Anaerobic biodegradability of leachate from MSW intermediate landfill

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Estudio de la biodegradabilidad anaeróbica de los lixiviados procedentes de los vertederos municipales de residuos sólidos

Estudi de la biodegradabilitat anaeròbica dels lixiviats procedents dels abocadors municipals de residus sòlids

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SUMMARY

Anaerobic digestion has been used for decades to treat leachate from municipal solid waste landfills. Due to the characteristics of this kind of effluent, the biodegradability results have not been as satisfactory as could be hoped. Consequently, this area has long been a focus of study. This paper contributes to knowledge of the characteristics of biodegradability of this kind of waste effluent, through application to a real case. Biodegradation performance was studied in depth using a range of physicochemical parameters and the amount of methane produced by various volumetric ratios of substrate:inoculum between 2:50 and 50:50 at 35 °C and atmospheric pressure by using anaerobic batch tests in 500 mL bottles. It has been observed an optimal situation for the ratios between 16:50 and 24:50 ratios with values of biodegradability COT 45% and 35% in COD. For smaller proportions it has detected the completion of the lack of readily biodegradable substrate. For higher proportions it is considered that the ammoniacal nitrogen has had an inhibiting effect on the anaerobic process.

Key words: Landfill leachate; anaerobic biodegradability; municipal solid waste (MSW).

RESUMEN

La digestión anaeróbica se ha utilizado durante décadas para tratar los lixiviados procedentes de los vertederos municipales de residuos sólidos. Debido a las características de este tipo de vertidos, los resultados de biodegradabilidad no han sido tan satisfactorios como lo que se esperaba. Como consecuencia de ello, este área ha sido durante tiempo un foco de estudio. Este informe contribuye al conocimiento de las características de biodegradabilidad de esta clase de residuos sólidos, mediante su aplicación a un caso real. El funcionamiento de la biodegradación se ha estudiado con profundidad usando una gama de parámetros fisicoquímicos y la cantidad de metano producida por diversas proporciones de sustrato: inoculo, entre 2:50 y 50:50. Se han utilizado ensayos o pruebas serológicas para frascos de 500 ml. Se ha observado una situación óptima para proporciones entre 16:50 y 24:50 con valores de biodegradabilidad COT del 45% y del 35% en COD. Para proporciones inferiores se ha detectado el fin de la falta de sustrato fácilmente biodegradable. Para mayores proporciones se considera que el nitrógeno amoniacal ha tenido un efecto inhibidor en el proceso anaeróbico.

Palabras clave: Lixiviado de vertedero; biodegradabilidad anaeróbica; residuos sólidos del vertedero municipal (MSW).

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RESUM

La digestió anaeròbica s'ha utilitzat durant dècades per tractar els lixiviats procedents dels abocadors municipals de residus sòlids. Degut a les característiques d'aquest tipus de abocadors, els resultats de biodegradabilidad no han sigut tan satisfactoris com el que s'esperava. Conseqüentment, aquest camp ha sigut durant temps objecte d'estudi. Aquest informe contribueix al coneixement de les característiques de biodegradabilitat d'aquesta classe de residus sòlids, mitjançant la seva aplicació a un cas real. El funcionament de la biodegradació s'ha estudiat amb profunditat fent servir una gama de paràmetres fisicoquímics i la quantitat de metà produïda per diverses proporcions de substrat: inoculo, entre 2:50 i 50:50. S'han utilitzat tests serològics per ampolles de 500 ml. S'ha observat una situació òptima per proporcions entre 16:50 i 24:50 amb valors de biodegradabilitat COT del 45% i del 35% en COD. Per proporcions inferiors s'ha detectat el final de la falta de substrat fàcilment biodegradable. Per proporcions més grans es considera que el nitrogen amoniacal ha tingut un efecte inhibidor en el procés anaeròbic.

Paraules clau: Lixiviat del abocador; biodegradabilitat anaeròbica; residus sòlids del abocador municipal (MSW).

1. INTRODUCTION

The management and treatment of municipal waste (MW) has evolved throughout the twentieth century and the first decade of the twenty-first century. However, dumping waste in landfill sites continues to be a common option. It was included as an alternative in Spain's Integrated National Waste Plan for the 2008–2015 period[1]. MW in Catalonia (Spain) contains around 40% organic matter[2], and approximately 95% is fermentable organic matter[3,4].

When MW is dumped in landfill, a process of anaerobic digestion takes place that is due largely to the organic fraction and has two outputs: biogas comprised mainly of CH_4 and CO_2, and leachate as a new waste product, which can still be considered a long-term environmental problem.

This leachate, which may cause physical damage and harm living beings[5], is formed from water in the MW itself and water from external sources that seeps through the landfill. It contains biological matter and a high number of chemical compounds. Many of the compounds are organic and are more or less biodegradable. However, the leachate may also contain substances that cause it to exhibit an extreme pH value, as well as heavy metals[6].

One way to treat these leachates is based on processes of anaerobic digestion, as this technique can be applied to effluents with a high concentration of organic matter[7], can be adapted to high concentrations of toxic substances[8,9], leads to low sludge production[10] and generates CH_4, which is a potential source of energy[11,12].

Anaerobic digestion consists on the biodegradation of organic substances, in the absence of free oxygen, to produce CH_4 and CO_2 and stabilise the remaining organic matter. This process has been successfully applied to different kinds of urban[13,14], agricultural[15] and industrial[16,17,18,19] wastewaters. In addition, leachates from MW landfills have been treated, leading up to 80 - 90% reductions in organic matter[8,20,21]. Table 1 summarizes the characteristics of leachates according to the age of the landfill[22].

Table 1: *Leachate classification based on the age of the urban solid waste landfill*[22]

	Young landfill (< 1 year)	Intermediate landfill (1-5 years)	Old landfill (> 5 years)
pH	<6.5	6.5-7.5	>7.5
COD (g/L)	>15	3-15	<3
BOD/COD	0.5-1	01.-0.5	<0.1
TOC/COD	<0.3	0.3-0.5	>0.5
$N\text{-}NH_4^+$ (mg/L)	<400	400	>400
Heavy metals (mg/L)	>2	<2	<2
Organic compounds	80% VFA	5-30% VFA+HA+FA	HA+FA
Results:	No degradation	Partial degradation	Stabilized
VFA = Volatile fatty acids; HA = Humic acids; FA = Fulvic acids			

Normally, waste effluent is characterised by simply determining certain physicochemical and chemical parameters. Factors related to microbial activity are generally not considered, except for biochemical oxygen demand (BOD), which is used in studies of aerobic biodegradability but not in those of anaerobic biodegradability, as oxygen is not consumed in this case[23,24].

The most commonly used method for studying anaerobic biodegradability is the biodegradability and toxicity test, which has been applied to different kinds of effluents[16,18,19]. The test consists of determining how much a substrate can be biodegraded through an anaerobic process. To achieve this, the production of CH_4 from organic components of the effluent is measured when it is exposed to the action of anaerobic sludge[20,21,25]. CH_4 production is related to the anaerobic biodegradability of the effluent and its toxic effects on the microbial groups that are responsible for the anaerobic process[26].

In this study, the objective is to assess the level of degradation in order to analyse the feasibility of applying an anaerobic process to treat leachate from an intermediate landfill in Catalonia (Spain), to set the necessary operating conditions for an anaerobic digester.

MATERIALS AND METHODS

Reactors and system for measuring methane

Biodegradability tests[25,27] (Figure 1) were carried out in 500 mL serum bottles with a high-purity nitrogen (99.999%) volume fraction of 30%. Once the bottles had been inoculated, they were sealed with rubber stoppers and aluminium seals and incubated at a temperature of 35 °C ± 2 °C and shaken every day.

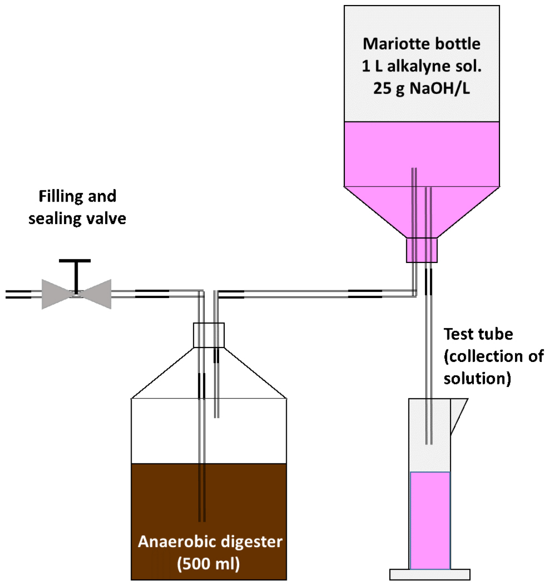

Figure 1: *Experimental setup for biodegradability anaerobic test*

Biogas production is calculated using a system comprised of a Mariotte bottle containing 1 L of an alkaline solution (25 g NaOH/L) to absorb the CO_2 that is generated and to allow the continuous, direct measurement of the CH_4 that is produced by hydraulic displacement. As the CO_2 contained in the biogas is absorbed in the alkali and forms sodium hydrogen carbonate, the volume of the alkaline solution that is displaced is equivalent to the volume of CH_4 produced, measured in laboratory conditions, and can be collected in a graduated test tube for measurement.

Substrate

Leachate from a MW landfill that had been in operation for over 25 years was used as the substrate. It was selected for its complexity and the problem it currently poses for landfill[1] since it is the main landfill in Catalonia in the process of closure. The leachate was characterised[28] by analysing the following parameters: pH, total solids (TS), total volatile solids (TVS), total dissolved solids (TDS), total organic carbon (TOC), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), ammonium nitrogen content and metals. The alkalinity and density of the sample have also been analyzed.

The substrate was characterised by measuring the above parameters in three assays (except for the concentration of metals, which was determined in one sole measurement). The results are shown in Table 2.

The leachate in this study had a high COD of which approximately 60% corresponded to soluble COD. The leachate has a low content of easily-biodegradable organic material due to the extended period of time that it has remained in the landfill site. This accounts for the difference observed between the values for BOD_{5total} and COD_{total}.

The pH was slightly above neutral, which would be expected in an operating landfill site[29]. The metal content was below the limits that are considered toxic[26].

Table 2: *Physicochemical characterisation of the substrate and inoculum*

Substrate					
Parameter		**Number of assays**	**Minimum value**	**Maximum value**	**Average value**
Alkalinity	CO_3^{2-} (ppm)	3	1,109	1,149	1,127 ± 23
	HCO_3^- (ppm)	3	24,735	24,900	24,813 ± 94
TOC (ppm)		4	2,301	2,629	2,483 ± 154
COD total (ppm)		9	9,821	13,398	11,542 ± 919
COD soluble (ppm)		6	6,125	6,890	6,466 ± 250
BOD_5 total (ppm)		3	1,942	2,003	1,970 ± 35
Density (kg/L)		7	1.088	1.0093	1.0090 ± 0.0002
$N\text{-}NH_4^+$ soluble (ppm)		7	1,988	2,165	2,072 ± 57
pH		9	8.02	8.37	8.24 ± 0.12
TS (%)		7	1.29	1.31	1.30 ± 0.01
VS (%TS)		7	29.1	29.4	29.2 ± 0.1
TS soluble (%)		3	1.25	1.26	1.26 ± 0.01
VS soluble (%TS soluble)		3	27.1	27.5	27.3 ± 0.2
Metals (µg/g)	Zn	1	---	---	0.39
	Cu	1	---	---	0.34
	Ni	1	---	---	0.31
	Cr	1	---	---	0.45
	As	1	---	---	0.18
	Hg	1	---	---	< 0.02
	Cd	1	---	---	< 0.02
	Pb	1	---	---	< 0.2
Inoculum					
Parameter		**Number of assays**	**Minimum value**	**Maximum value**	**Average value**
TOC (ppm)		3	6,798	6,894	6,831 ± 62
COD total (ppm)		5	22,473	23,465	22,952 ± 336
COD soluble (ppm)		3	2,970	3,194	3,066 ± 131
Density (kg/L)		5	0.9772	0.9822	0.9802 ± 0.0020
$N\text{-}NH_4^+$ soluble (ppm)		3	1,022	1,037	1,030 ± 7
pH		6	7.40	7.43	7.42 ± 0.01
TS (%)		3	2.50	2.51	2.50 ± 0.01
VS (%ST)		3	60.4	60.5	60.4 ± 0.1
TS soluble (%)		3	0.31	0.34	0.33 ± 0.002
VS soluble (%TS soluble)		3	39.4	40.1	39.8 ± 0.4

Inoculum

The inoculum was digestate derived from an anaerobic digester of sludge from wastewater, which could develop bacterial flora capable of degrading the substrate through an anaerobic process. This inoculum contained 2.5% TS; with 60.5% TVS out of the TS. The COD was 22,952 ppm and the soluble COD was 3,066 ppm. It had a pH of 7.42. Table 1 shows the characteristics of the inoculum that were analysed.

Metal concentration values are not given, as they were all below the detection thresholds. This indicates that metals were not present in the inoculum that was used.

Trial method

The 500 mL reactors were filled with 250 mL of the inoculum and the substrate in different volumetric ratios of substrate:inoculum, varying between 2:50 and 50:50; a buffer solution of carbonate (1 g/L of $NaHCO_3$) to maintain a pH of around 7; and water to equal the volume in all reactors. In addition, a blank reactor containing no substrate was used to determine the net production of methane due to the inoculum.

All the reactors were set up three times. However, in each of the three sets of mixtures, one reactor was sacrificed to determine the pH, TOC, COD, the VFA, the TVS and the concentration of soluble $N\text{-}NH_4^+$ at the start of the trial (start time) with the objective to validate the partial results of the combination of substrate and inoculum in the different proportions. The two remaining reactors for each ratio were monitored for approximately 30 days, and the methane production was measured periodically, the experimental results have been validated by comparing both samples. At the end of the trial, the reactors were opened to determine the final values of pH, TOC, COD, VFA, TVS and soluble $N\text{-}NH_4^+$.

Analytical methods

The proportion of methane in the gas that was obtained was quantified by a Shimadzu GC-9A gas chromatograph, equipped with a thermal conductivity detector and using helium as a carrier gas. The TOC analyses were carried out using a Carlo Erba 1500 elemental analyser, with a thermal conductivity detector. The concentration of VFA in the liquid phase was obtained using a Hewlett-Packard 5890A model gas chromatograph with a flame ionisation detector and using nitrogen as the carrier gas.

In order to determine the concentration of $N\text{-}NH_4^+$, the ammonia-selective electrode method was used (model Orion 95-10). The samples were filtered and rendered alkaline to a pH of 10–12 in order to detect all the free dissolved ammonia[28]. The concentration of $N\text{-}NH_4^+$ was monitored for the inoculum proportions: substrate 16:50, 24:50 and 36:50 to analyze the influence of this parameter over the time of experimentation.

The physicochemical analyses for determining other control parameters were carried out using standard methods[28].

Indexes of methanation and biodegradability

The index of biodegradability (%BD) is the fraction of the substrate that can be biodegraded anaerobically[30]. This index is useful for comparing the substrate:inoculum ratios that were considered[30,31]. The indexes of methanation (%M) and acidification (%A) can be used to compare the efficiency of these processes and study the influence of other factors, such as the presence of inhibitory or toxic substances[30]. Both indexes are determined using COD data.

The index of methanation (%M) estimates the fraction of initial organic matter that has been converted into CH_4 (see equation 1)[30,32]:

$$\%M = \frac{COD_{CH_4}}{COD_{initial}} \times 100 \qquad (1)$$

The index of acidification (%A) estimates the fraction of initial organic matter that has been converted into VFA, including that converted into CH_4 (see equation 2)[30,32]:

$$A = \frac{COD_{CH_4} + COD_{VFA}}{COD_{initial}} \times \qquad (2)$$

The index of biodegradability (%BD) is calculated from the COD that is eliminated by the process of anaerobic degradation and the fraction of COD present in the system in the form of VFA[31] (see equation 3)[30,32]:

$$A = \frac{COD_{CH_4} + COD_{VFA}}{COD_{initial}} \times \qquad (3)$$

where the difference between $COD_{initial}$ and COD_{final} includes the different intermediate compounds that are generated and not only the content in CH_4.

RESULTS AND DISCUSSION

VFA and pH

VFA are intermediate compounds in the anaerobic digestion process, and their formation is essential to ensure the subsequent production of methane. However, the accumulation of VFA leads to inhibition of the biodegradation process.

The concentrations of VFA obtained at the end of each of the experiments were totally insignificant. In many cases, the values did not reach the minimum detection limits. Consequently, in no case was the process inhibited by VFA[33].

At the same time, the pH values obtained were slightly alkaline, which substantiates the lack of VFA and means that the condition of a pH of around 7 was met, which is essential for validation of biodegradability tests[25]. In addition, the ideal pH for correct functioning of the methanogenic phase is considered to be in the range of 6.5–8.0[34].

Ammonium nitrogen and heavy metals

Due to the inhibitory characteristics of ammonium nitrogen ($N\text{-}NH_4^+$) in anaerobic digestion processes, its concentration was studied in the substrate:inoculum ratios that were prepared (Figure 2). A rise in the initial concentration of $N\text{-}NH_4^+$, due to de hydrolytic activity and the ammonification of organic nitrogen, was observed when the amount of leachate in the mixture was increased. In addition, it was found that substrate:inoculum ratios above 28:50 had concentrations of $N\text{-}NH_4^+$ above 1,200 ppm, which could be indicative of a possible inhibitory effect on the anaerobic digestion process[34,35,36].

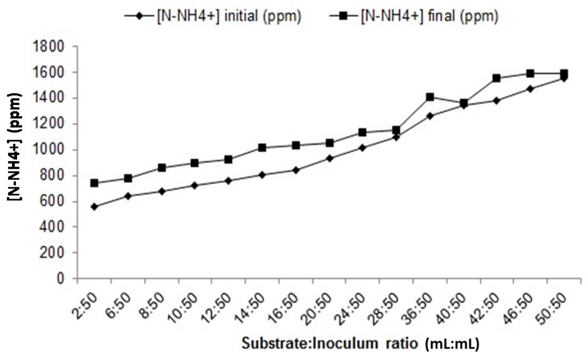

Figure 2. *Initial and final nitrogen comparison for different volumetric ratios of Substrate:Inoculum*

An increase was also observed in the concentration of $N\text{-}NH_4^+$ as the substrate:inoculum ratio increased. This was due to a greater solubility of the nitrogen contained in the substrate originating from the solid MW.

The interim analyses carried out for the substrate:inoculum ratio 36:50 (Figure 3) show that the production of biogas was greatest when the concentration of $N\text{-}NH_4^+$ was lower. After the first 150 hours, the period during which degradation of the most easily biodegradable material takes place, a decrease in the production of CH_4 is observed at a $N\text{-}NH_4^+$ concentration of around 1,350 ppm, indicating that an possible inhibitory effect has been produced by $N\text{-}NH_4^+$; this value is not far removed from other proposals in the referenced literature for similar substrates[37,38,39]. Finally, on reaching an $N\text{-}NH_4^+$ concentration of 1,400 ppm, a sharp drop in the production of CH_4 was observed.

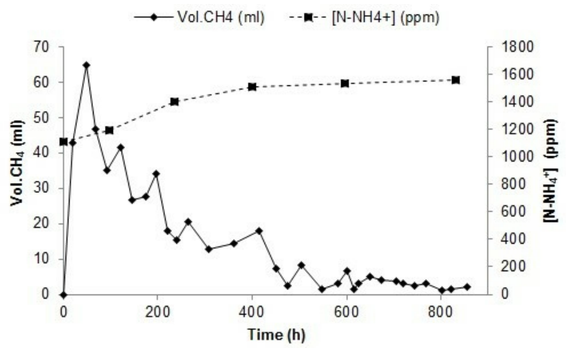

Figure 3. *Concentration of $N\text{-}NH_4^+$ for the volumetric ratio Substrate:Inoculum 36:50*

Following this, constant production was observed until the ammonium nitrogen concentration reached around 1,400 ppm, at which moment methane production fell significantly[38,39].

An inhibitory effect due to the presence of heavy metals was ruled out, as thresholds of toxicity were not reached[26].

TOC and COD

A comparison between the start and end values of TOC for each of the substrate:inoculum volumetric ratios showed that, in all cases, the organic matter that was initially provided by the substrate was biodegraded. An optimum range of biodegradability, in terms of TOC reduction, was observed between the ratios of 14:50 and 24:50. The highest level of elimination was found to be 45% (Figure 4). These data suggest the presence of an inhibitory or very low biodegradability component in landfill leachate and further experiments would be required to define it.

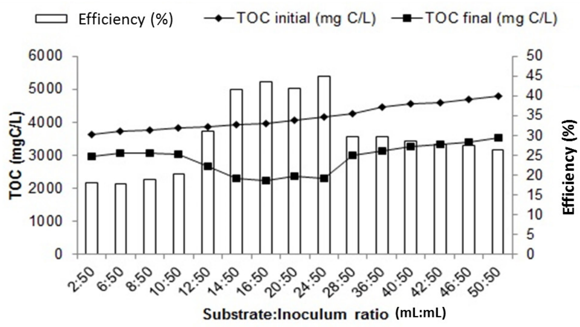

Figure 4. *TOC biodegradability and biodegradability efficiency for different volumetric ratios of Substrate:Inoculum*

The same situation of biodegradability that was observed for TOC was seen in the start and end values of COD, and in the associated biodegradability efficiency. Maximum biodegradability was found at a substrate:inoculum ratio of around 24:50, with a performance of 33.4% of COD, a value that is not very high, due to the fact that complex landfill leachates were used that are difficult to biodegrade[40,41].

Methane production

As the CO_2 is absorbed by the NaOH solution, the measured biogas fraction corresponds to CH_4 production, which was measured daily.

For the daily methane production three different behaviours were observed depending on the volumetric substrate:inoculum ratio that was studied: from 50:50 to 36:50, from 28:50 to 16:50, and from 14:50 to 6:50.

In experiments carried out with a high leachate load (Figure 5) from 36:50 to 50:50, after a few days of high methane production, the values dropped gradually until production was practically non-existent. This indicates that the limit of biodegradability had been reached and only matter that is very difficult to biodegrade in an anaerobic process was left, or that inhibition had occurred[7,42]. Specifically, inhibition should be considered when the concentration of ammonium nitrogen is above 1,200 ppm, as indicated in the section entitled 'Ammonium nitrogen and heavy metals'.

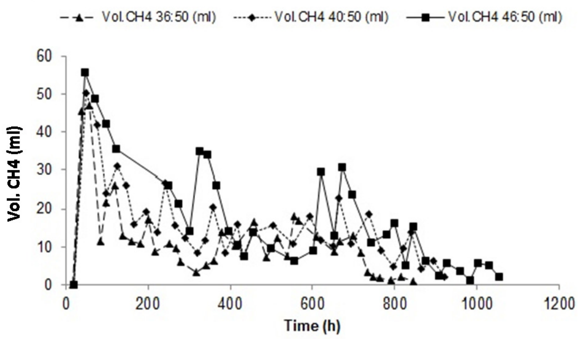

Figure 5. *Daily CH_4 production for high Substrate:Inoculum volumetric ratios*

Figure 5 also shows that CH_4 was produced at the greatest rate in the first few hours of the process. By comparison with methane production in the blank reactor without leachate, we can observe that this phase corresponds to the degradation of the organic matter that is most easily biodegraded in the inoculum.

The oscillations in the production of methane indicate that the biodegradation process took place in phases. First, the solubilised organic matter was consumed. Then, the organic matter that is more difficult to biodegrade was successively solubilised hydrolytically. This successive process occurred in cycles. In each cycle, less CH_4 was produced and, therefore, the degree of solubilisation was lower. Therefore, initially, the phase that controls the process is methanation, but as the process advances over time, the control phase becomes the hydrolysis of the substrate.

CH_4 production was highest in the second interval of substrate:inoculum ratios that was analysed, from 28:50 to 16:50, and theoretical maximum values of CH_4 output were obtained. The theoretical maximum biogas production, determined on the basis of TOC (under normal conditions), was estimated at 4.6 mL biogas/mL leachate, with a volumetric proportion of CH_4 of 60%[42]. Other experiments independent of this work allow a theoretical maximum methane production value to be determined with greater precision[38]. The biodegradation process was considered completed once the maximum production had been obtained in an experiment, or after 30 days.

In this range of substrate:inoculum ratios, the same situations were found as described above. That is, the rate of methane production was initially very high. Subsequently, the rate decreased, and phases of hydrolytic solubilisation and methanation alternated until the organic matter in the leachate was (theoretically) totally consumed. Total leachate consumption was reached in around 900 hours.

In this interval of substrate:inoculum ratios, the initial and final values of ammonium nitrogen were not above the inhibition threshold of 1,200 ppm in any case[37].

Finally, in the ratios with a low organic load, from 14:50 to 6:50, behaviour equivalent to that described for proportions between 28:50 and 16:50 was observed. The phases of hydrolytic solubilisation and methanation alternated until all of the organic matter was consumed. The time until total degradation of the substrate was around 300 hours.

The maximum accumulated experimental CH_4 production per unit of initial volatile solid of substrate was determined by estimating the behaviour of the anaerobic digestion process at infinite time (B_0)[43].

Figure 6 shows the efficiency of biodegradation (B/B_0) according to the maximum experimental CH_4 production (B). Low CH_4 production was associated with the leachate, as expected in complex substrates that are difficult to biodegrade. At the lowest substrate:inoculum ratios, between 2:50 and 24:50, the values remained almost constant. At substrate:inoculum ratios higher than 24:50, the methane production decreased considerably, which again indicates a situation of inhibition, as some substrate remained to be biodegraded.

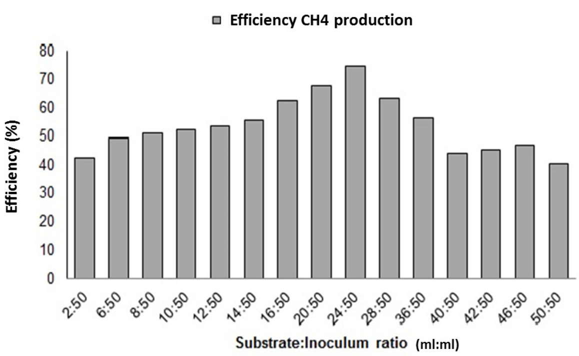

Figure 6. *Efficiency according to the maximum experimental CH_4 production*

Volatile solids contained in the leachates were not highly biodegraded compared to other organic waste[11,44,45,46]. This could be due to the presence of organic compounds that can inhibit the anaerobic process. However, it is more likely to be due to the refractory nature of these compounds[7,42,46].

In the study of accumulated production for the 10:50, 24:50 and 46:50 substrate:inoculum ratios (Figure 7) for the three potential cases (reduction of production due to a lack of substrate at 10:50, optimum situation of maximum biodegradability at 24:50, and inhibition or hydrolysis rate drop at 46:50), a first phase was observed in which the behaviour was almost the same in all cases. After the first 300 hours, each of the ratios began to behave in a specific way. From this time on, the experiment with the 24:50 ratio had considerably higher accumulated CH_4 production, which indicates that optimum biodegradability occurred in the intermediate ratios.

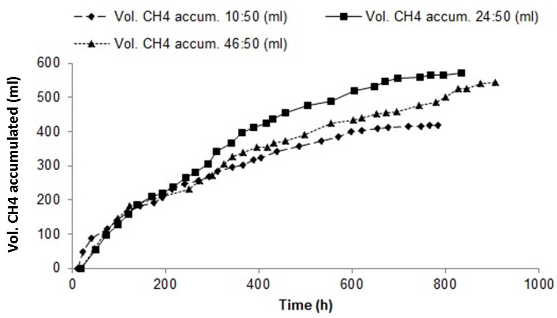

Figure 7. *Accumulated CH4 production for the 10:50, 24:50 and 46:50 volumetric ratios*

The fact that methane production was appreciable during last working days in the experiments with high ratio substrate:inoculum, suggest some type of inhibition. Free ammonia nitrogen has not been analysed, so that its value can not be compared with the reported inhibitory threshold range, from 400 to 1000 mg/L[47]. But similar tests recently performed with food waste as a substrate, lead to the same methane production tendency and reach similar ammonium nitrogen values without observed ammonia inhibition[48]. The authors found methanogenic inhibition only for high VFA concentration that did not occur in present work due to the low amount of VFA remaining. It has been thought about the accumulation of toxic materials, but it has been discarded because in present work biogas production did not stop completely[49]. On the other hand, in order to increase the efficiency of a possible continuous treatment of leachate by means of anaerobic co-digestion, pretreatments and/or acclimatization of biomass could be applied[50,51].

Mature leachates are known to be rich in stable organic matter[52]. It has been reported that biogas yield reaches a maximum of about 10% leachate and then decreases by increasing the percentage of leachate in the co-digestion of mature leachate with a mixture 60:40 sewage sludge:digested sludge[53]. In the present work, as indicated by the BOD_5/COD ratio (0.2) and the content of volatile solids (3.8 g/L), leachate is still not mature and contains more biodegradable organic matter. This biodegradable organic matter provided by leachate could be responsible for moving the maximum in the efficiency of the elimination of TOC and in the formation of CH_4 to values of around 40% leachate.

Indexes of methanation and biodegradability

Based on the equations (1), (2) and (3), the indexes of biodegradability, acidification and methanation have been determined for the different substrate:inoculum ratios.

The highest biodegradability, expressed in %BD, was found in the 24:50 ratio of substrate:inoculum, which is in agreement with previous results for optimum biodegradation (Figure 8).

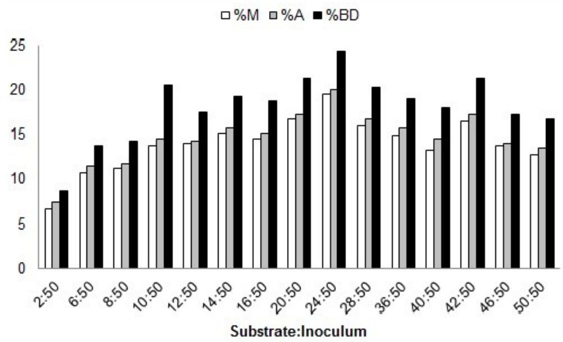

Figure 8. *Indexes of methanation (%M), acidification (%A) and biodegradability (%BD)*

Little difference was observed between the indexes of acidification and methanation, which indicates that the control phase was hydrolysis of the organic matter. This suggests that the methanogenic bacteria were capable of consuming almost all of the VFA formed and the system was limited by the lack of hydrolytic activity, that is, by the lack of solubilisation of organic matter.

CONCLUSIONS

The biodegradation of a landfill leachate has been investigated at different proportion mixtures with digestate derived from a wastewater anaerobic digester as inoculum, at 35 °C and atmospheric pressure. Volumetric ratios substrate:inoculum used were between 2:50 and 50:50. All ratios has shown biodegradation in anaerobic conditions, but higher biodegradation has been found for ratios from 14:50 to 24:50 attending to TOC elimination (around 40%). Regarding DQO, higher elimination has been found in the case of 24:50 ratio (near to 35%). This ratio also has been shown a higher methane production and higher indexes of methanation, acidification and biodegradability. When substrate ratios increase, complex compounds accumulated, and hydrolysis become the limiting step.

REFERENCES

1. Ministry of Environment and Rural and Marine Affairs –MERMA–, Government of Spain *Plan Nacional Integrado de Residuos 2008-2015*. Boletín Oficial del Estado nº49, Madrid, 2009.
2. Ministry of Environment and Rural and Marine Affairs –MERMA–, Government of Spain Gestión de biorresiduos de competencia municipal: Guía para implantación de la recogida separada y tratamiento de la fracción orgánica. Madrid, 2013.
3. Sans, R.; Álvarez, D.; Forné, C.; Puig, M.D. Caracterización de los residuos sólidos urbanos del municipio de Terrassa. *Residuos* **1999**, *48*, 58-61.
4. Agència de Residus de Catalunya. *Memòria de l'Agència Catalana de Residus*. Departament de Territori i Sostenibilitat (Generalitat de Catalunya), Barcelona, 2010.
5. Rodríguez, J.L.; Iza, J.; Ilardia, J.L. *Pretratamiento de lixiviados en vertedero controlado por digestión anaeróbica*. Actas del IV Congreso de Ingeniería Ambiental, Bilbao, 1995.
6. Renoua, S.; Givaudana, J.G.; Poulaina,S.; Dirassouyanb, F.; Moulinc, P. (2008). Landfill leachate treatment: Review and opportunity. *J. Hazard. Mat.* **2008**, *150(3)*, 468–493.
7. Fiestas, J.A.; León, R.; García, A.J.; Fernández, F.R.; Sainz, J.A. Aplicación de los procesos anaerobios en la depuración de las aguas residuales industriales. *Ing. Quim.* **1981**, *147*, 8589.
8. Alkalay, D.; Guerrero, L.; Lema, J.M.; Méndez, R.; Chamy, R. Anaerobic treatment of municipal landfill leachates: The problem of refractory and toxic components. *World J. Microbiol. Biotechnol.* **1998**, *14*, 309-320.
9. Chen, Y.; Cheng,J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.* **2008**, *99(10)*, 4044-4064.
10. Tsonis, S.P.; Grigoropolus, S.G. Anaerobic treatability of olive oil mill wastewater. *Water Sci. Technol.* **1993**, *28*, 34-44.
11. Amona, T.; Amona, B.; Kryvouruchkoa, V.; Machmüllera, A.; Hopfner-Sixta, K.; Bodirozaa, V.; Hrbekb, R.; Friedelb, J.; Pötschc, E.; Wagentristld, H.; Screinere, M.; Zollitschf, W. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresour. Technol.* **2007**, *98(17)*, 3204-3212.
12. Peng, Y. Perspectives on technology for landfill leachate treatment. *Arab. J. Chem.* **2017**, 10(52), 2567-2574.
13. Monroy, O.; Fama, G.; Meraz, M.; Montoya, L.; Macaric, H. Anaerobic digestión for wastewater treatment in Mexico: State of the technology. *Water Res.* **2000**, *34(6)*, 1803-1816.
14. Brennan, R.B.; Clifford, E.; Devroedt, C.; Morrism, L.; Healy, M.G. Treatment of landfill leachate in municipal wastewater plants and

impacts on effluent ammonium concentration. *J. Environ. Manage.* **2017**, *188*, 64-72.
15. Ward, A.J.; Hobbs, P.J.; Holliman, P.J.; Jones, D.L. Optimisation of the anaerobic digestion of agricultural resources. *Bioresour. Technol.* **2008**, *99(17)*, 7928-7940.
16. Kleerebezem, R.; Macarie, H. Treating industrial wastewater: Anaerobic digestion comes of age. *Chem. Eng.* **2003**, *110*, 56-64.
17. Rincón, N.; Chacín, E.; Marín, J.; Torrijos, M.; Moletta, R.; Fernández, N. Anaerobic biodegradability of water separated from extracted crude oil. *Environ. Technol.* **2003**, *24*, 963970.
18. Fernández, M.; Abalos, A.; Crombet, S.; Caballero, H. Ensayos de biodegradabilidad anaerobia de aguas residuales generadas en una planta refinadora de aceite de soja. *Interciencia* **2010**, *35(8)*, 600-604.
19. Van Lier, J.B.; Van der Zee, F.P.; Fritjers, C.T.M.J.; Ersahin, M.E. Celebrating 40 years anaerobic sludge bed reactors for industrial watewater treatment. *Rev. Environ. Sci. Bio.* **2015**, *14(4)*, 681-702.
20. Timur, H.; Ozturk, I. Anaerobic sequencing batch reactor treatment of landfill leachate. *Water Res.* **1999**, *33(15)*, 3225-3230.
21. Calli, B., Mertoglu, B., Inanc, B. Landfill leachate management in Istanbul: applications and alternatives. *Chemosphere* **2005**, *59*, 819-829.
22. Renou, S.; Givaudan, J.G.; Poulain, S.; Dirassouyan, F.; Moulin, P. Landfill leachate treatment: review and opportunity. *J. Hazard. Mat.* **2008**, *150*, 468-493.
23. Shelton, D.; Tiedje, J. General method for determining anaerobic biodegradation potential. *Appl. Environ. Microb.* **1984**, *47(4)*, 850-857.
24. Angelidaki, I.; Alves, M.; Bolzonella, D.; Borzacconi, L.; Campos, J.L.; Guwy, A.J.; Kalyuzhnyi, S.; Jenicek, P.; Van Lier, J.B. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci. Technol.* **2009**, *59(5)*, 927-34.
25. Owen, W.; Stuckey, D.; Healy, J.; Young, L.; McCarty, P. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res.* **1979**, 1, 485-492.
26. Lin, Ch. Effect of heavy metals on volatile fatty acid degradation in anaerobic digestion. *Water Res.* **1992**, *26(2)*, 177-183.
27. Soto, J.M.; Méndez, R.; Lema, J.M. Determinación de la toxicidad y biodegradabilidad anaerobia de aguas residuales. *Tecnología del agua* **1992**, *92*, 70-81.
28. APHA, AWWA, WEF. *Standard Methods for the examination of water and wastewater.* 21th ed. Am. Public HLTC Assoc. Washington, D.C. 2005.
29. Tejero, I.; Szanto, M.; Fantelli, M.; Díaz, R. Caracterización y tratabilidad de los lixiviados del vertedero de Meruelo. *Revista Técnica de Medio Ambiente* **1991**, *25*, 111-118.
30. Lesteur, M.; Bellon-Maurel, V.; Gonzalez, C.; Latrille, E.; Roger, J.M.; Junqua, G.; Steyer, J.P. Alternative methods for determining anaerobic biodegradability: A review. *Process Biochem.* **2009**, *45(4)*, 431-440.
31. Méndez, M.S.; Lema, J.M. Methanogenic and non-methanogenic activity tests. Theoretical basis and experimental set up. *Water Res.* **1993**, *27(8)*, 1361–1376.
32. Elbeshbishy, E.; Nakhla, G. Batch anaerobic co-digestion of proteins and carbohydrates. *Bioresour. Technol.* **2012**, *116*, 170-178.
33. Ahring, B.K.; Sandberg, M.; Angelidaki, I. Volatile fatty acids as indicators of process imbalance in anaerobic digestion. *Appl. Microbiol. Biotechnol.* **1995**, *43(3)*, 559-565.
34. Dai, X.; Hu, Ch.; Zhang, D.; Dai, L.; Duan, N. Impact of a high ammonia-ammonium-pH system on methane-producing archaea and sulfate-reducing bacteria in mesophilic anaerobic digestion. *Bioresour. Technol.* **2017**, *245(A)*, 598-605.
35. Torres, R.; Llabrés, P.; Mata-Álvarez, J. Temperature effect on anaerobic digestion of wheat Straw in a one phase system at different inoculum concentration. *Agric. Ecosys. Environ.* **1995**, *54*, 55-66.
36. Cartes, J.; Neumann, P.; Hospido, A.; Vidal, G. Life cycle assessment of management alternatives for sludge from sewage treatment plants in Chile: does advanced anaerobic digestion improve environmental performance compared to current practices? *J. Mater. Cycles Waste Manage.* **2017**, *20*, 1530–1540.
37. Berrueta, J.; Castrillón, L. Efectos del $N\text{-}NH_4^+$ sobre el tratamiento anaerobio de lixiviados de vertedero. *Ing. Quim.* **1997**, *336*, 121-125.
38. Robbins, J.E.; Gerhardt, S.A.; Kappel, T.J. Effects of total ammonia on anaerobic digestion and an example of digestor performance fro, cattle manure-protein mixture. *Biolog. Wastes* **1989**, *27*, 1-4.
39. Jokela, J.P.Y.; Vavilin, V.A.; Rintala, J.A. Hydrolisis rates, methane production and nitrogen solubilisation of grey waste components during anaerobic degradation. *Bioresour. Technol.* **2005**, *96(4)*, 501-508.
40. Kulikowska, D.; Klimiuk, E. The effect of landfill age on municipal leachate composition. *Bioresour. Technol.* **2008**, *99(13)*, 5981-5985.
41. Lou, Z.; Dong, B.; Chai, X.; Song, Y.; Zhao, Y.; Zhu, N. Characterization of refuse landfills leachates of three different stages in landfill stabilization process. *J. Environ. Sci.* **2009**, *21(9)*, 1309-1314.
42. Iwami, A.; Iwai, A.; Inamori, Y.; Sudo, R. Treatment of a landfill leachate containing refractory organics and ammonium nitrogen by the microorganism-attached activated carbon fluidized bed process. *Water Sci. Technol.* **1992**, *26*, 9-11.

43. Chen, T.H.; Hashimoto, A.G. Kinetics of methane fermentation. *Biotechnol. Bioeng.* **1978**, *8*, 269-272.
44. Torres, R.; Llabres, P.; Mata-Álvarez, J. Moisture and HRT influence on the two-phase anaerobic digestion of solid agricultural wastes. *Biomass Bioenergy* **1988**, *23(5)*, 256-263.
45. Pavan, P.; Traverso, P.G.; Battistoni, P.; Cecchi, F.; Mata-Álvarez, J. *Two-phase anaerobic digestion of sourced OFMSW: Performance and kinetic study*. Water Sci. Tecnhnol. **2000**, *41*, 111-118.
46. Zhang, W.; Wei, Q.; Wu, S.; Qi, D.; Li, W.; Zuo, Z.; Dong, R. Batch anaerobic co-digestion of pig manure with dewatered sewage sludge under mesophilic conditions. *Appl. Energ.* **2014**, *128*, 175-183.
47. Stams, A.J.; Oude Elferink, S.J.; Westermann, P. Metabolic interactions between methanogenic consortia and anaerobic respiring bacteria. *Adv. Biochem. Eng. Biot.* **2003**, 81: 31-56.
48. Li, Y.; Jin, Y.; Borrion, A.; Li, J. *Influence of feed/inoculum ratios and waste cooking oil content on the mesophilic anaerobic digestion of food waste. Waste Manag.* **2018**, *73*, 156164.
49. Jiang, J.; Li, L.; Cui, M.; Zhang, F.; Liu, Y.; Liu, Y.; Long, J.; Guo, Y. Anaerobic digestion of kitchen waste: The effects of source, concentration, and temperature. *Biochem. Eng. J.* **2018**, *135*, 91-97.
50. Contrera, R.C.; Lucero Culi, M. J.; Morita, D. M.; Domingues Rodrigues, J. A.; Zaiat, M.; Schalchd, V. Biomass growth and its mobility in an AnSBBR treating landfill leachate. *Waste Manag.* **2018**, *82*, 37–50.
51. Gagliano, M. C.; Gallipoli, A.; Rossetti, S.; Braguglia, C. M. Efficacy of methanogenic biomass acclimation in mesophilic anaerobic digestion of ultrasound pretreated sludge. *Environ. Technol.* **2018**, *39(10)*, 1250-1259.
52. Huo, S.; Xi, B.; Yu, H.; Liu, H. Dissolved organic matter in leachate from different treatment processes. *Water Environ J.* **2009**, *23(1)*, 15–22.
53. Berenjkar, P.; Islam, M.; Yuan, Q. Co-treatment of sewage sludge and mature landfill leachate by anaerobic digestion. *Int. J. Environ. Sci. Te.* **2018**, *100*: 1-10.