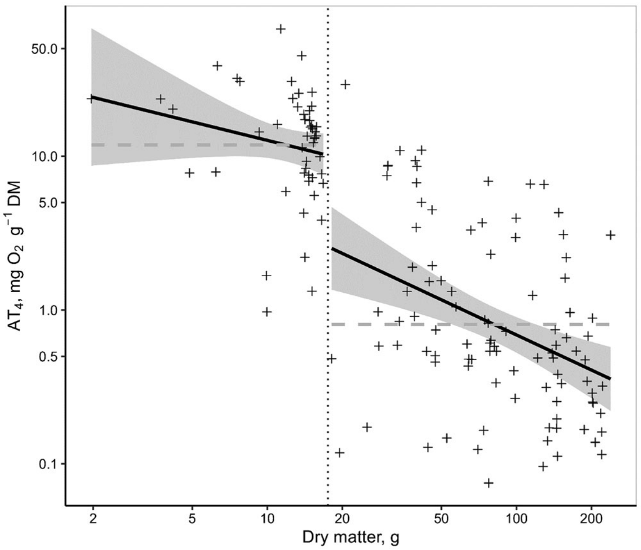

FIGURE 3 Respiration activity (AT_4) respiration index versus sample dry mass, in two groups (below and above 17.5 g). Solid lines correspond to the fitted models, ribbons show the model confidence interval, and the dashed lines present the average value for AT_4 in each group.

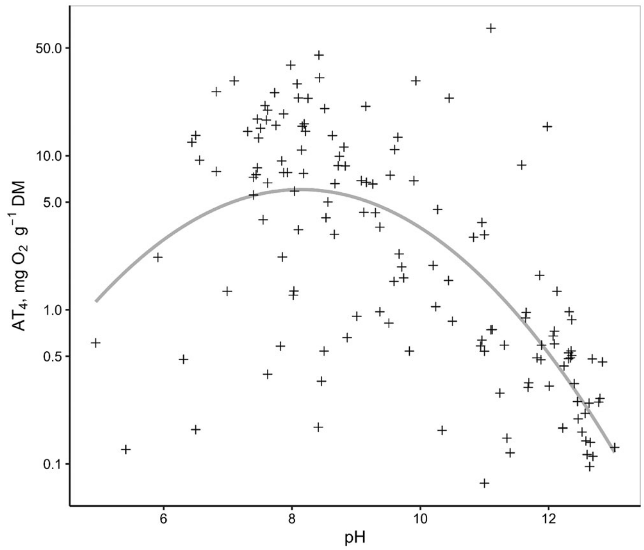

FIGURE 4 Respiration activity (AT_4) respiration index versus pH.

According to these results, a sample dry mass no larger than 17.5 g (or around 20 g) should be preferred for a sensitive biodegradability determination. So, given the fact that higher sample sizes generate lower AT_4, selecting a sample dry mass around 20 g would prevent us from underestimating the biodegradability value of waste and its environmental impacts such as landfill gas emissions.

We also found that pH is a major factor affecting AT_4 determination. Figure 4 shows the relationship between biodegradability and the pH of the sample leaching eluate. A quadratic trend can be seen with an optimum oxygen consumption in an intermediate pH range. The highest degradation was obtained in samples with pH values between 6.5 and 10.5. The lowest respirometric activities were found at extreme pH, especially in alkaline samples (>12). Like in many biotic processes, the pH influences the rate of biodegradation through its effect on microbial consortia diversity and enzyme-catalyzed reaction control. Biodegradation can occur under a variety of pH because different bacterial species flourish within different pH ranges, with the optima for most microorganisms falling between pH 6.5 and 8.5 (Boyd & Shelton, 1984). Several studies state that alkaline or slightly acid sample pH enhances biodegradation, while acidic environments pose limitations to degradation (Pawar, 2015; Singh et al., 2003; Sturová & Walker, 2006). Usually, pH values between 6.5 and 8.0 are considered optimum for oil degradation (Vidali, 2001). In the same sense, Xu (2012) found

TABLE 7 Fitted models for the natural log of respiration activity (AT_4).

(a) Samples with dry matter below 17.5 g ($R^2 = 0.05, F = 0.74, p = 0.53, n = 50$)					
	Coefficient	**Error**	***T***	***p*-value**	
Intercept	3.830059	5.191593	0.738	0.464	
pH	−0.426148	0.287925	−1.480	0.146	
pH^2	−0.024563	1.129084	−0.022	0.983	
log(MASS_DRY)	−0.001465	0.061668	−0.024	0.981	
(b) Samples with dry matter above 17.5 g ($R^2 = 0.43, F = 23, p = 3 \times 10^{-11}, n = 95$)					
	Coefficient	**Error**	***t***	***p*-value**	
Intercept	−7.51555	2.62971	−2.858	0.00528	**
pH	−0.37745	0.16585	−2.276	0.02521	*
pH^2	2.27635	0.50727	4.487	$2.10 \cdot 10^{-5}$	***
log(MASS_DRY)	−0.13100	0.02608	−5.024	$2.51 \cdot 10^{-6}$	***

Note: For detailed information, please refer the Supporting information.
*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$

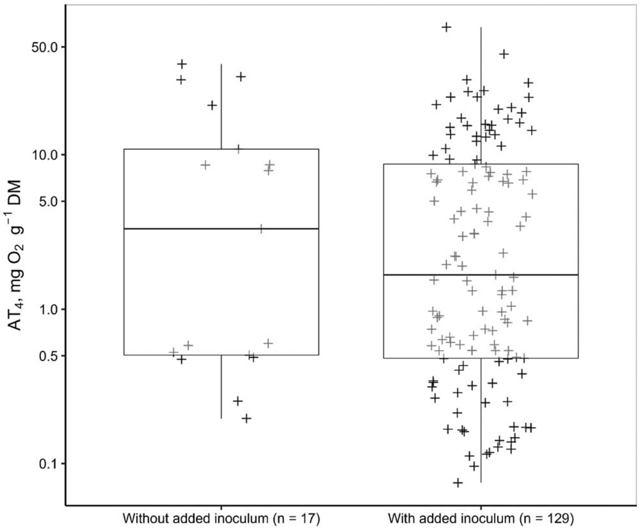

FIGURE 5 Respiration activity (AT_4) respiration index versus presence of inoculum.

that some bacteria isolated from petroleum-contaminated soil being able to degrade over 70% of petroleum at pH 7 and 9.

3.3 | Influence of microbial inoculum addition

Because of the complex nature of hazardous waste, the hypothesis was that native bacteria could be inhibited by potential toxic compounds in the industrial samples, and therefore the supply with supplemental organisms might accelerate the degradation of industrial organic matter (Castro-Aguirre et al., 2018). However, as shown in Figure 5, there is not an apparent difference between AT_4 industrial waste in samples without added inoculum ($n = 17$) and the results of the measurements with the addition of an inoculum ($n = 129$). A Mann–Whitney test cannot reject the location equivalence between determinations with and without inoculum ($p = 0.51$). The test results conclude that there are no significant differences (with 95% confidence) between the measures obtained. This means that the addition of a supplemental bacterial consortium from an industrial activated sludge does not seem to change the biodegradability values of a hazardous solid waste. In other words, the tested samples have sufficient autochthonous microbial population to generate the enzymes to catalyze the oxidation of organic material without the need of providing an acclimated microbial seed. In contrast, there are other types of wastes, namely, disinfected industrial wastes or wastes that have been treated to a high temperature, that contain negligible bacterial population to perform the test and they would need to be seeded with a population of microorganisms to exert an oxygen demand (Dhall et al., 2012).

3.4 | Limitations and further work

Although this study demonstrates the importance of pH on the biodegradability of hazardous solid waste determination, further research is needed to confirm the effects of adjusting the pH to mitigate the problem observed at extreme pHs. In this sense, there is still much to be gained from developing a deeper understanding of the effects and responses to extreme pH in microbe respiration. Moreover, particular challenges arise for biodegradability processes when the proton concentration is high (acidic or low pH), generating organic acid by-products through redox balancing reactions.

Additionally, it would be important to investigate the implications of creating conditions that better simulate biodegradation processes within the landfill deposition environment. Moreover, supplementary research is needed to optimize the sample weight in order to increase the oxygen diffusion into the interior of the material and consequently not underestimate the biodegradability value.

4 | CONCLUSIONS

The biological stability of solid waste is one of the main issues related to the evaluation of the long-term emission potential and the environmental impact of waste management. For safe landfill disposal, an accurate approach to meet organic stability goals is needed. The methodology applied in this study describes the biodegradability assessment of hazardous industrial solid waste following a modified AT_4 method. This determination allows the selection of stable, non-reactive solid waste for hazardous waste acceptance at landfills (European Commission, 2003), avoiding biodegradable organic matter disposal and enabling the landfill process to be compliant with the requirements of the Directive 1999/31/EC on landfill waste.

Our results show that AT_4 is independent of the key chemical parameters LOI, TOC, COD, BOD, and the ratio BOD_5/COD. For an optimal determination of AT_4, special consideration should be made in terms of sample mass and pH. Extreme pHs and larger samples could result in lower AT_4 respiration index estimations. The addition of supplemental inoculum does not seem to be relevant for the analysis.

On the basis of the conducted research, it can be concluded that this methodology can be used to effectively estimate hazardous industrial solid waste biodegradability, independently to other biodegradability proxy measurements. This measure is important to avoid dumping organic matter in landfills and the generation of greenhouse gases, contributing to reduce negative impact of landfill disposal toward a sustainable waste management.

AUTHOR CONTRIBUTIONS

M. Auset: Conceptualization; investigation; methodology; supervision; writing—original; writing—review and editing. **L. Margarit**: Investigation; methodology; writing—original draft. **J. Cuadros**: Data curation; formal analysis; software. **L. Fernández-Ruano**: Data curation; formal analysis; software. **M. Claramunt**: Investigation; methodology. **X. Mundet**: Funding acquisition; project administration; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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