Advances in Environmental Technology 3 (2021) 195-208

Iranian Reasearch Organization for Science and Technology (IROST)

Advances in Environmental Technology

Journal home page: https://aet.irost.ir/

# Co-disposal of bottom ash and municipal solid wastes: chemical, microbiological behaviours and stability in anaerobic conditions

**Ghita Ait Baddi [a]*, Rabha Aissa [a], Bouchra Chebli[b], Fouad Achemchem [a, b], Btissam Mouria [a], Aaziz Ouatmane [c a], Théodore Bouchez [e]**

[a]*Equipe Bio-procédés et environnement, Laboratoire des Sciences de l'Ingénieur et le Management de l'Énergie, Ecole Supérieure de Technologie, Université Ibn Zohr, Agadir, Route Oued Ziz, Morocco*

[b]*Ecole Nationale des Sciences Appliqués d'Agadir, Université Ibn Zohr, Agadir, Morocco*

[c]*Faculté des Sciences et Techniques, Université Sultan Moulay Sliman, Béni –Mellal, Faculté des Sciences et Techniques, B.P : 523 Béni Mellal, Morocco*

[d]*Irstea, Hydrosystems and Bioprocesses Research Unit, 1 rue Pierre-Gilles de Gennes, F-92761 Antony, France*

ARTICLE INFO

*Article history:*
Received 23 June 2021
Received in revised form
6 December 2021
Accepted 8 December 2021

**Keywords:**
Leachates
Anaerobic conditions
Bottom ash
Methanosarscina
Trace elements

ABSTRACT

The present study deals with the possible pre-treatment of municipal solid wastes (MSW) in anaerobic conditions and their co-disposal with bottom ash. In this objective, the effect of the addition of bottom ash on organic matter degradation was checked using different analyses. The obtained results showed a great reduction of organic matter in the presence of bottom ash (53% of a solid fraction is degraded instead of 18% for the control after 26 days of degradation). Consequently, methane production was more important for the leachate from a bioreactor mixed with the bottom ash, which reached 70.10 L/Kg in comparison with 50L/Kg for leachate from the control. The characterization of leachates by molecular tools revealed that the microorganisms responsible for organic matter degradation in anaerobic conditions belong to the *Methanosarscina* genera, and the methanogenesis reaction was acetate-dependent methanogenesis. To better understand the role of bottom ash in the acceleration of methanogenesis, molecular tools were used to identify the microorganisms in the bottom ash leachate after a leaching test. The obtained results showed the absence of microorganisms in the bottom ash leachate. The quantification by atomic absorption spectrophotometry (AAS) of dissolved iron, nickel, and cobalt in the bottom ash leachate after the leaching test, showed that the amounts of these metals were 57.30, 0.035, and 0.006mM, respectively. Moreover, the quantification of iron, nickel, and cobalt in leachates from the bioreactor mixed with bottom ash and from the control showed that these amounts reached 94.78mM, 0.49mM, and 0.01mM after 26 days of degradation, and 99.40mM, 0.08mM, and 0.009mM, respectively, after 138 days of degradation. Consequently, it can be suggested that bottom ash must be co-landfilled with municipal solid

*Corresponding author: : +2126 66936463
E-mail: gaitbaddi@yahoo.fr
DOI: 10.22104/AET.2021.4988.1345

wastes at 9% as a suitable strategy, which will provide a more rapid chemical and microbiological stabilization of the municipal solid waste in landfills.

## 1. Introduction

Industrial development and the modern economy, in addition to demographic growth, have led to considerable production progress. Nevertheless, this growth has been accompanied by a worrying environmental degradation. The production of solid and liquid wastes has reached critical levels and is expected to increase more in the future due to rapid urbanization and industrialization [1]. In the European Union (EU), 2.5 billion tonnes of wastes are produced annually [2]. Sixty percent of this production is mainly represented by biodegradable wastes [2]. Moreover, around one-third of municipal wastes were sent to landfills in 2012 in the EU [3]. Other by-products, including bottom ash, which resulted from wastes incineration, are mostly destined for landfilling. Their use is very limited due to weak demand and economic repercussions [1]. Once these wastes are landfilled, a series of biologically mediated reactions are involved in refuse decomposition in anaerobic conditions. The performance of an anaerobic degradation is tied closely to the structure of the involved microbial community. Efforts to optimize anaerobic digestion processes to increase organic matter degradation and methane production during MSW landfilling have led scientists to adopt many procedures like leachate recirculation. Other strategies, such as mechanical–biological pre-treatment (MBP), are also proposed to minimize the environmental, health, and security problems resulting from landfilling [1,2] and to increase organic matter degradation and methane production. In the literature, the aspect of MSW co-landfilling with other compounds like bottom ash is rarely studied [1,4-7]. These works proposed the use of bottom ash as a soil covering, which can procure the potential positive effects on landfill practice. However, the knowledge about co-disposal in general and especially with bottom ash is still not fully known. Moreover, the assessment of the effect of using MBP on the microbial community structure has rarely been investigated. Fortunately, advances in microbial ecology have made a complete characterization of anaerobic digestion systems [8]. Indeed, one of the revolutionary techniques to study microbial ecology is fluorescence in situ hybridization (FISH). Another revolutionary and developed analysis is the automated ribosomal intergenic spacer analysis (ARISA), which allows for investigating with more diversity at the intraspecific level by inspecting the intergenic 16S–23S internally transcribed spacer sequences (ITS) within the rDNA operon. Another promising method is the determination of the concentrations and isotopic compositions (13C/12C) of leachate, which permits quantifying methane consumption. These approaches are shown to be more efficient, reliable, and less time-consuming, giving assessment to specific taxonomic groups and estimating richness and community composition. The objective of this study is to follow the evolution of the mixture MSW-Bottom ash (9% d.w.) to increase the biological activity and to reduce the post-closure monitoring of landfills expected over 30 years. For this purpose, the chemical and microbial changes that occurred in MSW leachate during pre-treatment by adding bottom ash (9% d.w.) in comparison with the control in anaerobic degradation were followed. The FISH, ARISA, isotopic, and cloning methods were used to identify the micro-organisms responsible for MSW anaerobic degradation during the pre-treatment. Also, the metals contained in the bottom ash leachate obtained after a leaching test and in leachates from bioreactors with and without bottom ash were analyzed by AAS.

## 2. Materials and methods

The MSW was shredded at 20mm and reconstituted according to the MODECOM method (Method of MSW Characterization) (Table1), representing the average MSW composition in France [9].

**Table 1.** The average composition of MSW in France [9].

| Waste Category | Waste | Quantity (g dry weight) |
|---|---|---|
| Putrescible fraction | Alfalfa | 1.16 |
| | Coffee | 0.80 |
| | Potato | 6.68 |
| | Chicken | 1.72 |
| | Bread | 1.16 |
| Paper | Packaging paper | 0.56 |
| | Journal and booklet | 1.44 |
| | Magazines | 1.88 |
| | Other | 2.52 |
| Paperboards | Plate Packaging | 1.40 |
| | Undulated Packaging | 2.32 |
| Composed | Complex Packaging | 0.48 |
| | Other | 0.08 |
| Textile | Packaging | 0.08 |
| | Other | 0.96 |
| Sanitary textile | Disposable diapers | 1.24 |
| Plastic | Polyolefins films | 2.68 |
| | PET bottles | 0.20 |
| | Polyolefins bottles | 0.36 |
| | PVC | 0.60 |
| | Polyesterin Packaging | 0.60 |
| Wood | | 1.28 |
| Glass | | 5.24 |
| Metals | Aluminium | 0.28 |
| | Iron, Copper | 1.36 |
| Non Classified « Incombustibles » | Sand | 2.72 |
| Special wastes (no toxic) | Drugs packaging | 0.20 |

40 g of MSW were disposed of in 1 L bioreactors closed with a septum and a screw cap, with 680mL of buffer solution (mixture of 6mL of $NaHCO_3$ 0,151M and 1.6mL of $K_2CO_3$ 0,032M in 1L of deionized water). A 330 mL headspace was maintained in each bioreactor. To establish anaerobic conditions in the bioreactors, headspaces were flushed with helium until the oxygen concentration fell below 0.5% before running the bioreactors. The experimental bioreactors were incubated at 35 °C (Figure 1). Control bioreactor B1 was filled with the reconstituted waste and the buffer solution; bioreactor B2 was inoculated with 3.25 g of bottom ash (9% of the dry weight of MSW) to accelerate the methanogenesis potential. This percentage is deduced from previous experiments that showed 9% is the ideal quantity that positively influenced methanogenesis.

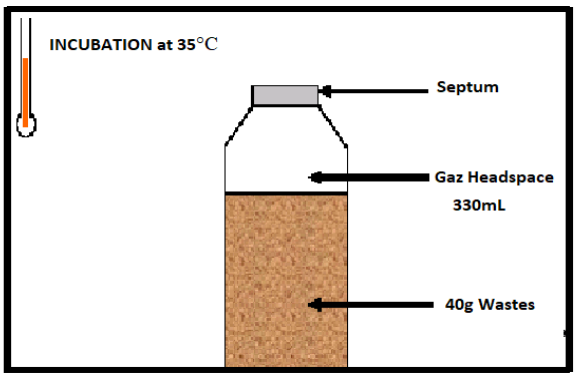


**Fig. 1.** Schematic diagram of the Bioreactor.

The monitoring of bioreactors 'evolution consisted to determine several analyses:

*2.1. Physico-chemical analyses*

The gas production was measured twice a week directly in the headspace using a manometer (Merical, 0 2000 mbar) equipped with a needle. The pressure in the headspaces was then equilibrated to atmospheric pressure. The biogas composition was analyzed by mGC Varian CP4900 chromatograph, fitted with four chromatographic columns. The latter was combined with thermal conductivity detectors (TCD); helium was the carrier gas. A commercial gas mixture carrying 0.5% $H_2S$, 3% $N_2O$, 40% $CO_2$, 50% $CH_4$, and 6.5% N2 (Air Products) was used. The oxygen calibration was done with air. The detection limit for all the gases was below 0.1% [10]. After analyzing the biogas, leachate samples were taken similarly, from every bioreactor (4mL) from their septum using a syringe, and the pH was immediately measured (a Mettler Inlab 427 probe). 2mL of leachate was centrifuged, and at the end, the raw (2mL), supernatants (2mL), and pellets leachate samples were kept at –20 °C. The amounts of Total Organic (TOC) and Inorganic Carbon (TIC) were analyzed in the leachate samples using BIORITECH700.

*2.2. Fixation for Fluorescence In situ Hybridization (FISH)*

The pellets of the centrifuged leachate (1mL) were washed with phosphate-buffered saline 1X (Sigma). After centrifugation, the pellets were re-suspended in 100 µL of phosphate-buffered saline PBS 1X (Sigma) and 300 µL de paraformaldehyde 20% (Sigma). After two hours of incubation at 4°C, these samples were centrifuged (13000 rpm, 10 min.). Then, the samples were fixed with 250 µL of PBS 1X and dehydrated with 250 µL of pure ethanol; then, kept at -20°C.

*2.3. FISH*

The 16S rRNA-targeted oligonucleotide probes manipulated in this study are Arch915, Eub338, EubII, EubIII, Euiry499, MSI414, and MS821 [11]. 10 µL of the fixed samples of leachate from bioreactors B1 and B2 were disposed of in wells of slides and dried in an incubator at 46 °C for 10 min. Once the wells on the slide were dried, they were dehydrated by soaking the slide in 50%, 80%, and 100% of ethanol for three minutes at a time, starting with 50% ethanol and ending with 100%. After the evaporation of ethanol, a hybridization buffer (180µL of NaCl (5M), 20µL of tris HCl (1M pH8), 600µL of distilled water, 2µL of SDS (10%), and 200µL of formamide was added. Also, a mixture of probes was added, and the slides were incubated at 46 °C for 3H [11]. Then, the slides were washed and incubated for 10 min. in a washing solution (1mL tris HCl, 2150µL of NaCl, 2500µL of EDTA (0,1M), 50 mL of distilled water, and 50 µL of SDS (10%)). To visualize probe positive signals, the slides were observed with a Zeiss Confocal Laser Scanning Microscope (CLSM, LSM510-META) equipped with three lasers (Argon 488 nm, Helium-Neon 543 nm, Helium-Neon 633 nm).

*2.4. Genomic DNA extraction*

DNA was obtained from the leachate of B2 and bottom ash according to [11].

*2.5. PCR amplification of 16S rDNA genes*

The 16S rDNA genes were amplified using primers specific for the domain Archaea 8aF 5′ TCY GGT TGA TCC TGC C 3′ and 1114aR 5′ GGG TCT CGC TCG TTR CC 3′ that were designed to encompass the Euryarchaeal, Crenarchaeal, Korarcheaeal, and Nanoarchaeal subkingdoms [11]. The PCR mixture contained 1mLof DNA, 12mLof the primers, 12mL of deoxynucleoside triphosphate mix (25 mM each) (Stratagene), 9mL MgCl2, 25 mM, 0.75mL of Taq commercial buffer (10x), 0.5 U Thermo-StartR DNA polymerase (ABgene), and sterile Millipore water to a volume of 150 mL. The PCR thermal profile was an initial denaturation at 94 °C for 10 min and 30 cycles consisting of denaturation at 94 °C for the 30s, primer annealing at 60 °C for one min, and extension at 72 °C for 1.5 min. The final elongation step was extended to seven min. A negative control without DNA was included in the experiments. The PCR products were analyzed by 1% (w/v) agarose gel-electrophoresis stained with ethidium bromide. The PCR products (1100 bp) were cut from the gel and purified by the Wizard PCR Clean-up Kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions.

*2.6. ARISA*

ARISA fingerprinting of bacterial communities was used to gain more information about the genetic structures of bacterial consortium in the presence and the absence of bottom ash 9%. As described above, this method exploited the polymorphism between the intergenic space (IGS) between rrs (ARNr16S coding) and rrl (ARNr 23S coding) genes. The polymorphism of IGS varied between 50 pb et 10380 pb for prokaryotes. The IGS of the DNA from different stages of degradation (0, 40, 80, 100, 160, 280 days) in leachates from the control (without bottom ash) and bottom ash bioreactors were amplified by using the primers 71R(5'-TCGGYGCCCGAGCCGAGCCATCC-3') and 915F (5'-GTGCTCCCCCGCCAATTCCT-3') [12]. One of the primers was labeled with the FITC fluorochrome, which permitted the separation of ARISA-PCR fragments by capillary electrophoresis on the bioanalyzer (Agilent DNA 7500 Kit (Agilent Technologies Inc., CA)). The ARISA profiles were analyzed using the Agilent software [13]. A principal components analysis (PCA) was realized on a matrix of the obtained results in the absence and presence of bottom ash in the leachates.

*2.7. Cloning and sequencing of 16S rDNA*

The cloning and sequencing of 16SrDNA were done according to [11].

*2.8. Isotopic composition analysis*

The carbon isotope composition of leachate was quantified with a gas chromatography-combustion-isotope ratio monitoring mass spectrometry (GC-CIRMS) system (MAT-252 FINNIGAN, Bremen), according to [18].

*2.9. Determination of metals concentration in leachates from B1 and B2 and in bottom ash leachate obtained after leaching test*

The leaching test was realized by adding 8mL of pure water (mQ) to 1g of bottom ash (ratio 1/8 g/mL w/v). The mixture was agitated for 24 H. After centrifugation at 8000 rpm for 10 min, the mixture was filtrated at 0.45m, and the HCL (1%) was added. The nickel (Ni), cobalt (Co), and iron (Fe) concentrations were determined by using AAS spectrophotometry. The Ni, Co, and Fe concentrations in the leachates from bioreactors B1 and B2 were determined by using AAS.

## 3. Results and discussion

*3.1. Evolution of physico-chemical parameters*

The effect of biological stabilization of MSW landfill leachate can be described by using various analytical variables such as Total Organic Carbon (TOC), Total Inorganic Carbon (TIC), carbon dioxide ($CO_2$), and methane ($CH_4$) formation [10]. The repartition of carbon fractions in both bioreactors with and without bottom ash in laboratory conditions is shown in Figure 2.

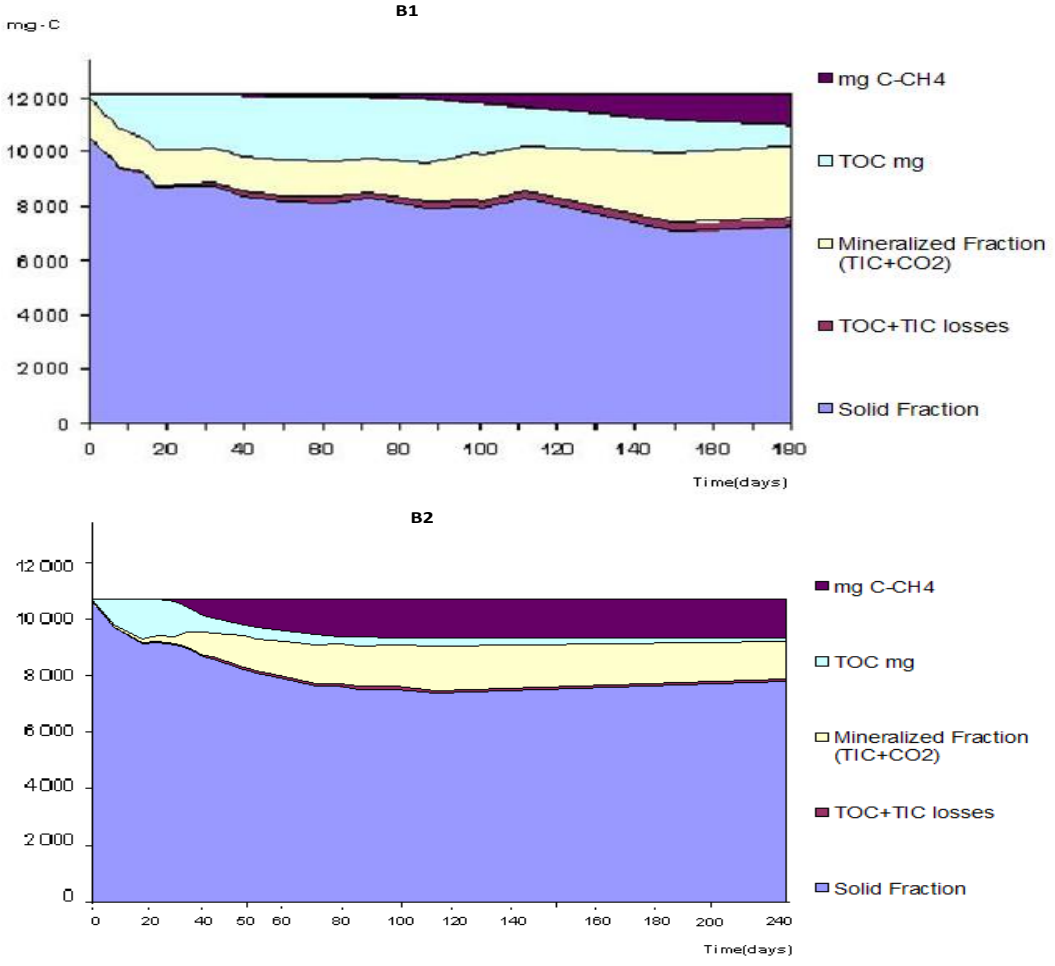


**Fig. 2.** Carbon repartition in the leachate from control bioreactor (B1) and from bioreactor of pre-treatment with bottom ash 9% (B2).

Once the anaerobic degradation started, the solid fraction decreased, especially in the case of bioreactor B2. Indeed, after only 26 days of degradation, 53% of the solid fraction degraded. And the value of the solid fraction was about 5144 mg in leachate from B2. However, in the case of leachate from the control, only 18% of a solid fraction degraded, and the value of the solid

fraction was about 8956mg after 26 days. The solid fraction degradation led to an increase of TOC, TIC, and $CO_2$ during the first phase of degradation, which is called the fermentative phase, in both bioreactors B1 and B2. This degradation was more important in the case of leachate from the bioreactor inoculated with bottom ash B2. Parallel to this evolution, the pHs started from 8.38 and dropped to 5.07 for B1 and 6.48 for B2 (Figure 3). The reduction of pH could be due to the liberation of volatile fatty acids (VFAs). In this respect, [15] attributed the reduction of pH to the accumulation of acidic fermentation intermediates. Additionally, [16] explained that in the fermentative phase, carbohydrates, proteins, and lipids are degraded to VFAs, which are metabolized to methane in the methanogenic step. In the present study, the latter step begins after 37 days of anaerobic degradation of leachate in bioreactor B2 and after 80 days in B1. Further, [17] explained that leachate contained a combination of inorganic, organic, and bacteriological compounds accompanied by waste going through biodegradation. The pH of the leachates increased to reach 7.65 for B2, indicating the beginning of the methanogenic environment and the subsistence values over 7.5 until the end of the degradation. However, the pH remained acid (5.67) for leachate from B1. The pH increase in the case of B2 was due mainly to the consumption of VFAs by the methanogenic populations. In this respect, [5] studied the possibility of the co-landfilling of 70% of pre-treated municipal solid wastes with 30% of bottom ash in anaerobic and aerobic conditions. They explained that the presence of bottom ash in the anaerobic reactors limited the duration of the acid phase, owing to the reduction of acid compounds and thus stimulated a more rapid transition into the methanogenic phase. The present study results regarding anaerobic conditions are in good agreement with the findings of the latter studies. Indeed, Figure 1 shows a marked increase in methane production in the case of pre-treatment with bottom ash B2, which reached 70.10 L/Kg STP. However, methane production reached 50 L/Kg (STP) in leachate from control B1. Consequently, the present results showed that the co-disposal of bottom ash directly with MSW at 9% (d.w.) accelerated the length of the acidogenic phase and improved the methanogenesis, which starts early and leads to important methane production. Therefore, the co-landfilling of the bottom ash at 9% with MSW allowed for a quick passage to the methanogenic phase and permitted the acceleration of landfill stability.

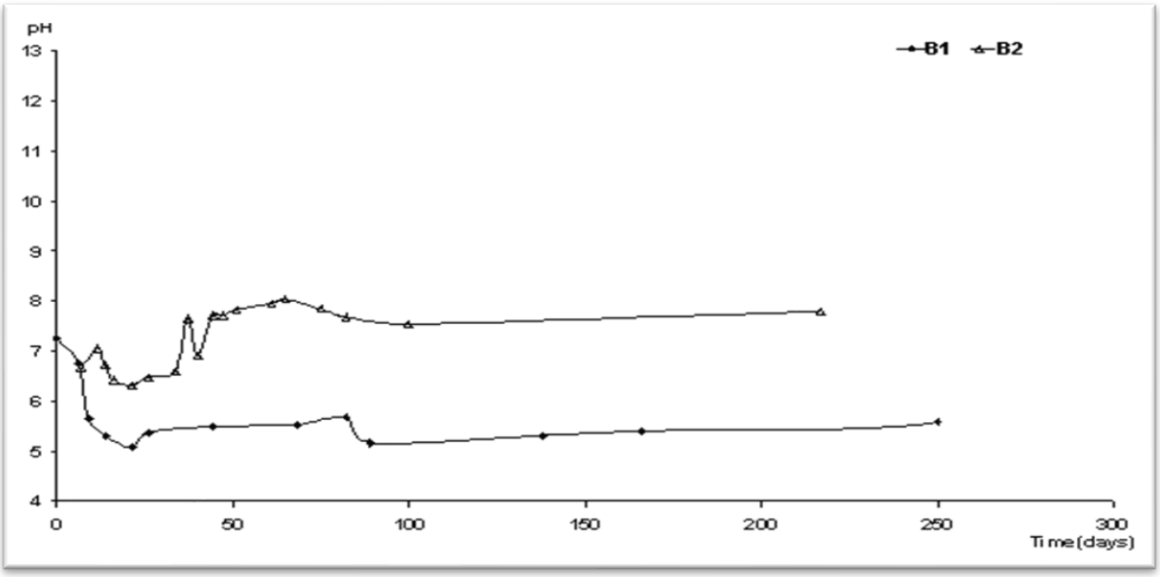


**Fig. 3.** pH leachate evolution during different stages of anaerobic degradation of bioreactors B1 and B3.

### *3.2. Characterization of microbial communities in leachates from bioreactors B1 and B2*

A Confocal Laser Scanning Microscopy (CLSM) was used to visualize the identified microorganisms during anaerobic degradation (Figures 4, 5, 6). The observation of the hybridization experiments using probe Arch915 Cy5 and Eub338 I, II, and III Cy3 at the initial stage and after 44 and 54 days respectively for B1 and B2 revealed that the microorganisms in these leachates belong to the archaea and bacteria domains. It is important to note that in the leachate from bioreactor B1, the bacteria were more developed after 44 days of degradation. However, the archaea were more developed after 54 days of degradation in the leachate from bioreactor B2. The use of the probes MS1414 Cy5 and Arc 915 Cy3 showed that archaea hybridized with MS1414, which confirmed their affiliation to the family of Methanosarcinaceae, and especially to the *Methanosarcina* species and close relatives. Similarly, the use of MS821 Cy3 and Eury 499 Cy5 (Figure 4) confirmed that these archaea belong to the order of Methanomicrobiales and to *Methanosarscina* gender. with the mixture of the probes MS821 Cy3 and Eury 499 Cy5 according to the FISH technique at 0 and 44 and 54 days of incubation, respectively. In this respect, [11] reported the existence of bacilli-like bacteria, which accompany Methanosarcinales and Methanomicrobiales archaes. The totality of the hybridization realized in the present work showed that *Methanoscarcina* microorganisms were well developed in the case of leachate from bioreactor B2 at 54 days, which confirmed the previous results obtained in the present study. After only 138 days of degradation, these microorganisms were still present in the leachate from bioreactor B2 but not very developed (Figure 5) as in the stages before. Additionally, these microorganisms were present in the leachate from bioreactor B1 after 138 days of degradation (Figure 6). Since the degradation in laboratory conditions takes 200 days in the presence of a buffer solution, we can conclude that bottom ash addition (9%) permitted an activation of *Methanoscarscina* microorganisms and a fast transition to the methanogenesis phase; therefore, the acceleration of stability of degradation in laboratory conditions (138 days). The ARISA results revealed 34 peaks as the high number, and the fragment length fluctuated from 50 to 10 380 bp. One important band was found and is more developed in the leachate samples in the presence of bottom ash 9% that varied between 920 and 930 bp (Figure 7). The comparison with the DNA of *Methanosarscina mazei* showed the presence of one band at 927 bp, which proved that in the presence of bottom ash 9%, the *Methanosarscina* were well developed. Additional peaks, which are characteristics of low fluorescence intensity, were observed and can be related to less dominant communities. Meanwhile, notable differences were detected between samples without bottom ash (control) and samples with bottom ash 9%. These results were confirmed by those of the PCA of ARISA profiles. Indeed, the ARISA results were converted into a model based on the presence and absence of peaks in each sample. A principal component analysis based on the obtained model was applied to compare the samples (Figure 8). The results obtained revealed the presence of mainly one group constituted by the last stages of degradation in the leachates with bottom ash 9% (40, 160, 280 days), together with the *Methanosarscina mazei*. The remaining stages of degradation in leachates in the absence, as in the presence of bottom ash at 9%, constituted other groups far from *Methanosarscina mazei*. A 16SrDNA library was created from the total-community genomic DNA extracted from the leachate using a universally conserved primer and an Archaea 16S rDNA-targeted primer [11]. Comparative inspection of the retrieved sequences showed that all the clones might be assembled in the Archaea domain [11]. Therefore, a maximum of the sequence types was combined with the genera inside the hydrogenotrophic Methanomicrobiales (*Methanoculleus*) and the methylotrophic and acetoclastic Methanosarcinales (*Methanosarcina*). These two dominant genera together accounted for 100% of the clones of the analyzed samples with bottom ash 9%. [17] highlighted the presence in landfill leachate of only two methanogenic genera, *Methanosarcina* and *Methanosaeta,* which are recognized to produce methane from acetate. Generally, *Methanosaeta spp*. has a less competitive edge over *Methanosarcina spp*. at high acetate concentrations [17]. Consequently, these authors assumed that *Methanosarcina* might be

supported by conditions in which a high load of organic matter conducts to the rapid accumulation of hydrogen and acetate in the landfill. However, these conditions are unfavourable for the slow-growing specialist *Methanosaeta*. In the present study, the obtained results were confirmed by the amounts and isotopic constitution of the leachate with 9% of bottom ash during different stages of degradation; the factor (αC) of isotopic fractionalization was also estimated to be 1.1 at the initial stage of degradation and dropped after only 40 days to reach 1.0015. This trend confirmed that methanogenesis is an acetate-dependent methanogenesis [18]. Based on these considerations, two theories were proposed to explain the role of bottom ash in the activation of *Methanoscarscina* microorganisms and the acceleration of methanogenesis. The first one consisted of the contribution to these compounds to add new microorganisms and\or to increase the amounts of the existing microorganisms. Recent studies of bottom ash from municipal solid wastes incineration [19,20] showed that it was not mineralized and still contained organic constituents. Further, [19] studied the influence of organic matter on bottom ash during the carbonation step. They explained that the remaining organic fraction in MSW bottom ash represented a probable source for chemical and microbiological processes and could give a substrate for microbial activity. Moreover, this microbial activity in bottom ash was due to the unburned organic matter in these compounds. To verify the hypothesis that bottom ash can contribute to adding some new microorganisms to the medium and\or to increase the amounts of the existed microorganisms, the FISH analysis was applied to the leachate from bottom ash obtained after the leaching test using the mixture of the probes MS821 Cy3, Arc915 Cy5, and Eub338 FITC (paragraph 2.2 and 2.3). The visualization by CLSM showed the absence of any microorganisms of Archaea and/or Bacteria. Genomic DNA from bottom ash and leachate from bioreactor B2 were extracted to confirm this result. The electrophoresis gel (Figure 9a) showed a unique band from DNA only in leachate from B2 and not in bottom ash, which can be correlated to the size of amplified DNA (approximately 1±0 kb). Similarly, the PCR amplification of 16S rRNA genes (Figure 9b) done with prokaryote-specific primers showed only a single band corresponding to the expected size of the amplified DNA (approximately 1±0 kb) for genomic DNA of the leachate of bioreactor B2 preparations on agarose gel electrophoresis. These results highlighted the absence of microorganisms and their activity in bottom ash, which are not in agreement with the works of [19,20]. A second theory is proposed to explain the methanogenesis acceleration in the presence of bottom ash (9%) and consists of the content of trace metals in bottom ash that are necessary for methanogenesis activity. Many studies [21,22,23,24,8,16] tried to explain the role of trace elements in methanogenesis. The data obtained from these studies showed that some metals such as Ni and Co are vital for methanogenesis because they are structural compounds of the cofactors vitamin B12 (Co) and factor F430 (Ni). The latter elements are key constituents of the enzyme system that catalyzed many reactions of the methanogenesis. Other authors [25] found that nickel is required by the methanogens, not only for the synthesis of factor F430 but also for incorporation into the protein fraction. Also, Co [26] reported that this element is also available in the carbon monoxide dehydrogenase (CODH) complex, which is in charge of the cleavage acetate and methylcob(III)alamin: coenzyme M methyltransferase, and consequently, is an important intermediate in methanogenesis. In the present paper, the quantification by AAS of dissolved iron (Fe), nickel (Ni), and cobalt (Co) in the leachate of bottom ash after the leaching test showed that these compounds were rich in Fe (57.30mM). However, the amounts of Ni and Co were 0.035mM and 0.006mM, respectively. Moreover, the quantification by AAS of the amounts of Fe, Ni, and Co in the leachates from B1 and B2 at different stages of anaerobic degradation (Figure 10a and 10b) showed that these amounts were more important in leachate from B2 compared to B1. Moreover, these amounts were more important in the methanogenic phase (26 days of degradation). Indeed, they reached 94.78mM for Fe, 0.49mM for Ni, and 0.01mM for Co in the case of leachate from B2. However, these amounts were about 99.40mM for Fe, 0.08mM for

Ni, and 0.009mM for Co after 138 days of degradation for leachate from B1. The concentrations in these elements hereby presented were not in good agreement with those reported by [26]. The latter authors reported that the optimal concentration of Co for the growth and activity of methanogenic microorganisms was about 0.05mg/L (0.84mM) and about 0.13mg/L (2.21mM) for Nickel in leachates for old landfills. The difference between the amounts of Fe, Ni, and Co and those found in our study may be due to the origin and age of the leachate, as well as to the stabilization processes. Otherwise, the decrease of the amounts of these metals could be due to the higher pH during the methanogenic phase, which enhances adsorption and precipitation, and a lower amount of dissolved organic matter that can complex the cations [26]. Although these amounts are inferior to those reported by the latter authors, they are in good agreement with the results of [27]. In this respect, [28] explained that the nickel-containing tetrapyrrole, cofactor 430 (F430), is of great importance to the methanogenesis reaction. Further, these authors reported that many catalytic mechanisms have been proposed for this reaction and can be fractionated into two basic models. In the first one, the basic intermediate points to a nickel methyl species. In the second model, a nickel-thiol bond is set up that subsequently results in the liberation of the methyl group as a methyl radical.

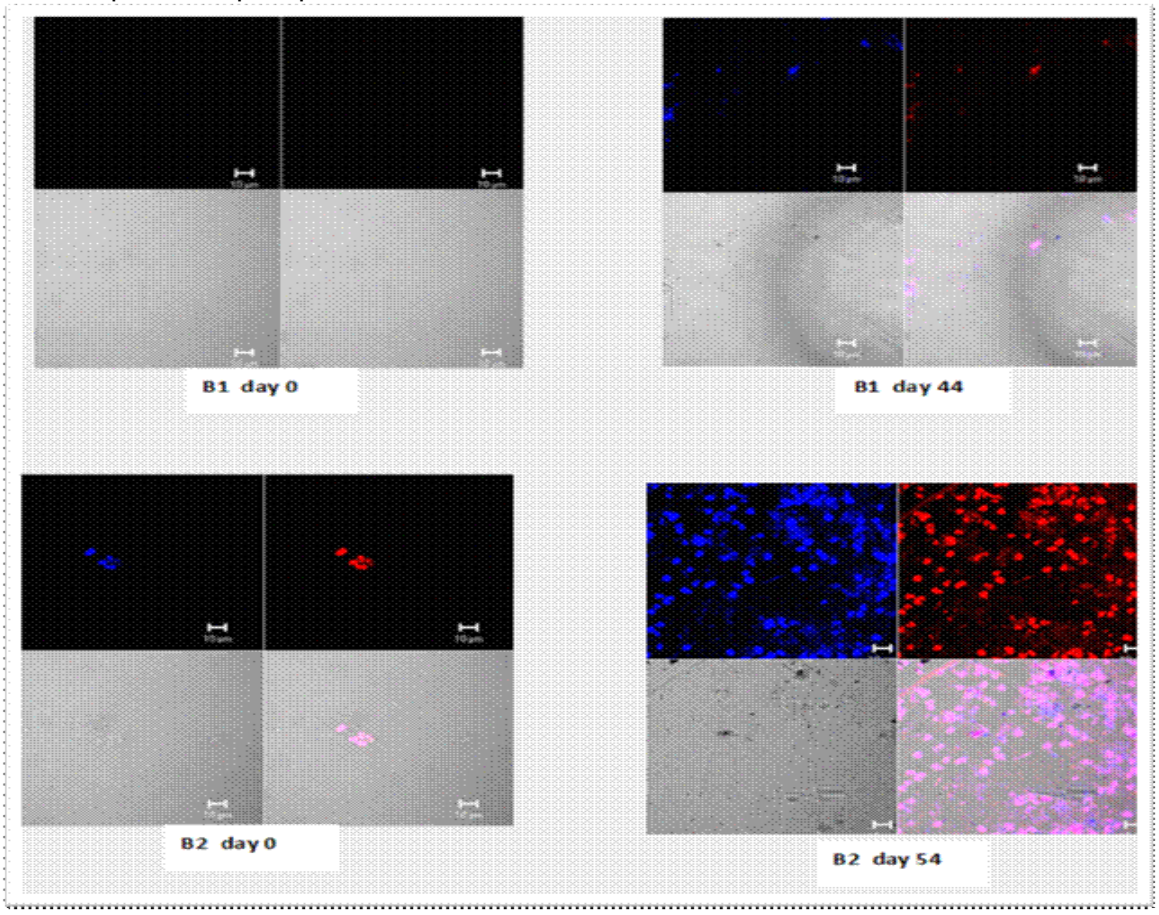


**Fig. 4.** Confocal Laser Scanning Microscopic observations of leachate from bioreactor B1 and B2 hybridized with the mixture of the probes MS821 Cy3 and Eury 499 Cy5 according to the FISH technique at 0 and 44 and 54 days of incubation, respectively.

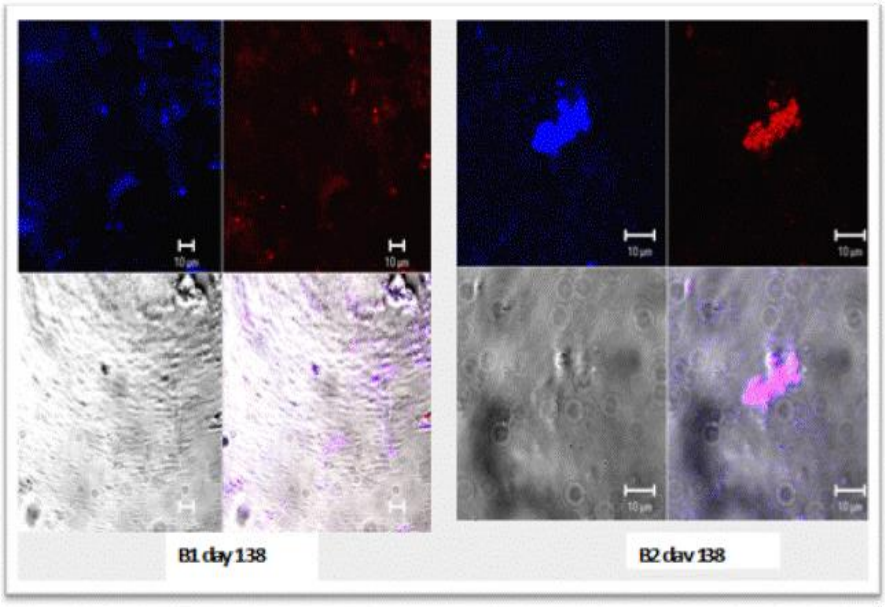


**Fig. 5.** Confocal Laser Scanning Microscopic results of leachate from bioreactors B1 and B2 hybridized with the mixture of the probes Arc915Cy5 and MS821Cy3 according to the FISH technique at 138 days of incubation.

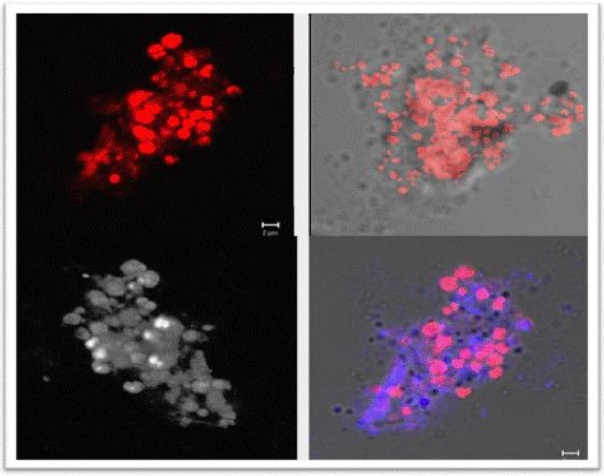

**Fig. 6.** Methanoscarcina leachate from bioreactors B1 and B2 hybridized with the mixture of the probes MS821 Cy3 and Eury 499 Cy5 according to the FISH technique at 54 days of degradation.

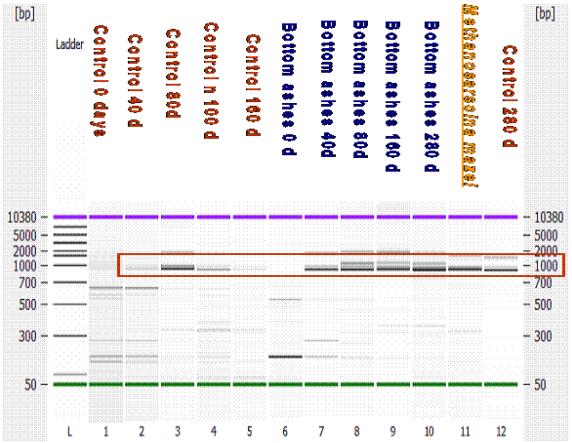


**Fig. 7.** ARISA (Automated Ribosomal Intergenic Spacer) profiles of DNA from leachates from control and bottom ash bioreactors at different stages of degradation (0, 40, 80, 100, 160, 280 days).

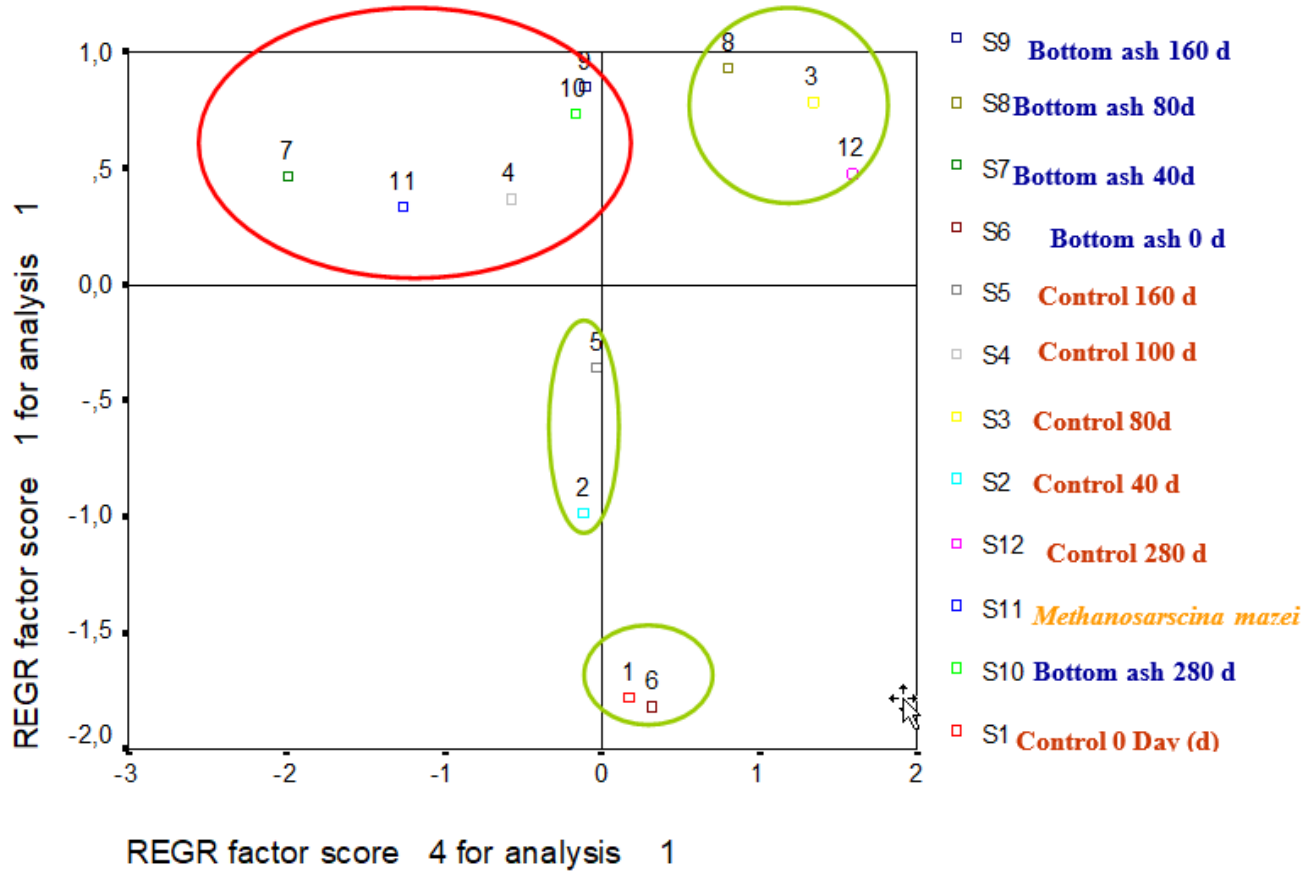


**Fig. 8.** PCA between the peaks of DNA in ARISA at different stages of degradation (0, 40, 80, 100, 160, 280 days) in leachates from the control and from bottom ash bioreactors.

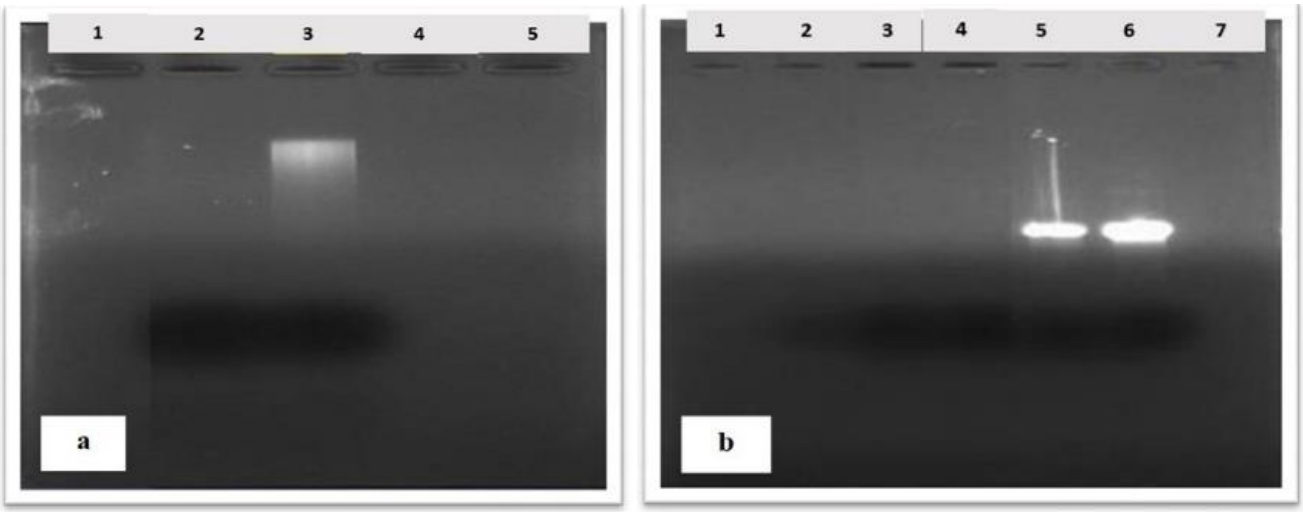


**Fig. 9.** Agarose gel electrophoresis of extracted genomic DNA (a) and PCR amplification products of 16SrRNA obtained using prokaryote-specific primers (b) from bottom ash (2 in Figure 9a and 4 in Figure 9b) and from pellets of B2 (3 in Figure 9a and 5 in Figure 9b). (NC and PC: negative and positive control are in lanes 2 and 6 respectively in Figure 9b).

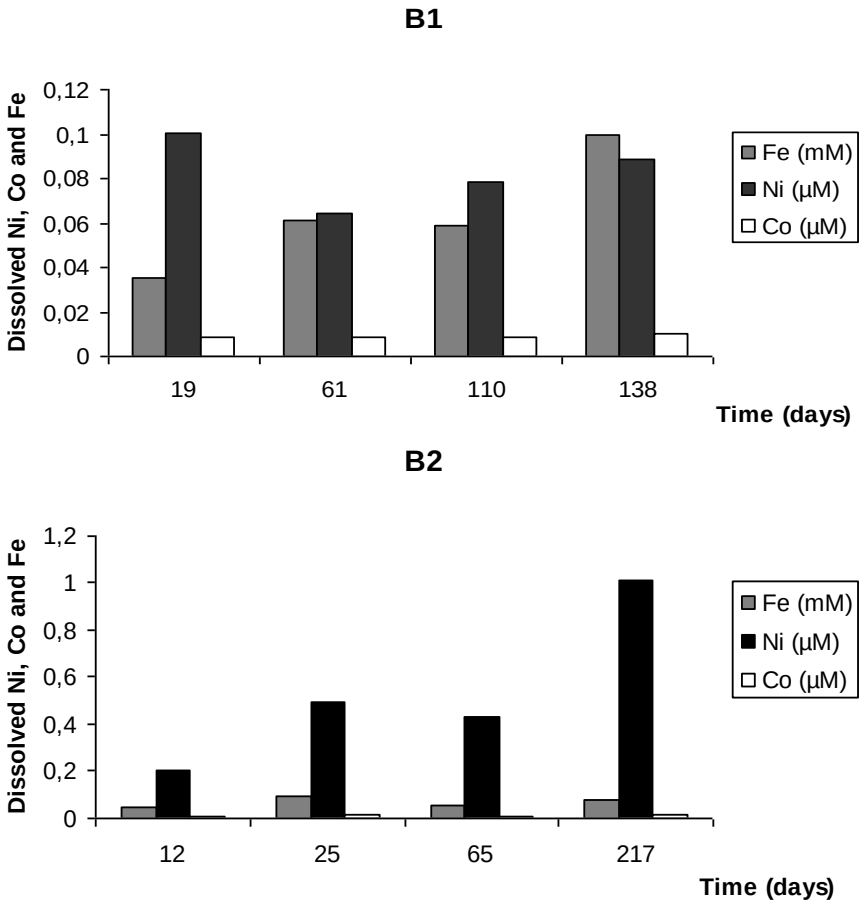


**Fig. 10.** Dissolved amounts of Ni, Co, and Fe in leachate from B1(a) and B2 (b) analysed by AAS.

## 4. Conclusions

The effect of chemical and microbiological behaviors of bottom ash (9% d.w.) co-disposed with municipal solid wastes during anaerobic degradation were studied and compared to a control without bottom ash in laboratory conditions. The results showed an important degradation of organic matter (53%) in the bioreactor with bottom ash in comparison with the control bioreactor (18%) after 26 days of degradation. Later, evolution led to an increase in methane production in the case of pre-treatment with bottom ash, which reached 70.10 L/Kg STP in comparison with 50L/Kg (STP) for the control. The use of FISH, ARISA, cloning, and isotopic analyses to study microorganisms in the presence and the absence of bottom ash showed that these microorganisms belong to *Methanoscarcina* genera and are more developed in the presence of bottom ash at 9% (d.w.). The results of quantification by AAS of the dissolved concentrations of Fe, Ni, and Co in the leachate of bottom ash after the leaching test showed that leachate from bottom ash was characterized by the amounts of Fe, Ni, and Co that are about 57.30mM, 0.035mM, and 0.006mM, respectively. This analysis showed that the inoculation with bottom ash increased the amounts of these compounds. Indeed, the amounts of Fe, Ni, and Co in leachate from B2 were 94.78mM for Fe, 0.49mM for Ni, and 0.01mM for Co after 26 days of degradation. However, these amounts were about 99.40mM for Fe, 0.08mM for Ni, and 0.009mM for Co for leachate from the control after 138 days of degradation. Therefore, the inoculation by bottom ash (9% d.w.) led to an increase in the amounts of Fe, Ni, and Co, which improved the microorganisms' activity. And this allowed for a faster transition to the methanogenesis phase and, consequently, methane production.

## Acknowledgments

The authors thank Pr. Benssaouia Amina for her help in the correction of English.

References

[1] Wang, Q., Ko, J. H., Liu, F., Xu, Q. (2021). Leaching characteristics of heavy metals in MSW and bottom ash co-disposal landfills. *Journal of hazardous materials, 416, 126042*.

[2] Pomberger, R., Sarc, R., Lorber, K. E. (2017). Dynamic visualisation of municipal waste management performance in the EU using Ternary Diagram method. *Waste management, 61*, 558-571.

[3] European Parliament Report (2017). Towards a circular economy – Waste management in the EU. *IP/G/STOA/FWC/2013-001/LOT 3/C3*. 140p.

[4] Lo, H. M. (2005). Metals behaviors of MSWI bottom ash co-digested Anaerobically with MSW. *Research conservation recovery, 43*, 263-280.

[5] Boni, M. R., Leoni, S., Sbaffoni, S. (2007). Co-landfilling of pretreated waste: Disposal and management strategies at lab-scale. *Journal of hazardous materials, 147* (1–2), 37-47.

[6] Lo, H. M., Chiu, H. Y., Lo, S. W., Lo, F. C. (2012). Effects of micro-nano and non micro-nano MSWI ashes addition on MSW anaerobic digestion. *Bioresource technology, 114*, 90-94.

[7] Wu, H., Wang, Q., Ko, J. H., Xu, Q. (2018). Characteristics of geotextile clogging in MSW landfills co-disposed with MSWI bottom ash. *Waste management, 78*, 164-172.

[8] Xu, H., Li, Y., Hu D., Zhao, Y., Chen, L., Zhou, L., Chen, G. (2021). Effect of microaerobic microbial pretreatment on anaerobic digestion of a lignocellulosic substrate under controlled pH conditions. *Bioresource technology, 328*, 124852.

[9] Ademe (French Environment and Energy Management Agency) "Les déchets en chiffres en France," 12p (2006).

[10] Ait Baddi, G., Antizar-Ladislao, B., Alcuta, A., Mazeas, L., Li, T., Duquennoi, C., Redon, E., Bouchez, T. (2013). Investigation of the degree of municipal solid waste stabilization during anaerobic degradation using fluorescence excitation-emission spectroscopy. *Environment engineering science, 30* (5), 232-240.

[11] Laloui-Carpentier, W., Li, T., Vigneron, V., Mazeas, L., Bouchez, T. (2006). Methanogenic diversity and activity in municipal solid waste landfill leachates. *Antonie van Leeuwenhoek, 89*, 423-434.

[12] Stahl, D. A., Amann, R. (1991). Development and application of nucleic acid probes.. In: Stackebrandt, E. and Goodfellow, M., Eds., Nucleic Acid Techniques in Bacterial Systematics. *John Wiley and Sons Ltd.*, Chichester, 205-248.

[13] Boulanger, A., Pinet, E., Bouix, M., Bouchez, T., Mansour, A., (2012). Effect of inoculum to substrate ratio (I/S) on municipal solid waste anaerobic degradation kinetics and potential. *Waste management, 32* (12), 2258-2265.

[14] AFNOR (1992). Norme AFNOR X31-210 "Déchets, essais de lixiviation," In: *Normalisation française*, 13 p.

[15] Barlaz, M. A., Schaefer, D. M., Ham, R. K. (1989). Bacterial population development and chemical characteristics of refuse decomposition in a simulated sanitary landfill. *Applied environment microbiology 10*, 55-65.

[16] Xu, Q., Qin, J., Yuan, T., Ko, J. H. (2020). Extracellular enzyme and microbial activity in MSW landfills with different gas collection and leachate management practices. *Chemosphere, 250*, 126264.

[17] Huang, L. N., Chen, Y. Q., Zhou, H., Luo, S., Lan, C. Y., Qu, L. H. (2003). Characterization of methanogenic Archaea in the leachate of a closed municipal solid waste landfill. *FEMS microbiology ecology, 46*, 2 171-177.

[18] Richnow, H. H., Meckenstock, R. U., Reitzel, L. A., Baun, A., Ledin, A., Christensen, T.H. (2003). In situ biodegradation determined by carbon isotope fractionation of aromatic hydrocarbons in an anaerobic landfill leachate plume (Vejen, Denmark). *Journal contaminants hydrology, 64,* (1-2) 59-72.

[19] Rendek, E., Ducom, G., Germain, P. (2006). Influence of organic matter on municipal solid waste incinerator bottom ash carbonation. *Chemosphere, 64*, 1212-1218.

[20] Rendek, E., Ducom, G., Germain, P. (2007). Assessment of MSWI bottom ash organic carbon behavior: A biophysicochemical approach. *Chemosphere,* 67, 8, 1582-7.

[21] Gonzalez-Gil, G., Kleerebezem, R., Lettinga, G. (1999). Effects of nickel and cobalt on kinetics

of methanol conversion by methanogenic sludge as assessed by on-line $CH_4$ monitoring. *Applied environment microbiology, 65* (4), 1789-1793.

[22] Zandvoort, M. H., Geerts R., Lettinga, G., Lens, P. N. L. (2003). Methanol degradation in granular sludge reactors at sub-optimal metal concentrations: role of iron, nickel and cobalt. *Enzyme microbial technology, 33*, 190-208.

[23] Zandvoort, M. H., Van Hullebusch, E. D., Gieteling, J., Lens, P. N. L. (2006). Granular sludge in full-scale anaerobic bioreactors: Trace element content and deficiencie. *Enzyme microbial technology, 39*, 337-346.

[24] Wang, J., Westerholm, M., Qiao, W., Mahdy, A., Wandera, S. M., Yin, D., Bi, S., Fan, R., Dong, R. (2019). Enhancing anaerobic digestion of dairy and swine wastewater by adding trace elements: Evaluation in batch and continuous experiments. *Water science technology, 80 (*9), 1662-1672.

[25] Diekert, G. U., Konheiser, K. Piechulla, R. K., Thauer, K. (1981). Nickel requirement and factor F430 content of methanogenic bacteria. *Journal bacteriology, 148*, 459-464.

[26] Christensen, B., Christiansen, T., Gombert, A. K., Thykaer, J., Nielsen, J. (2001). Simple and robust method for estimation of the split between the oxidative pentose phosphate pathway and the Embden-Meyerhof-Parnas pathway in microorganisms. *Biotechnology bioengeneering, 74*(6), 517-523.

[27] Jansen, S., Gonzalez-Gil, G., Van Leeuwen, H. P. (2007). The impact of Co and Ni speciation on methanogenesis in sulfidic media—Biouptake versus metal dissolution. *Enzymatic microbiology technology, 40*, 823–830.

[28] Yang, S., Winkel, M., Wagner, D., Liebner, S. (2017). Community structure of rare methanogenic archaea: insight from a single functional group. *FEMS microbiology, 93*(11), 126.